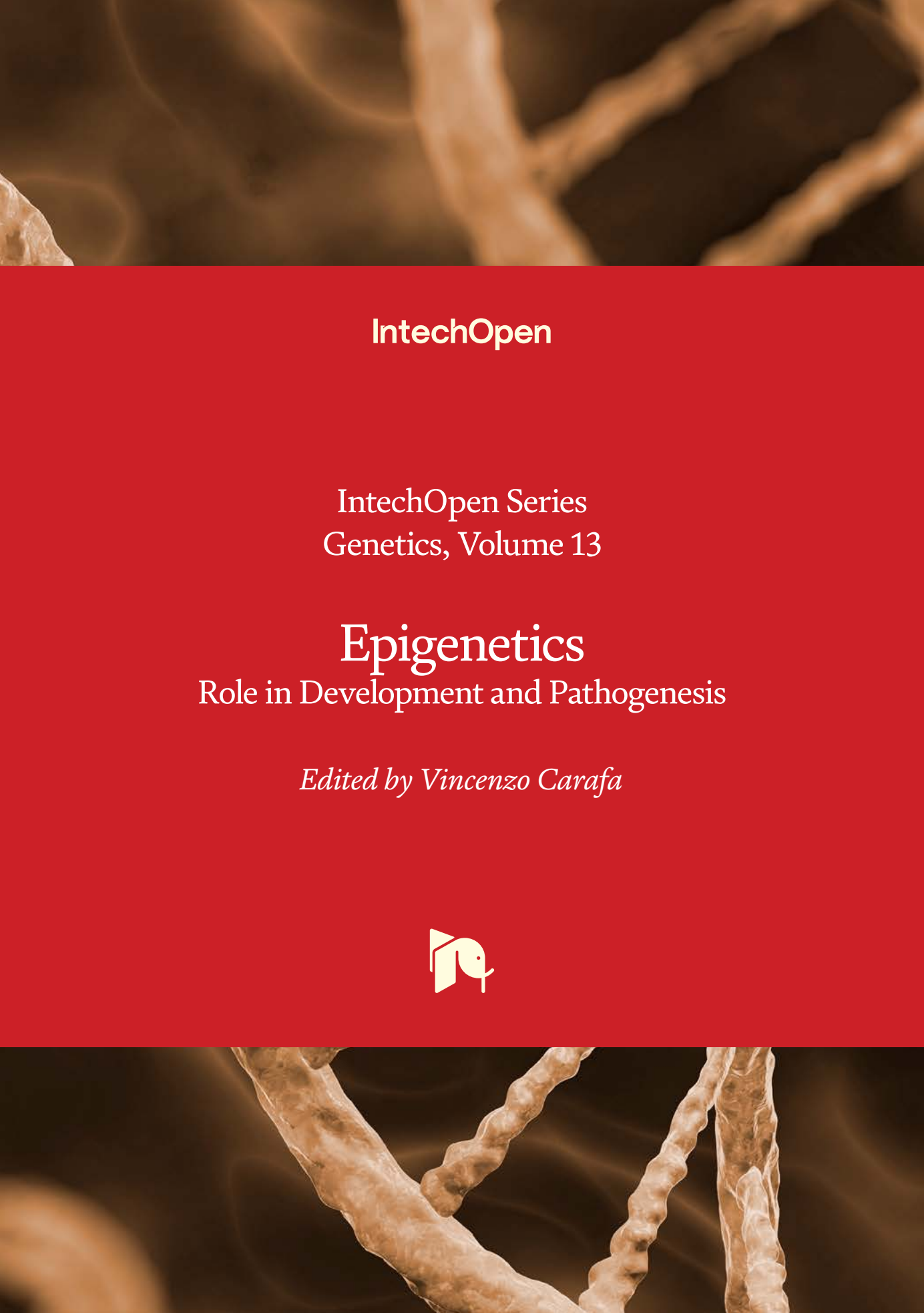




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Epigenetics

Role in Development and Pathogenesis

Edited by Vincenzo Carafa



Epigenetics - Role in Development and Pathogenesis

Edited by Vincenzo Carafa

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IntechOpen Book Series

Genetics

Volume 13

Aims and Scope of the Series

“Genetics,” which has been proud of its tradition since Mendel presented his research results in 1865, initially progressed quite slowly due to simple observational approaches of individuals and groups. However, the discovery of double-stranded DNA by Watson and Crick about 70 years ago triggered rapid progress in life sciences, including genetics, which was primarily conducted using *Escherichia coli* and bacteriophages infecting *E. coli*. Subsequently, genetics has achieved remarkable developments, such as understanding genetic disorders, including cancers, through research on the biogenesis and differentiation of plants and animals. The two topics of this book series - Human Genetics, and Genomics - will address important areas of advancement in genetics.

Human Genetics: After fundamental genetics, initially studied with the main goal of revealing the functions of individual genes and proteins, genetics expanded from understanding the genetic system itself to understanding many infectious diseases caused by bacteria and viruses. Consequently, human beings are now overcoming infectious diseases by developing medicinal chemicals, including antibiotics and vaccines. However, genetic disorders remain challenging to cure up to now. Nevertheless, even the cure for them, including various cancers, is coming closer to reality due to the rapid progress of human genetics. In this way, the welfare of human life continues to improve, and even longevity, which was once a dream, has been achieved to some extent in recent years.

Genomics: On the other hand, the understanding of the comprehensive interrelationship of whole genes or whole proteins functioning in one organism has become possible now, as research has entered the era of genomics, owing to the rapid progress of base sequence analysis and bioinformatics. The development of genomics has further made it possible to understand the evolutionary processes of organisms through comparative studies among the genomes of many organisms.

This book series will discuss the findings obtained during the advancement of human genetics and genomics. It is also expected that this series will trigger the formation of a better world composed of human beings and all other organisms on Earth through discussions of research results obtained under the development of general genetics.

Meet the Series Editor



Kenji Ikehara graduated from the Department of Industrial Chemistry, Faculty of Engineering, Kyoto University in 1968. He received his B. Eng. (1968) and subsequently earned M. Eng. (1970) and D. Eng. (1976) degrees from Kyoto University. He began his career as a research associate in the Faculty of Science at the University of Tokyo before moving on to become an associate professor in the Faculty of Science at Nara Women's University. He was later promoted to professor and subsequently served as the dean of the Faculty of Science at Nara Women's University. Additionally, he held the position of director at the Nara Study Center of the Open University of Japan. For approximately 15 years, he focused his research on sporulation initiation of *Bacillus subtilis*. Later, he shifted his focus to the origins and evolutionary processes of microbial genes, the genetic code, proteins, and life. He has proposed several hypotheses, including the GC-NSF(a) hypothesis on the origin of genes, the GNC-SNS hypothesis on the genetic code, the protein 0th-order structure hypothesis on the origin of proteins, and the [GADV]-protein world hypothesis (GADV hypothesis) on the origin of life. Furthermore, he served as the local chair of the International Conference, Origin 2014, held in Nara in 2014.

Meet the Volume Editor



Vincenzo Carafa is an Associate Professor of General Pathology at Università degli Studi della Campania “L. Vanvitelli.” He holds a Ph.D. in Signal Transduction Pathology and a master’s in Forensic Genetics. His research focuses on epigenetics and programmed cell death (PCD) mechanisms in cancer, leading to the identification of a novel SIRT inhibitor with tumor-selective properties. He has authored over 70 publications (H-index: 31) and participated in different EU and national projects. Prof. Carafa is active in teaching, mentoring, and editorial roles, serving on the board of Cells (MDPI). He has received multiple research grants and awards for his scientific contributions.

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Preface

Over the past few decades, the concept of epigenetics has gained increasing prominence in our understanding of human physiology and pathology.

Epigenetics is the study of heritable changes in gene expression that occur without altering the DNA sequence; these epigenetic modifications (epi-modifications) constitute a fundamental regulatory layer essential for cellular function and organismal development.

This book was assembled with a simple goal: to offer a coherent view of epigenetic regulation as a unifying language across disciplines that are often treated as autoimmunity, inflammation, cardiovascular and neurovascular disease, infection biology, and cancer separately. Across these fields, a recurring question emerges: What is the role of epigenetics? Epigenetic mechanisms provide plausible mechanistic answers because they govern chromatin accessibility and transcriptional competence, and they are exquisitely sensitive to context. Throughout the chapters, readers will encounter the major epigenetic platforms DNA methylation, histone modifications, and non-coding RNAs, viewed not as isolated phenomena but as an integrated regulatory network. The molecular logic is shared: specialized enzymes write, read, and remove epigenetic marks, while additional factors remodel chromatin architecture and influence higher-order genome organization. Together, these systems shape gene expression programs that determine cellular identity, plasticity, and the ability to respond appropriately to stress.

Several key themes are well described in this collection. Autoimmune disease and macrophage remodeling illustrate how epigenetic states can tune immune thresholds, reinforce tolerance or break it, and create persistent inflammatory “set-points.” In these contexts, epigenetics is not merely descriptive; it becomes an explanatory framework for chronicity, how transient cues can be converted into durable transcriptional behavior within immune cells and within tissues exposed to ongoing inflammatory pressure. Heart development and cardiovascular pathology are presented through an epigenetic lens that connects developmental gene control with maladaptive remodeling in adult disease. Neurovascular injury (stroke) highlights a different kind of plasticity: rapid epigenetic shifts occurring after acute damage, shaping both inflammatory cascades and repair programs. These contributions emphasize timing and cell type as critical determinants of an epigenetic change that can be protective in one setting and harmful in another. Cancer-related chapters explore epigenetic dysregulation as both driver and consequence of malignant evolution. The collection spans classic chromatin dysfunction in leukemia to the intersection of inflammation, metastasis, and therapy resistance in ribosomopathies, and it also considers environmental disruption in endometrial cancer. Importantly, these chapters do not treat “epigenetics” as a single pathway: they show how altered methylation patterns, histone-mark redistribution, chromatin remodeling, and non-coding RNA circuits can converge on shared phenotypes, such as immune

evasion, invasive behavior, and treatment adaptation, while still offering disease-specific vulnerabilities. Lactylation-driven modulation positions metabolism at the center of chromatin regulation, particularly relevant to vascular aging and chronic inflammatory states. Exercise-induced microRNA changes address a translationally attractive question: whether lifestyle interventions can reshape regulatory RNA networks in ways that matter for inflammation-associated carcinogenesis. Host–pathogen dynamics extend the discussion beyond non-communicable disease, emphasizing that infections can reprogram host epigenetic machinery, while hosts deploy chromatin-based defense strategies, an evolutionary dialogue written, in part, onto chromatin. Finally, this collection reflects the practical relevance of epigenetics: its relative reversibility. Unlike fixed genetic lesions, epigenetic alterations can, in principle, be modified, and this has fueled a growing therapeutic effort to “rewrite” aberrant regulatory states. The promise of pharmacoepigenomics extends beyond new drugs to better stratification, improved biomarkers, and a more realistic path toward personalized interventions for diseases shaped by gene–environment interplay. We hope readers will use these chapters in two ways: as focused, state-of-the-art reviews within each disease area, and as a connected map of how epigenetic regulation links inflammation, metabolism, environment, and cellular identity.

The take-home message is quite clear: epigenetics is not an accessory to genetics, but one of the primary mechanisms through which biology transforms exposure into function and dysfunction into disease.

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Section 1

Epigenetics and Immune
Regulation

Introductory Chapter: Epigenetics and Its Involvement in Disease

Vincenzo Carafa

1. Introduction

Over the past few decades, the concept of epigenetics has gained increasing prominence in our understanding of human physiology and pathology. Traditionally, molecular biology focused primarily on DNA sequence as the sole repository of genetic information, emphasizing that mutations such as point mutations, deletions, chromosomal translocations, or gene amplifications were the key drivers underlying the onset and variability of human diseases. However, it has become increasingly clear that gene expression is not dictated solely by the nucleotide sequence but is also regulated by dynamic and reversible modifications that do not alter the underlying DNA code, many of which arise in response to environmental stimuli [1]. In this context, epigenetics is defined as the study of heritable changes in gene expression that occur without alterations in the DNA sequence. These epigenetic modifications (epi-modifications) represent a fundamental regulatory layer essential for cellular function and organismal development. The main recognized epigenetic mechanisms include DNA methylation, histone modifications, and non-coding RNAs (ncRNAs), all of which dynamically and often reversibly modulate gene activity. Each mechanism influences chromatin accessibility and structure, thereby regulating gene transcription and subsequent protein expression. Importantly, these processes act in close synergy, forming a complex epigenetic regulatory network.

Unlike genetic mutations, epigenetic modifications are highly susceptible to environmental influences and can vary throughout an individual's life in response to nutritional, toxic, inflammatory, or behavioral factors [2]. Central to these processes are specific enzymes commonly categorized as writers, readers, and erasers. Writers add chemical marks, such as methyl or acetyl groups, to histones or DNA; readers recognize and interpret these marks to modulate transcriptional activity; erasers remove these modifications, rendering the processes largely reversible. Additional regulatory factors, often termed movers, shapers, and insulators, physically remodel chromatin or act as structural and functional barriers, further contributing to epigenetic regulation [3].

1.1 Epigenetic involvement in pathology

Epigenetic modifications play key roles not only in gene expression but also in maintaining genomic stability, directing cellular differentiation, and mediating responses to environmental signals [4]. Dysregulation of these mechanisms has been increasingly linked to different pathological conditions, including autoimmune

diseases, neurological disorders, cardiovascular diseases, and notably, cancer. For instance, in cancer, aberrant DNA methylation patterns can silence tumor suppressor genes or activate oncogenes, promoting uncontrolled cell proliferation. Chromatin structural alterations may also facilitate epithelial-mesenchymal transition, enhancing tumor invasiveness and metastatic potential.

Similarly, in diabetes, recent studies have identified DNA methylation changes in critical tissues such as pancreatic islets, adipose tissue, and skeletal muscle, suggesting an epigenetic component in disease onset and progression. These changes can affect insulin production, secretion, and cellular sensitivity, highlighting the intersection between genetics and the environment, where factors like diet and lifestyle directly shape the epigenetic landscape.

Neurodegenerative diseases, including Alzheimer's and Parkinson's, are also associated with epigenetic alterations, such as DNA methylation and histone acetylation changes, impacting gene expression related to neuronal plasticity, apoptosis, and toxic protein accumulation. Cardiovascular diseases similarly exhibit epigenetic dysfunction, with modifications influencing genes involved in blood pressure regulation, lipid metabolism, and inflammation. Notably, histone modification changes have been linked to cardiac hypertrophy and atherosclerosis development.

Major psychiatric disorders, such as autism spectrum disorder, bipolar disorder, schizophrenia, and major depressive disorder, are complex conditions arising from the interplay of multiple genes. Epigenetic regulation, influenced by environmental factors including diet, gut microbiota, and infections, plays a crucial role in their pathogenesis. For example, specific nutrients and intestinal microbes can modify DNA methylation and histone marks, impacting gene expression relevant to brain function [5].

2. Purpose and goals

The aim of this collection is to gather a wide range of scientific studies that explore the role of epigenetics in various pathologies. In recent years, it has become increasingly evident that epigenetic alterations contribute to the development and progression of numerous diseases, including cancer, neurodegenerative disorders, inflammatory diseases, autoimmune conditions, and metabolic syndromes. This underscores the interest in compiling and presenting diverse works to address the topic from multiple perspectives. The chapters will describe the specific role of epigenetics in individual diseases or will focus on experimental approaches and/or the molecular mechanisms involved.

Understanding the role of epigenetics in pathology opens new avenues for early diagnosis, disease monitoring, and the development of targeted and personalized therapies. Through this collection, we aim to provide an updated and comprehensive overview of the scientific evidence linking epigenetic mechanisms to several pathological contexts, thereby promoting greater understanding and encouraging further research in this rapidly evolving field.

3. Conclusion(s)

A particularly exciting aspect of epigenetics is its potential reversibility, offering promising therapeutic opportunities. The ability to “rewrite” aberrant epigenetic

profiles has spurred the development of a new generation of epigenetic drugs designed to restore proper protein function. This emerging field of “pharmacoepigenomics” aims to develop therapies that can “reprogram” the cellular epigenome and correct pathological alterations. Thus, epigenetics not only expands our understanding of disease mechanisms but also paves the way for personalized therapeutic strategies. In summary, a deeper understanding of the molecular mechanisms underlying epigenetic processes represents a pivotal step toward unraveling cellular and pathological complexity. Epigenetics has evolved from being a mere complement to classical genetics into an essential framework for comprehending cellular identity regulation, gene-environment interactions, and the etiology of numerous complex diseases.

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References

- [1] Deans C, Maggert KA. What do you mean, “epigenetic”? *Genetics*. 2015;**199**(4):887–896. DOI: 10.1534/genetics.114.173492
- [2] Anjaria P, Asediya V, Nayak J, Koringa P. The epigenetic landscape: How environmental cues shape gene expression. *Epigenomics*. 2023;**15**(5):267–270. DOI: 10.2217/epi-2023-0112
- [3] Biswas S, Rao CM. Epigenetic tools (The Writers, The Readers and The Erasers) and their implications in cancer therapy. *European Journal of Pharmacology*. 2018;**837**:8–24. DOI: 10.1016/j.ejphar.2018.08.021
- [4] Farsetti A, Illi B, Gaetano C. How epigenetics impacts on human diseases. *European Journal of Internal Medicine*. 2023;**114**:15–22. DOI: 10.1016/j.ejim.2023.05.036
- [5] Zhang L, Lu Q, Chang C. Epigenetics in health and disease. *Advances in Experimental Medicine and Biology*. 2020;**1253**:3–55. DOI: 10.1007/978-981-15-3449-2_1

Epigenetic Mechanisms in Autoimmune Diseases

Xiaojing Nie, Yuxian Tang, Yi Chen, Yunfan Zhang, Junyan Chen, Yan Deng, Shuqi Zheng, Min Liu, Mingqiang Li and Zhongxin Liu

Abstract

Autoimmune diseases refer to conditions where the immune system of the body responds to self-antigens, leading to damage or dysfunction of its own tissues. Epigenetics refers to the three-level regulatory mechanism at the transcriptional, translational, and protein metabolic levels without altering the base information on DNA. There is evidence that abnormal epigenetic events can be used as biomarkers for autoimmune diseases. Biomarkers based on DNA methylation, histone modification, and non-coding RNA are closely related to the early diagnosis, therapeutic evaluation, and prognosis of autoimmune diseases such as systemic lupus erythematosus (SLE) and IgA vasculitis (IgAV). In the future, the combination of epigenetic maps with clinical practice can promote the safe and efficient transformation of epigenetic drugs from basic research to clinical application.

Keywords: autoimmune diseases, epigenetic regulation, SLE, IgAV, DNA methylation, histone modification, non-coding RNAs

1. Introduction

Autoimmune diseases refer to a category of disorders in which the immune system of the body breaks immune tolerance to its own components, thereby attacking its own organs, tissues, or cells and causing damage. They are characterized by an imbalance in the immune system, loss of immune tolerance to self-antigens, abnormal attacks on the body's cells and tissues, and impaired self-non-self discrimination [1]. Autoimmune diseases have a high incidence and mortality rate [2], with over 10% of the population potentially affected, and the overall prevalence shows an increasing trend over time. The incidence and prevalence of different autoimmune diseases vary, and they are associated with age, gender, ethnicity, and other demographic characteristics [3]. Moreover, epidemiological studies have shown that environmental factors and genetic susceptibility are risk factors for the loss of tolerance [4].

The formation process of immune tolerance involves multiple mechanisms and their interactions, due to which such diseases present significant heterogeneous characteristics in clinical practice [5]. Some autoimmune diseases affect a certain type of tissue throughout the body, such as blood vessels, cartilage, or skin – for example, systemic lupus erythematosus (SLE) [6]. Other autoimmune diseases affect specific organs, such as primary biliary cholangitis (PBC) [7]. However, the diagnosis of these diseases currently mainly relies on a series of clinical and laboratory criteria and the treatment is primarily based on immunotherapy, lacking early biomarkers and individualized management tools.

Autoimmune diseases have a high incidence and mortality rate [5], with over 10% of the population potentially affected, and the overall prevalence showing an increasing trend over time. The incidence and prevalence of different autoimmune diseases vary, and they are associated with age, gender, ethnicity, and other demographic characteristics [6, 7].

At present, an increasing number of research results show that epigenetic mechanisms play a key role in the occurrence and development of autoimmune diseases. Studies have shown that a large number of epigenetic changes in DNA are associated with the loss of immune tolerance in autoimmune diseases [8]. For instance, the DNA methylation patterns of various cells in patients with SLE have undergone significant changes, specifically including hypomethylation in the promoter and enhancer regions of the CD40 ligand [9] and hypermethylation of interferon-induced protein 44-like genes in CD4⁺ T cells [10]. Not only that, for lupus patients in the active stage, the overall acetylation levels of histone H3 and H4 in CD4⁺ T cells are relatively low [11]. Meanwhile, the levels of various microRNAs (miRNAs) in the peripheral blood and CD4⁺ T cells of the patients also decreased significantly [12–14]. Other examples include hypermethylation of the insulin gene in type 1 diabetes (T1D) [15] and the CD4⁺ T cell promoter in systemic sclerosis (SSc) patients [16], hypomethylation expression of peptidyl-arginine deiminase 2 and Src homologous domain phosphatase 1 in patients with multiple sclerosis (MS) [17], the therapeutic effect of patients with rheumatoid arthritis (RA) treated with histone deacetylase inhibitors was observed [18], acetylation modification of histone H4 exists in the promoter region of the AQP5 gene in patients with Sjogren's syndrome (SS) [19], overall high acetylation of histone H4 in SSc patients [20], and miRNA signaling in T1D, MS, SS, ulcerative colitis (UC), and psoriasis [21–23]. The above studies have shown that compared with healthy persons, the genomes of patients with autoimmune diseases exhibit epigenetic changes, which are the earliest events before the clinical manifestations of the disease and are closely related to the disease phenotype and activity.

2. Epigenetics regulates the immune system

The core components of epigenetics mainly include DNA methylation, histone modification, and non-coding RNA, etc., as shown in **Figure 1**.

DNA methylation is the most classic epigenetic modification method. In the human genome, 5-methylcytosine (5mC) is the most common form of methylation [24]. DNA methylation can precisely guide the functional differentiation of T cells. The differentiation of naive CD4⁺ T cells into Treg

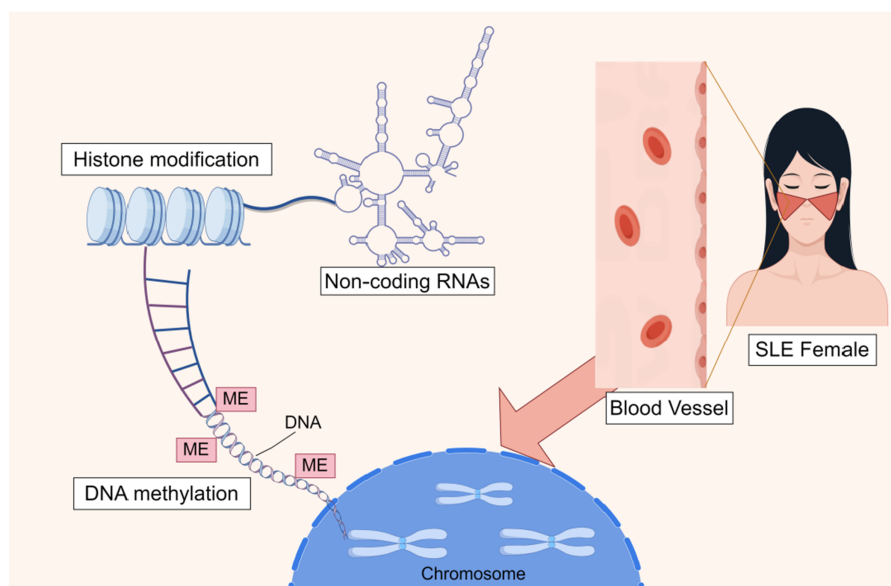


Figure 1.
 The core components of epigenetics. Created in Figdraw.

requires the induction of the transcription factor Foxp3, and the TSDR region of the Foxp3 locus needs to be continuously hypomethylated to ensure the stable expression of Foxp3. If DNMT1 is overexpressed, it will lead to methylation of TSDR, thereby blocking the differentiation of Treg [25]. During B cell development, DNA methylation dynamically regulates the activation or silencing of key genes to ensure the normal development and maturation of B cells. For example, Shi et al. demonstrated that DNA methylation inhibits the activity of the Pax5 promoter, thereby causing B cell development disorders [26]. In addition, the research by Barwick et al. confirmed that when the transferase genes DNMT3a and DNMT3b responsible for the methylation of nascent DNA in B cells are absent (i.e. DNMT3 deficiency), it will affect the normal development and maturation process of B cells [27].

Histone modification refers to a series of modifications carried out on the amino acid residues of histones, including histone methylation, acetylation, phosphorylation, ubiquitination, etc. This modification is involved in a variety of cellular activities and has an impact on them, specifically including the activation and inactivation of transcription, chromosome packaging, and the repair of DNA damage. Liu et al. [28] detected the histone modification characteristics at different stages of Th1 and Th2 cell differentiation, thereby identifying some sites that share both common H3K4me1 modification features and cell-specific H3K4me1 modification features. These sites imply that they might be the regulatory elements in the active or silent state of the Th cell differentiation transcription program. In addition, transcription factor binding site analysis showed that the promoter regions marked by H3K18la are rich in ETS transcription factor binding sites, whereas the promoter regions marked by H3K9la are rich in zinc finger transcription factor binding sites. This indicates that H3K18la and H3K9la play important roles in the transcriptional activation of CD8⁺ T cells [29]. Sun et al. [30] also found that in activated CD8⁺ T cells, histone lactylation is mainly H3K18la and H3K9la, and the

genes regulated by histone modification are not the same as those regulating the fate and function of CD8⁺ T cells.

Non-coding RNA is an RNA molecule that is not translated into protein and regulates gene expression through base complementary pairing or binding to proteins. The most important regulatory non-coding RNAs include miRNA, siRNA, piRNA, and lncRNA, which play multiple roles in regulating gene expression [31]. lncRNA is involved in various pathophysiological processes such as cell proliferation, differentiation, and apoptosis, and its expression changes with different stimulating factors and cell types. Qiao et al. [32] discovered through microarray and RT-qPCR array techniques that a large number of dysregulated lncRNAs are produced in polarized macrophages, affecting the developmental processes of respiratory system inflammation, fibrosis, immune response, and tumors. In addition, it has been shown that miR-155 affects the level of SHIP1 in dendritic cells. The reduction of SHIP1 levels leads to excessive proliferation of T cells and promotes immune tolerance [33]. The biogenesis of miRNA is directly regulated by DNA methylation and targets multiple DNMTs, thereby regulating DNA methylation [34].

Autoimmune diseases are characterized by dysfunction of the immune system and abnormal activation of autoantigens, which, in turn, cause damage to tissues and organs [8]. Epigenetics has been confirmed to be an important factor involved in the pathogenesis of autoimmune diseases. From the perspective of epigenetic mechanisms, many internal and external environmental risk factors, such as smoking, nutritional status, viral infections, and exposure to chemical substances, may all have an impact on the pathogenesis of autoimmune diseases [35]. Epigenetic modifications of B cells play a regulatory role in the selection, activation, and differentiation processes of autoreactive B cells. Therefore, abnormal epigenetic regulation may lead to the abnormal proliferation of pathogenic B cell subsets, thereby inducing autoimmune diseases [36]. T cell dysfunction is the pathogenic mechanism of most autoimmune diseases. Studies have shown that abnormal epigenetic regulation can lead to imbalanced differentiation of T cell subsets, uncontrolled activation, and functional abnormalities, ultimately resulting in autoimmune diseases [37].

Generally speaking, DNA methylation mainly occurs on the CpG islands in the promoter regions of most genes. Low methylation can activate gene transcription, while high methylation can inhibit gene transcription [38]. In recent years, the analysis of the whole-genome methylation map has found that low methylation of T lymphocytes is closely related to autoimmune diseases. The combined effect of environmental and genetic factors can cause abnormal DNA methylation, and this abnormality will, in turn, affect the expression of genes related to leukocyte function-related antigen 1 [39]. Moreover, the formation of neutrophil extracellular traps is fundamentally involved in the pathogenesis of autoimmune diseases. Andrés et al. [40] found that during the formation of neutrophil extracellular traps, histone-citrullinated peptides are released into the blood, inducing the formation of autoantibodies in the blood, indicating that histone citrullination is related to autoimmune diseases.

In summary, epigenetics plays a “bridge” role in autoimmune diseases by regulating immune tolerance, immune cell differentiation, and inflammatory factor secretion: it is influenced by genetic background (such as the polymorphism of epigenetic regulatory genes) and responds to environmental factor stimulation, ultimately leading to the activation of autoreactive immune

cells and tissue damage. This mechanism not only reveals the essence of autoimmune diseases but also provides a clear theoretical basis for targeted intervention (such as DNA methylation regulators and histone modification enzyme inhibitors).

3. Epigenetics is used in clinical diagnosis and efficacy prediction

Epigenetic modifications, by precisely regulating gene expression, can directly act on key biological processes such as cell proliferation, differentiation, apoptosis, and immune response, thus becoming an important molecular link connecting genetic susceptibility and environmental factors [41]. With the development of epigenomic technologies, epigenetics has been increasingly applied in clinical translation, mainly in the early diagnosis, therapeutic efficacy evaluation, and prognosis of autoimmune diseases.

In terms of DNA methylation, SLE patients show epigenetic dysregulation across the entire genome [42], characterized by a global hypomethylation pattern [35], especially in immune-related gene regions. Moreover, the promoter of the interferon-induced protein 44-like (IFI44L) gene in CD4⁺ T cells is hypomethylated, enhancing the interferon signaling pathway and boosting the immune response, indicating that the DNA methylation of the IFI44L promoter can be a biomarker for diagnosing SLE [43]. Furthermore, in RA patients, the reduced level of DNA methyltransferase in synovial fibroblasts leads to hypomethylation, inducing an invasive synovial fibroblast (SF) phenotype and the production of pro-inflammatory cytokines such as IL-6, thereby promoting RA progression [44]. Similarly, in IgA vasculitis (IgAV) patients, the decreased activity of the MAPK/ERK signaling pathway is associated with the downregulation of DNMT1 expression, which may cause abnormal expression of inflammation-related genes and promote disease progression [45]. In the salivary gland epithelial cells of patients with SS, a decrease in DNA methylation levels can be observed, and this decline is associated with lymphocyte infiltration and the production of anti-SSB/La antibodies [46]. The DNA methylation of these specific genes may serve as biomarkers for the diagnosis of autoimmune diseases.

Regarding histone modifications, Miceli-Richard et al. proposed that in CD4⁺ T cells of patients with SLE, the acetylation levels of histone H3 and H4 are generally in a low state, among which the acetylation level of histone H3 is negatively correlated with the activity of the disease [47]. The function of TNF- α -induced protein 3 (TNFAIP3) is to negatively regulate the NF- κ B inflammatory signaling pathway, thereby inhibiting the inflammatory response. In the CD4⁺ T cells of patients with SLE, there is a decreased level of trimethylated lysine 4 (H3K4me3), a transcriptional activity marker of histone H3, in the promoter region of the TNFAIP3 gene. This change causes a significant decrease in the mRNA expression level of TNFAIP3, which in turn leads to the overexpression of IFN- γ and IL-17, ultimately intensifying the inflammatory response and promoting the occurrence and development of SLE. This abnormal histone modification can be used as a biomarker for the clinical diagnosis of SLE [43]. However, in IgAV patients, histone H3 acetylation in peripheral blood mononuclear cells (PBMCs) is significantly enhanced and is associated with elevated expression levels of IL-6, GATA-3, IL-13, TIM-1, IL-4, and CXCL4,

participating in the immune response of IgAV [48]. Furthermore, studies have also shown that the labeling levels of histone 3 H3K4me3 and acetylated lysine 27 (H3K27ac) in IFN response genes in monocytes of patients with SSc increase to varying degrees, further promoting the expression of these genes. Notably, the expression of MX1 in the lesional skin of patients with SSc was reported to correlate with a decreased lung function and a more severe phenotype associated with increased mortality [49]. Wang et al. found that histone H3K9 hypomethylation was observed in B cells of patients with SSc and CD4+ T cells of patients with SLE. However, SSc patients also have overall histone H4 excessive acetylation, and the level of this excessive acetylation is positively correlated with the activity of the disease [20]. The above-mentioned research suggests that abnormal changes in histone modifications can serve as potential biomarkers for disease diagnosis and prognosis assessment.

Non-coding RNAs (such as miRNAs) also show diagnostic potential. miRNAs can bind to the 3' untranslated region of mRNAs, inhibiting translation or degrading mRNAs, thereby suppressing protein production and participating in the post-transcriptional regulation of gene expression [50]. In patients with autoimmune diseases such as SLE and Crohn's disease, the expression level of miR-125a in peripheral blood CD4+ T cells decreases, which can affect effector T cells (Teffs). It inhibits the secretion of multiple factors (including STAT3, IFN- γ , IL-13, etc.). Not only that, in the peripheral blood of SLE patients, miR-34a may also be related to the activity of the disease – its expression level is positively correlated with multiple serum disease indicators such as rheumatoid factor, anti-hemolytic O antibody, and erythrocyte sedimentation rate [43]. Other miRNAs, such as miR-7, are involved in the overactivation of B cells in SLE patients and are related to the production of autoantibodies; the upregulation of miR-21 can lead to excessive activation of T cells and participate in the immune regulation of SLE [51]. In patients with IgAV, the degree of gastrointestinal involvement is negatively correlated with the expression levels of miRNA-155-5p and/or miRNA-146a-5p in the skin, while the severity of skin purpura is positively correlated with the expression level of miRNA-223-3p in the skin. These abnormally expressed miRNAs may serve as biomarkers for IgAV [52]. According to the latest research, urinary exosomal miRNAs are associated with IgAV renal injury. The analysis combining clinical parameters suggests a significant correlation between hsa-miR-383-5p and urinary protein levels. hsa-miR-383-5p, hsa-miR-3065-5p, hsa-miR-450b-5p, and hsa-miR-25-3p may mediate the pathogenesis of IgAV nephritis (IgAVN) by targeting fibrotic and inflammatory pathways. Among them, hsa-miR-383-5p in exosomes is likely to become a novel non-invasive biomarker for evaluating the disease status of IgAV nephropathy (IgAVN), which provides a new direction for the non-invasive diagnosis and treatment of IgAVN [53].

In addition to its wide application in non-invasive diagnosis, epigenetics also has new application potential in predicting disease prognosis, recurrence risk, organ involvement, and treatment response. The onset, diagnosis, and treatment of SLE are closely related to epigenetics. Research has found that there are abnormal methylation levels in the promoter regions of immune-related genes in patients with SLE, specifically manifested as a hypomethylation state, and this state can lead to overexpression of genes, thereby intensifying abnormal immune activation [41, 42]. Therefore, it can be used as a biomarker for predicting disease

risk and progression. In terms of predicting treatment response, the methylation status of specific genes is related to drug sensitivity (such as glucocorticoids and biologics), providing a reference for the formulation of personalized treatment plans [46, 47]. In addition, hypomethylation of interferon-related genes and changes in the proportion of CD4⁺ T cell subsets can indicate the risk of recurrence, facilitating early intervention [50]. The reversibility of epigenetic modifications provides a new direction for treatment; drugs such as DNA methyltransferase inhibitors can correct abnormal gene expression, and formulating treatment plans based on patients' epigenetic characteristics can improve efficacy and reduce side effects [51].

Currently, research on the use of epigenetics to predict the efficacy and prognosis of IgAV is still in its early stages, but there have been some initial findings. Patients with IgAV have abnormal ERK signaling pathway function, which is closely related to the down-regulation of DNA methyltransferase DNMT1 expression [54]. H3 acetylation in PBMCs of patients is enhanced, and the H3K4 methylation level in IgA nephritis patients is elevated [52]. Luo et al. [53] demonstrated that in children with IgAV, HAT and histone methyltransferase levels increase, while histone deacetylase and demethylase levels decrease, leading to abnormally active transcription; in patients with kidney involvement, the mRNA and protein levels of IL-13, IL-6, and IL-4 are significantly elevated; in CD4⁺ T cells, the promoter and enhancer regions of IL4 are rich in H3 acetylation and H3K4me3, and the expression of TIM-1 also increases, while IFNG, which encodes the Th1 cell cytokine IFN γ , does not show similar changes. The overall increase of specific open chromatin markers and the overexpression of helper T cell 2 (Th2)-related genes in IgAV suggest that Th2 cells may be the key effector cells in the pathogenic mechanism of IgAV.

In summary, epigenetics is currently crucial for the early diagnosis, efficacy evaluation, and prognosis in autoimmune diseases. In the future, it is necessary to further integrate epigenetic maps with clinical data to promote the safe and efficient translation of epigenetic drugs from basic research to clinical applications.

4. Current limitations and challenges

Although the epigenetic mechanisms of autoimmune diseases have great potential for clinical translation, they still face many challenges. The epigenetic mechanisms of autoimmune diseases are extremely complex, involving multiple genes, various epigenetic modifications, and interactions among numerous cell types. Epigenetic alterations in different cell types are intertwined, forming a complex regulatory network, making it feel like looking for a needle in a haystack to parse the specific pathogenic mechanisms. This multi-dimensional complexity greatly increases the difficulty of research, and researchers need to comprehensively integrate data from different levels and deeply explore the causal relationships among various factors to gradually unveil the mysteries of the disease. Environmental factors can also induce autoimmune diseases through epigenetic mechanisms, further increasing the complexity of the mechanism.

Epigenetic biomarkers have great prospects in disease diagnosis and monitoring, but their clinical promotion is hindered by issues such as the lack of

standardized detection techniques, low correlation between results from different platforms, high detection costs, sample heterogeneity, and low early sensitivity. Some epigenetic modifications, such as specific histone modification isoforms, are extremely difficult to detect, and existing detection methods are unable to accurately identify and quantitatively analyze them. Even relatively mature DNA methylation detection techniques face challenges. In addition, epigenetic drugs may trigger significant immune responses, increasing the risk of infection or tumors, so further development is needed to improve safety levels. In the field of precision treatment, although core challenges such as off-target effects, stability, and delivery efficiency still exist, the rapid development of molecular modification and nanoparticle technologies is gradually breaking through these limitations.

5. Summary and outlook

In recent years, with many breakthroughs in epigenetic research on autoimmune diseases, we have gradually realized that the normal functioning of the immune system is inseparable from epigenetic regulation. A large number of studies have confirmed that in autoimmune diseases such as RA, SLE, and multiple sclerosis, epigenetic regulatory methods such as DNA methylation, histone modification, and non-coding RNA play a key role in the development and differentiation processes of B cells, T cells, and dendritic cells, as well as the realization of their activation functions. This suggests that epigenetic dysregulation is an early driving force and common mechanism of autoimmune diseases. Based on this, epigenetic biomarkers are expected to significantly enhance the level of clinical diagnosis and treatment by enabling early diagnosis, conducting risk stratification, and promoting the development of therapeutic approaches. They will gradually reshape the individualized clinical management model.

Although epigenetic research has revealed that DNA methylation dysregulation, abnormal histone modification, and non-coding RNA regulatory disorders are the early core driving mechanisms of autoimmune diseases, there is still a long way to go to deeply reveal the interaction network between epigenetics and autoimmune diseases and to translate this research results into clinical practice for the diagnosis and treatment of autoimmune diseases. This brings new challenges and opportunities to epigenetic research. Developing effective biomarkers, establishing clinically applicable epigenetic analysis techniques, creating highly selective epigenetic regulatory drugs, and determining whether epigenetic biomarker-guided therapeutic transformation truly improves the survival rate and quality of life of patients are the key issues we face before applying epigenetics to clinical practice.

Conflict of interest

The authors declare no conflicts of interest.

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References

- [1] Bieber K, Hundt JE, Yu X, et al. Autoimmune pre-disease. *Autoimmunity Reviews*. 2023;**22**(2):103236.
- [2] Pisetsky DS. Pathogenesis of autoimmune disease. *Nature Reviews Nephrology*. 2023;**19**(8):509–524.
- [3] Yu C, Gershwin ME, Chang C. Diagnostic criteria for systemic lupus erythematosus: A critical review. *Journal of Autoimmunity*. 2014;**48–49**:10–13.
- [4] Wang L, Wang FS, Chang C, et al. Breach of tolerance: Primary biliary cirrhosis. *Seminars in Liver Disease*. 2014;**34**:297–317.
- [5] Wang L, Wang FS, Gershwin ME. Human autoimmune diseases: A comprehensive update. *Journal of Internal Medicine*. 2015;**278**(4):369–395.
- [6] Conrad N, Misra S, Verbakel JY, et al. Incidence, prevalence, and co-occurrence of autoimmune disorders over time and by age, sex, and socioeconomic status: A population-based cohort study of 22 million individuals in the UK. *The Lancet*. 2023;**401**:1878–1890.
- [7] Leung PS, Wang J, Naiyanetr P, et al. Environment and primary biliary cirrhosis: Electrophilic drugs and the induction of AMA. *Journal of Autoimmunity*. 2013;**41**:79–86.
- [8] Danieli MG, Casciaro M, Paladini A, et al. Exposome: Epigenetics and autoimmune diseases. *Autoimmunity Reviews*. 2024;**23**(6):103584.
- [9] Vordenbäumen S, Rosenbaum A, Gebhard C, et al. Associations of site-specific CD4(+)-T-cell hypomethylation within CD40-ligand promotor and enhancer regions with disease activity of women with systemic lupus erythematosus. *Lupus*. 2021;**30**:45–51.
- [10] Zhao M, Zhou Y, Zhu B, et al. IFI44L promoter methylation as a blood biomarker for systemic lupus erythematosus. *Annals of the Rheumatic Diseases*. 2016;**75**:1998–2006.
- [11] Hu N, Qiu X, Luo Y, et al. Abnormal histone modification patterns in lupus CD4+ T cells. *The Journal of Rheumatology*. 2008;**35**:804–810.
- [12] Luo Q, Fu B, Zhang L, et al. Decreased peripheral blood ALKBH5 correlates with markers of autoimmune response in systemic lupus erythematosus. *Disease Markers*. 2020;**2020**:8193895.
- [13] Guo G, Wang H, Shi X, et al. Disease Activity-Associated Alteration of mRNA m(5) C Methylation in CD4 (+) T Cells of Systemic Lupus Erythematosus. *Frontiers in Cell and Developmental Biology*. 2020;**8**:430.
- [14] Luo Q, Rao J, Zhang L, et al. The study of METTL14, ALKBH5, and YTHDF2 in peripheral blood mononuclear cells from systemic lupus erythematosus. *Molecular Genetics & Genomic Medicine*. 2020;**8**:e1298.
- [15] Fradin D, Le Fur S, Mille C, et al. Association of the CpG methylation pattern of the proximal insulin gene

promoter with Type 1 diabetes. *PLoS One*. 2012;**7**:e36278.

[16] Wang YY, Wang Q, Sun XH, et al. DNA hypermethylation of the forkhead box protein 3 (FOXP3) promoter in CD4⁺ T cells of patients with systemic sclerosis. *British Journal of Dermatology*. 2014;**171**:39–47.

[17] Kumagai C, Kalman B, Middleton FA, et al. Increased promoter methylation of the immune regulatory gene SHP-1 in leukocytes of multiple sclerosis subjects. *Journal of Neuroimmunology*. 2012;**246**:51–57.

[18] Young DA, Lakey RL, Pennington CJ, et al. Histone deacetylase inhibitors modulate metalloproteinase gene expression in chondrocytes and block cartilage resorption. *Arthritis Research and Therapy*. 2005;**7**:R503–12.

[19] Yamamura Y, Motegi K, Kani K, et al. TNF- α inhibits aquaporin 5 expression in human salivary gland acinar cells via suppression of histone H4 acetylation. *Journal of Cellular & Molecular Medicine*. 2012;**16**:1766–1775.

[20] Wang Y, Yang Y, Luo Y, et al. Aberrant histone modification in peripheral blood B cells from patients with systemic sclerosis. *Clinical Immunology*. 2013;**149**:46–54.

[21] Xu WD, Pan HF, Li JH, et al. MicroRNA-21 with therapeutic potential in autoimmune diseases. *Expert Opinion on Therapeutic Targets*. 2013;**17**:659–665.

[22] Pauley KM, Cha S, Chan EK. MicroRNA in autoimmunity and autoimmune diseases. *Journal of Autoimmunity*. 2009;**32**(3–4):189–194.

[23] Qu Z, Li W, Fu B. MicroRNAs in autoimmune diseases. *BioMed Research International*. 2014;**2014**:527895.

[24] Chen K, Zhao BS, He C. Nucleic acid modifications in regulation of gene expression. *Cell Chemical Biology*. 2016;**23**(1):74–85.

[25] Bai L, Hao X, Keith J, et al. DNA methylation in regulatory T cell differentiation and function: Challenges and opportunities. *Biomolecules*. 2022;**12**(9):1282

[26] Shi Y, Zhu Z, Chen Q, et al. DNA methylation regulates B cell activation via repressing Pax5 expression in teleost. *Frontiers in Immunology*. 2024;**15**:1363426.

[27] Barwick BG, Scharer CD, Martinez RJ, et al. B cell activation and plasma cell differentiation are inhibited by de novo DNA methylation. *Nature Communications*. 2018;**9**:1900.

[28] Liu S, Cao Y, Cui K, et al. Regulation of T helper cell differentiation by the interplay between histone modification and chromatin interaction. *Immunity*. 2024;**57**:987–1004.e1005.

[29] Raychaudhuri D, Singh P, Chakraborty B, et al. Histone lactylation drives CD8(+) T cell metabolism and function. *Nature Immunology*. 2024;**25**:2140–2151.

[30] Sun R, Chi H. Metabolic-epigenetic rewiring in CD8(+) T cells via lactate-dependent histone lactylation. *Nature Immunology*. 2024;**25**(11):1980–1982.

[31] Li Y. Modern epigenetics methods in biological research. *Methods*. 2021;**187**:104–113.

- [32] Qiao X, Ding Y, Wu D, et al. The roles of long noncoding RNA-mediated macrophage polarization in respiratory diseases. *Frontiers in Immunology*. 2022;**13**:1110774.
- [33] Lind EF, Millar DG, Dissanayake D, et al. miR-155 upregulation in dendritic cells is sufficient to break tolerance in vivo by negatively regulating SHIP1. *The Journal of Immunology*. 2015;**195**:4632–4640.
- [34] Wang S, Wu W, Claret FX. Mutual regulation of microRNAs and DNA methylation in human cancers. *Epigenetics*. 2017;**12**(3):187–197.
- [35] Zhang Z, Zhang R. Epigenetics in autoimmune diseases: Pathogenesis and prospects for therapy. *Autoimmunity Reviews*. 2015;**14**(10):854–863.
- [36] Xiao F, Rui K, Shi X, et al. Epigenetic regulation of B cells and its role in autoimmune pathogenesis. *Cellular and Molecular Immunology*. 2022;**19**:1215–1234.
- [37] Bellanti JA, Li D. Treg cells and epigenetic regulation. *Advances in Experimental Medicine and Biology*. 2021;**1278**:95–114.
- [38] Rahmani M, Talebi M, Hagh MF, et al. Aberrant DNA methylation of key genes and acute lymphoblastic leukemia. *Biomedicine & Pharmacotherapy*. 2018;**97**:1493–1500.
- [39] Richardson BC. Role of DNA methylation in the regulation of cell function: Autoimmunity, aging and cancer. *The Journal of Nutrition*. 2002;**132**(8):2401s–2405s.
- [40] Andrés M, García-Gomis D, Ponte I, et al. Histone H1 post-translational modifications: Update and future perspectives. *International Journal of Molecular Sciences*. 2020;**21**:5941.
- [41] Wu H, Deng Y, Feng Y, et al. Epigenetic regulation in B-cell maturation and its dysregulation in autoimmunity. *Cellular and Molecular Immunology*. 2018;**15**:676–684.
- [42] Hedrich CM. Epigenetics in SLE. *Current Rheumatology Reports*. 2017;**19**(9):58.
- [43] Mu S, Wang W, Liu Q, et al. Autoimmune disease: A view of epigenetics and therapeutic targeting. *Frontiers in Immunology*. 2024;**15**:1482728.
- [44] Karouzakis E, Gay RE, Gay S, et al. Increased recycling of polyamines is associated with global DNA hypomethylation in rheumatoid arthritis synovial fibroblasts. *Arthritis & Rheumatism*. 2012;**64**:1809–1817.
- [45] Sugino H, Sawada Y, Nakamura M. IgA vasculitis: Etiology, treatment, biomarkers and epigenetic changes. *International Journal of Molecular Sciences*. 2021;**22**(14):7538.
- [46] Konsta OD, Le Dantec C, Charras A, et al. Defective DNA methylation in salivary gland epithelial acini from patients with Sjögren's syndrome is associated with SSB gene expression, anti-SSB/LA detection, and lymphocyte infiltration. *Journal of Autoimmunity*. 2016;**68**:30–38.
- [47] Miceli-Richard C. Epigenetics and lupus. *Joint Bone Spine*. 2015;**82**(2):90–93.
- [48] Luo S, Liang G, Zhang P, et al. Aberrant histone modifications in peripheral blood mononuclear cells from patients with Henoch-Schönlein

purpura. *Clinical Immunology*. 2013;**146**:165–175.

[49] van der Kroef M, Castellucci M, Mokry M, et al. Histone modifications underlie monocyte dysregulation in patients with systemic sclerosis, underlining the treatment potential of epigenetic targeting. *Annals of the Rheumatic Diseases*. 2019;**78**:529–538.

[50] Fasano S, Milone A, Nicoletti GF, et al. Precision medicine in systemic lupus erythematosus. *Nature Reviews Rheumatology*. 2023;**19**:331–342.

[51] Chi M, Ma K, Li Y, et al. Immunological involvement of MicroRNAs in the key events of systemic lupus erythematosus. *Frontiers in Immunology*. 2021;**12**:699684.

[52] Hočevár A, Tomšič M, Pižem J, et al. MicroRNA expression in the affected skin of adult patients with IgA vasculitis. *Clinical Rheumatology*. 2019;**38**:339–345.

[53] Zhang Y, Yang H, Chen Y, et al. Construction and diagnostic efficacy assessment of the urinary exosomal miRNA - mRNA network in children with IgA vasculitis nephritis. *The FASEB Journal*. 2025;**39**:e70492.

[54] Milillo A, Molinaro C, Costanzi S, et al. Defective activation of the MAPK/ERK pathway, leading to PARP1 and DNMT1 dysregulation, is a common defect in IgA nephropathy and Henoch-Schönlein purpura. *Journal of Nephrology*. 2018;**31**:731–741.

Epigenetic Regulation in Macrophage Functional Remodeling

Yu-Qi Yang, Yi-Yang Hu, Hong-Yan Qin and Jun-Long Zhao

Abstract

Macrophages are the primary immune cells constituting the innate immune system, with their main defensive functions including phagocytosis of apoptotic cells, clearance of harmful substances, and resistance to pathogenic microbial infections. They play a crucial regulatory role in maintaining organismal homeostasis and normal development. Over the past half-century, extensive research has been dedicated to uncovering the diverse roles and mechanisms of macrophage activation under physiological and pathological conditions. Since macrophages are distributed across most solid organs in mammals, their differentiation lineages and functional states are influenced by various tissue microenvironmental factors, which, to some extent, pose challenges to macrophage research. This chapter provides a detailed exposition of the activation/polarization types, functional states, and roles of macrophages in different pathological processes in recent years. It also reviews the regulatory mechanisms involving epigenetic factors (such as histone modifications and micro-RNAs) and molecular regulatory networks that govern the different activation/polarization states of macrophages.

Keywords: macrophage, epigenetic regulation, histone modification, DNA methylation, RNA modification, immunotherapy, signaling pathways

1. Introduction

Macrophages, a core component of the innate immune system, act as critical “guardians” against pathogens and in maintaining homeostasis. They perform indispensable roles in physiological and pathological processes through phagocytosis of apoptotic cells, clearance of foreign substances and pathogens, and modulation of immune responses – functions integral to life activities from embryonic development to chronic disease progression.

Over the past half-century, advancements in immunology and molecular biology have revealed that macrophages are not functionally homogeneous. Under the influence of tissue microenvironments, they exhibit remarkable plasticity, activating/polarizing into distinct phenotypes: classical pro-inflammatory (M1), anti-inflammatory/reparative (M2), regulatory macrophages (rMf), and oxidative

phospholipid-induced Mox macrophages. Each subtype plays unique roles: M1 macrophages secrete pro-inflammatory cytokines (e.g., tumor necrosis factor alpha [TNF- α], interleukin-1-beta [IL-1 β]) to eliminate pathogens during acute infections, while M2 macrophages secrete anti-inflammatory factors (e.g., IL-10, transforming growth factor beta [TGF- β]) to promote tissue repair in later stages.

Yet, challenges remain. Macrophages are widely distributed (e.g., liver Kupffer cells, brain microglia) and differ by tissue origin (embryonically derived vs. bone marrow-derived). Key questions persist: how do microenvironmental signals (cytokines, metabolites, pathogens) precisely regulate polarization, and what are the mechanisms in diseases like atherosclerosis, cancer, and neurodegeneration?

Recent advances in epigenetics and signaling networks have uncovered regulatory mechanisms. Histone modifications, DNA methylation, and non-coding RNAs (ncRNAs, e.g., micro-RNAs) influence gene expression and phenotype maintenance. Crosstalk among pathways (Notch, nuclear factor kappa B [NF- κ B], JAK-STATs, interferon regulatory factors [IRFs]) forms a complex network controlling activation states – insights that lay the groundwork for macrophage-targeted therapies.

This chapter will outline the major macrophage polarization types and their roles in pathology, focusing on epigenetic regulation (histone modifications, DNA methylation, micro-RNAs) and key signaling pathways. It will also explore applications in disease pathogenesis, providing a reference for understanding macrophage biology and developing interventions.

2. Activation/polarization states of macrophages and interaction with the microenvironment

2.1 Macrophage activation/polarization phenotypes

2.1.1 Physiological functions of macrophages

Under physiological conditions, macrophages maintain tissue homeostasis by clearing apoptotic cells and debris, regulating angiogenesis, and promoting tissue remodeling through extracellular matrix production. They also exhibit tissue-specific functions: microglia in the brain mediate postnatal synaptic pruning to support learning and memory, splenic red pulp macrophages recycle iron by phagocytosing erythrocytes, and peritoneal macrophages induce intestinal IgA production via interactions with B1 cells. These specialized functions are regulated by microenvironmental cues, such as heme-induced Spic expression or retinoic acid-mediated GATA6 activation.

Regarding functional regulation, conventional views suggest intrinsic differences between tissue-resident macrophages (trM ϕ) and bone marrow-derived macrophages – trM ϕ are long-lived and promote tissue repair, whereas bone marrow-derived macrophages exhibit heightened NF- κ B activation and proinflammatory responses [1, 2]. However, recent studies integrating enhancer profiling and transplantation experiments demonstrate that microenvironmental signals dominate phenotypic determination over cellular origin. For instance, peritoneal macrophages transplanted into the lung adopt an alveolar macrophage-like transcriptional profile while retaining partial original functionality. This indicates a dynamic equilibrium between microenvironment-driven plasticity and origin-dependent rigidity, the precise regulatory mechanisms of which require further investigation.

2.1.2 Functional regulation and polarization of macrophages

Macrophages encounter diverse and complex inflammatory microenvironments in peripheral tissues and various organs. Their functional diversity can largely be attributed to their ability to adopt distinct phenotypes in response to different microenvironmental cues. Upon sensing external inflammatory signals, macrophages undergo significant changes in their epigenetic profiles, transcriptional expression patterns, and cytokine secretion profiles to adapt to the local inflammatory milieu and mount an appropriate response. This remarkable functional plasticity of macrophages in response to varying stimuli is defined as polarization, which is classified into different subtypes. Indeed, the involvement of macrophages in regulating host defense, tissue development, and energy metabolism serves as strong evidence of their polarization.

Macrophage polarization is a complex functional remodeling process involving the interplay between microenvironmental stimuli and intrinsic gene expression profiles, including cytokines, growth factors, metabolites, and pathogen-derived molecules. Based on the inducing agents and immune responses, a fundamental dichotomous classification divides macrophage polarization into two major types: classically activated (M1) macrophages, which exhibit a proinflammatory phenotype, and alternatively activated (M2) macrophages, which exert anti-inflammatory effects (**Figure 1**). While macrophage polarization can be clearly defined in *in vitro* experiments and certain acute infection models *in vivo*, growing evidence suggests that the induction and regulation of macrophage polarization cannot be fully explained by this simplistic paradigm. Moreover, the initial polarization state is reversible upon environmental changes. In complex, multistage immune responses, some macrophage subsets display physiological functions that cannot be strictly categorized as either M1 or M2. Researchers propose that these heterogeneous macrophages may represent transitional states between M1 and M2 polarization, suggesting that macrophage activation exists along a continuum rather than as a strict M1–M2 dichotomy. Additionally, some scholars argue that many more macrophage phenotypes with diverse functions remain undiscovered, playing complex roles in inflammatory responses, tissue repair, and wound healing [3].

The shift in macrophage polarization depends on the type, concentration, and duration of cytokines or inflammatory mediators in the microenvironment. *In vitro* or *in vivo*, M1 macrophage activation relies on Toll-like receptor (TLR) ligands (e.g., microbial lipopolysaccharide, LPS) and Th1-associated cytokines such as interferon gamma (IFN- γ). These classically activated macrophages typically exhibit proinflammatory properties, upregulating inducible nitric oxide synthase (iNOS) to enhance NO production and releasing chemokines like CXCL10 to promote pathogen phagocytosis and killing. They also secrete proinflammatory cytokines (e.g., IL-12, IL-23, IL-15, TNF- α) to drive Th1 responses. Additionally, M1 macrophages highly express MHC class II molecules and co-stimulatory molecules (CD80/CD86), enhancing antigen presentation and cytotoxic cell activation. In contrast, M2 polarization is induced by Th2 cytokines (IL-4/IL-13) or immune complexes, leading to an anti-inflammatory phenotype. M2 macrophages express arginase-1 (Arg1), suppressing NO synthesis, and secrete chemokines (CCL17, CCL22) as well as anti-inflammatory mediators (IL-10, TGF- β). They also upregulate markers such as scavenger receptors (SR), mannose receptor (MR), Fizz1, and Ym1, promoting tissue repair, organ fibrosis, parasite clearance, and immune regulation. Beyond IL-4 stimulation, researchers have found that IL-10, glucocorticoids, and apoptotic cell debris can also induce an M2-like polarization, resulting in an immunosuppressive

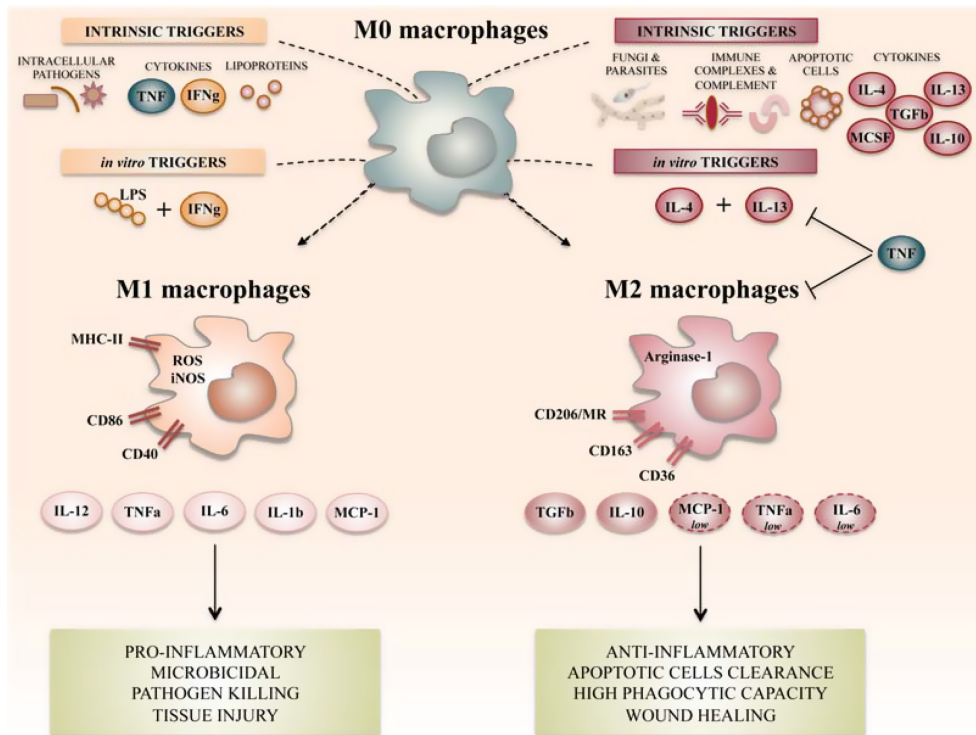


Figure 1.

Macrophage activation/polarization types. Summary of the main macrophage polarization states of activated macrophages. Different stimuli and signaling pathways have been described as inducers of M1-like or M2-like activation states, of which the most widely referenced ones are summarized here. M1-like or M2-like polarization has been reported in humans as being related to distinct defensive or healing schemas. Many roles have been ascribed to these polarization status, of which pro- and anti-inflammatory macrophage potentiation has for a long time been classically associated to the M1-like/M2-like-like dichotomy. LPS: lipopolysaccharide; MR: mannose receptor; TNF: tumor necrosis factor; IFN γ : interferon gamma; IL: interleukin; MCP: monocyte chemoattractant protein; TGF: transforming growth factor; MCSF: macrophage colony stimulating-factor; ROS: reactive oxygen species; iNOS: inducible nitric oxide synthase; MHC: major histocompatibility complex.

phenotype. M1 and M2 macrophages exhibit distinct functional and metabolic characteristics: M1 macrophages display strong proinflammatory and antitumor activity, relying on glycolysis for energy, whereas M2 macrophages facilitate tissue repair and immune suppression, primarily utilizing oxidative phosphorylation [3].

Beyond the classical M1 and M2 phenotypes, emerging research has identified novel macrophage subsets with distinct functional profiles in various disease contexts. Regulatory macrophages (rMf) represent a recently characterized subpopulation that plays a pivotal role in modulating both innate and adaptive immune responses. Following LPS stimulation, these cells exhibit robust IL-10 production and develop into suppressor macrophages, contributing to tolerance induction in solid organ transplantation and xenogeneic immune responses. Notably, dehydrogenase/reductase 9 (DHRS9) has been identified as a specific molecular marker for this unique macrophage subset. Importantly, the polarization dynamics of macrophages are significantly influenced by interactions with immune cells (e.g., lymphocytes) and stromal components (e.g., fibroblasts, mesenchymal stem cells), although these regulatory mechanisms require further elucidation.

2.2 Macrophage activation/polarization and inflammation

Macrophages participate in various inflammatory and immune responses *in vivo*. Due to their diverse polarization states, they play distinct regulatory roles in processes such as tissue repair, fibrosis, allergic reactions, and homeostasis.

2.2.1 Macrophage activation/polarization in infectious inflammation

Macrophages serve as the first line of defense against pathogens. Pathogen-associated molecular patterns (PAMPs) are recognized by pattern recognition receptors (PRRs) on macrophages – including TLRs, Nod-like receptors (NLRs), RIG-I-like receptors (RLRs), C-type lectin receptors (CLRs), and nucleic acid sensors – triggering antimicrobial effector functions, recruitment of innate immune cells, activation of adaptive immunity, and initiation of inflammatory responses.

During infection, both tissue-resident macrophages (trM ϕ) and bone marrow-derived macrophages (BMDM) contribute to pathogen clearance through dynamic polarization. M1-polarized macrophages dominate early stages, enhancing phagocytosis, antigen presentation, and type I inflammatory responses via cytokines (e.g., TNF- α , IL-1 β , IL-6) and chemokines (e.g., CXCL1, CCL2) [4]. As infection resolves, macrophages shift to an M2 phenotype, secreting TGF- β and IL-10 to promote tissue repair. This M1-to-M2 transition is essential for balancing pathogen elimination and damage resolution [5].

Evidence indicates that macrophages exhibit distinct polarization states under different infectious conditions. During bacterial infection, macrophages recognize pathogens via PRRs, leading to M1 polarization. This is accompanied by the production of pro-inflammatory cytokines and mediators – such as TNF- α , IL-1 β , IL-6, IL-12, and NO – which contribute to pathogen clearance. However, certain intracellular bacterial pathogens have evolved sophisticated strategies to suppress M1 polarization and promote a shift toward an M2-like phenotype, thereby dampening inflammatory responses and evading host immunity. For example, after being phagocytosed by macrophages, *Mycobacterium tuberculosis* can be restrained by M1-polarized cells through the secretion of factors like TNF- α and CXCL18, along with the suppression of IL-10 expression, limiting intracellular bacterial proliferation. Interestingly, *M. tuberculosis* can also inhibit IFN- γ transcription in host macrophages, attenuating M1 polarization and inflammatory responses. Similarly, infections with digestive pathogens, such as *Helicobacter pylori*, are associated with aberrant macrophage polarization, leading to recurrent and persistent inflammation.

During viral infections, macrophage polarization is also subject to manipulation. Although the mechanisms by which viruses alter polarization to evade immunity are not fully understood, it is generally accepted that, upon viral recognition, macrophages internalize the virus, present viral peptides to T cells, and facilitate the production of IFN- γ and other molecules that recruit effector immune cells and induce inflammatory responses to control viral replication – processes that rely on M1 polarization. However, some viruses, such as hepatitis B (HBV) and hepatitis C (HCV), interfere with normal macrophage development and functional specialization. HBV and HCV inhibit monocyte-to-macrophage differentiation via TLR2 signaling. Additionally, HCV activates STAT family signaling, resulting in a mixed M1/M2 macrophage cytokine expression profile and functional dysregulation of both M1-like and M2-like macrophages in chronic hepatitis C patients.

2.2.2 Macrophage activation/polarization in chronic inflammation

Beyond infectious diseases, macrophage polarization plays a critical role in diverse chronic non-infectious conditions, including neurodegenerative disorders, metabolic diseases, fibrotic pathologies, and cardiovascular disorders. These conditions initiate sterile inflammatory responses through the recognition of damage-associated molecular patterns (DAMPs), though their regulatory mechanisms remain less understood compared to infectious inflammation [6].

Atherosclerosis serves as a prototypical example of macrophage polarization's involvement in chronic inflammatory diseases. The disease process involves chemokine-mediated recruitment of monocytes to the arterial intima, where exposure to CSF1 and oxidized LDL (ox-LDL) drives their differentiation into macrophages and subsequent transformation into foam cells. While initial lipoprotein uptake may appear beneficial, these foam cells exacerbate disease progression through cytokine and inflammatory mediator secretion, ultimately promoting necrosis or apoptosis. Lipid components within atherosclerotic plaques differentially regulate macrophage polarization, with cholesterol crystals activating NLRP3 inflammasome to induce M1 polarization, while 9-oxononanoyl cholesterol promotes M2 polarization [7].

Clinical investigations reveal distinct macrophage polarization patterns between symptomatic and asymptomatic atherosclerotic plaques. M1 macrophages predominantly localize to lipid cores of symptomatic plaques, whereas M2 macrophages are more abundant in asymptomatic plaques, suggesting a protective role. Current therapeutic approaches demonstrate limited efficacy in modulating macrophage activity, though PPAR- γ agonists show promise by specifically promoting M2 polarization. Emerging evidence highlights the potential of certain *Lactobacillus* strains to modulate M1/M2 ratios through inhibition of ox-LDL uptake, underscoring the therapeutic potential of probiotics. While macrophage-targeted therapies remain challenging, precise modulation of their polarization represents a promising strategy for treating chronic inflammatory diseases like atherosclerosis [8].

2.2.3 Macrophage activation/polarization and the inflammation–cancer transition

Under physiological conditions, the inflammatory response resolves upon elimination of injurious stimuli, transitioning into a highly active and precisely regulated homeostatic state. However, in the presence of certain persistent or low-grade stimuli – such as chronic excessive inflammation in target organs – the response may fail to resolve, leading to nonresolving inflammation. Malignancy is a classic example of nonresolving inflammation; nearly all human cancers are associated with inflammatory processes or inflammatory precancerous lesions. As key innate immune cells, macrophages play a critical regulatory role in the transition from inflammation to cancer.

Substantial evidence indicates that tumor-associated macrophages (TAMs) significantly contribute to tumorigenesis and progression. First, solid tumors are often infiltrated by macrophages, and multiple mouse models have established a clear link between macrophage infiltration and progressive tumor growth. Second, across various human cancers – including breast, cervical, bladder, and prostate cancer – high TAM density correlates with poor prognosis. Specific depletion of

TAMs in tumor tissues impedes tumor cell survival, a finding consistently supported by studies using TAM dysfunction models, providing direct evidence for the pro-tumorigenic role of TAMs. Transcriptomic profiling reveals that TAMs resemble M2-polarized macrophages, characterized by an IL-12^{low}/IL-10^{high} cytokine profile and upregulation of pro-tumor factors such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and COX2, which underlie their tumor-promoting functions.

However, emerging research has refined the understanding of TAM identity and functionality. Owing to the complexity and dynamic nature of the tumor microenvironment, TAMs often comprise a heterogeneous population with varying degrees of M1 and M2 polarization. Although TAMs are commonly described as having an M2-like phenotype, pro-inflammatory M1-like TAMs have also been identified. Spatial heterogeneity exists within tumors: in breast cancer models, well-oxygenated regions are enriched with MHC-II^{hi} macrophages expressing M1 markers and anti-angiogenic chemokines, while hypoxic regions contain M2-like TAMs that promote angiogenesis. Temporally, early-stage tumors are dominated by M1-like TAMs that release inflammatory cytokines and reactive oxygen/nitrogen species. These M1-like TAMs may initially restrain tumor growth by enhancing inflammation and activating cytotoxic T and NK cells, but they also contribute to genomic instability and increased mutation rates. As tumors progress, TAMs shift toward an M2-like phenotype, exhibiting immunosuppressive functions such as inhibition of cytotoxic T cells, promotion of regulatory T cell expansion, stimulation of angiogenesis and metastasis, and enhancement of cancer stemness and therapy resistance [9].

Given the pivotal role of TAM polarization in tumor progression, strategies targeting TAM reprogramming have gained research interest. Stimulation with factors such as CSF2 or IL-12 can skew TAMs toward an anti-tumor M1 phenotype. Additionally, low-dose radiation has been shown to promote TAM repolarization to an M1-like state, contributing to tumor vascular normalization.

3. Epigenetic regulation of macrophage activation/polarization

3.1 Physiological functions of macrophages

Macrophage function is critically regulated by histone modifications, which alter chromatin structure and gene expression. These epigenetic marks – including methylation, acetylation, and newer forms like lactylation – dictate transcriptional accessibility and are essential in macrophage polarization [1].

M1 macrophages display open chromatin with high acetylation, promoting inflammatory gene expression. In contrast, M2 macrophages have repressive methylation marks and condensed chromatin. In tumors, the microenvironment reprograms histone modifications in TAMs, suppressing anti-tumor genes via reduced H3K4me and increased H3K27me, thereby promoting an immunosuppressive M2-like phenotype [10].

Enzymes such as methyltransferases (e.g., Smyd2), demethylases (e.g., Jmjd3), acetyltransferases (HATs), and deacetylases (HDACs) fine-tune polarization through locus-specific modifications. For example, HDAC3 is required for both M1 and M2 polarization, making HDAC inhibitors promising therapeutic tools [11] (Figure 2).

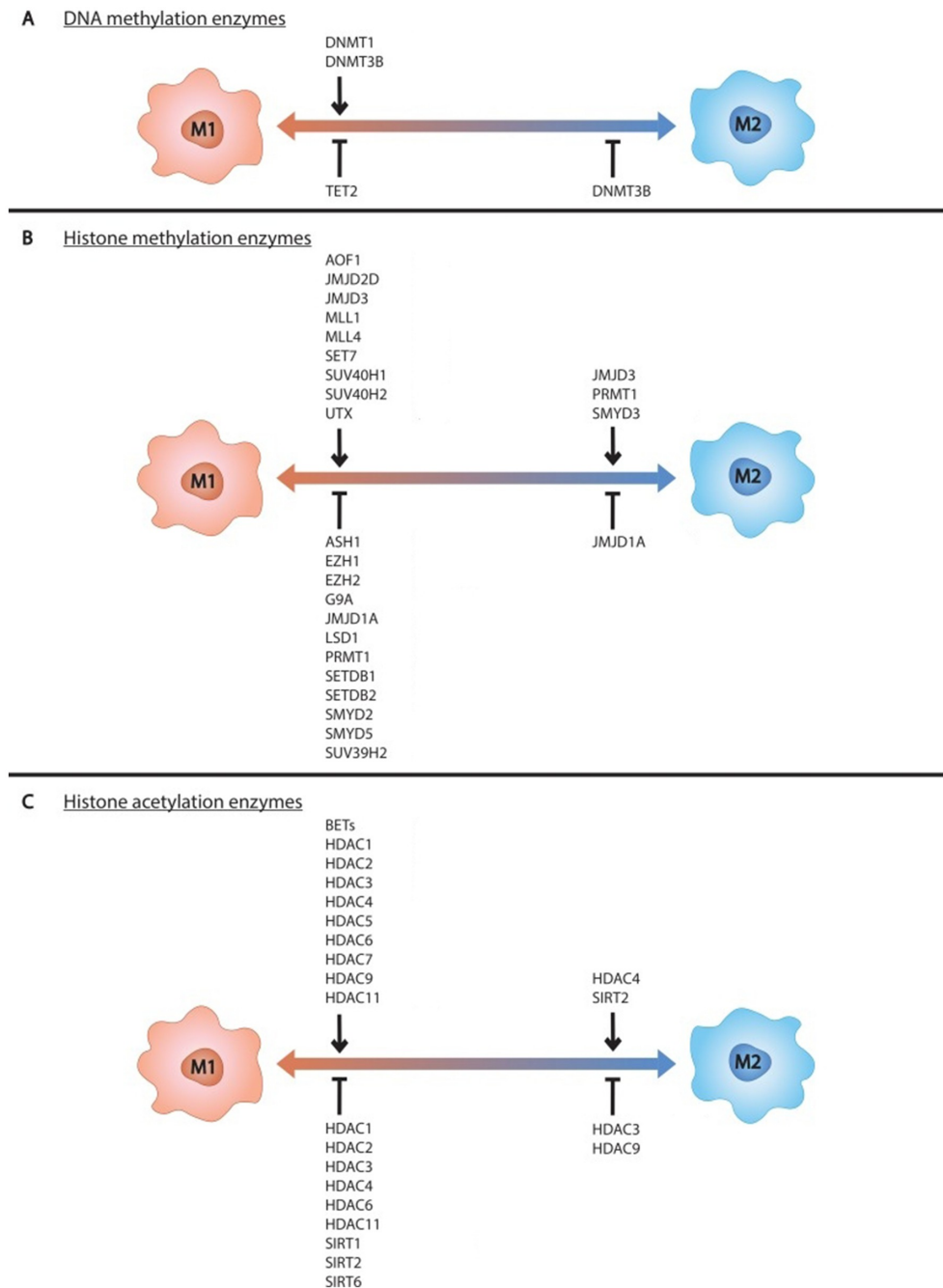


Figure 2. DNA methylation and histone modification. Epigenetic enzymes known to control (A) DNA methylation, (B) histone methylation, and (C) histone acetylation in macrophages are listed above the left side of the spectrum if they promote M1 polarization and below if they inhibit M1 polarization. Those known to control M2 polarization are listed likewise on the right side of the spectrum.

Critically, histone modifications confer memory: macrophages retain epigenetic imprints from prior stimuli, resisting repolarization. This durability underscores the challenge of sustaining macrophage reprogramming in disease therapy and highlights the need to understand microenvironment-specific epigenetic regulation.

3.2 DNA methylation and macrophage activation/polarization

DNA methylation, the primary chemical modification of chromatin DNA, involves the covalent addition of a methyl group to cytosine residues in CpG dinucleotides, catalyzed by DNA methyltransferases (DNMTs). Hypermethylation typically leads to chromatin condensation and gene silencing. Substantial evidence indicates that aberrant DNA methylation patterns significantly influence macrophage biological behavior.

DNMTs catalyze DNA methylation and repress gene expression. DNMT1 and DNMT3b are upregulated in M1 macrophages and promote proinflammatory polarization. For example, DNMT3b methylates the PPAR- γ promoter, inhibiting its expression and driving M1 polarization in adipose tissue macrophages. Similarly, DNMT1-mediated hypermethylation contributes to atherosclerosis by silencing anti-inflammatory genes. Inhibiting DNMTs (e.g., with Aza or curcumin) promotes M2 polarization and ameliorates inflammation in injury and disease models [12].

Conversely, DNMT3a is highly expressed in M2 macrophages and is associated with the silencing of M1-related genes. It methylates the promoter of Galectin-3, an M2 marker involved in angiogenesis. Demethylation restores its expression. Additionally, transcription factors like KLF4 – which promotes M2 polarization – can bind methylated DNA via unique motifs, enabling methylation-resistant gene regulation, thus adding another layer to epigenetic control in macrophage polarization [13].

DNMT1 and DNMT3a/b mediate maintenance and *de novo* DNA methylation, respectively, which stabilize macrophage polarization and enable functional remodeling. Although the hierarchical relationship between DNA methylation and histone modifications remains unresolved, both mechanisms synergistically and indispensably regulate macrophage polarization.

3.3 Micro-RNAs and macrophage activation/polarization

The ncRNAs, particularly long non-coding RNAs (lncRNAs) and micro-RNAs (miRNAs), serve as crucial epigenetic regulators in macrophage biology. miRNAs, ~22-nucleotide small RNAs, mediate post-transcriptional gene silencing by binding to the 3' untranslated regions (3' UTRs) of target mRNAs. These molecules orchestrate complex regulatory networks that enhance cellular robustness, playing pivotal roles in macrophage differentiation, maturation, and polarization.

Extensive studies have demonstrated dynamic miRNA expression profiles during macrophage polarization. Functionally, these miRNAs can be categorized into pro-inflammatory (M1-polarizing) and anti-inflammatory (M2-polarizing) subtypes. Among them, miR-155 emerges as the most extensively characterized regulator of M1 polarization. It potentiates inflammatory responses by targeting negative regulators SOCS1 and SHIP1, thereby enhancing AKT and cytokine signaling pathways. Genetic evidence shows that miR-155 deficiency impairs immune

responses and promotes M2 polarization, whereas its overexpression drives myeloid hyperplasia. In disease contexts, miR-155 contributes to atherosclerosis progression through the SOCS1–STAT3–PDCD4 axis and inhibits M2 polarization by suppressing IL-13R α 1 and SMAD2 expression [14]. Conversely, M2-associated miRNAs, including miR-181a and let-7c, attenuate inflammation by targeting KLF6/C/EBP α and other anti-inflammatory mediators. In the central nervous system, microglia-specific miR-124 alleviates neuroinflammation by modulating the C/EBP α –PU.1 signaling axis. Additionally, miRNAs such as miR-146 and miR-21 establish negative feedback loops to fine-tune TLR signaling pathways, highlighting their roles in immune homeostasis.

These findings underscore the central importance of miRNA networks in macrophage polarization and their therapeutic potential for inflammatory diseases. However, the complexity of miRNA-mediated regulation warrants further investigation to elucidate precise mechanistic insights.

4. Activation/polarization states of macrophages and interaction with the microenvironment

4.1 Notch signaling pathway and macrophage activation/polarization

4.1.1 Notch signaling pathway

The Notch signaling pathway is an evolutionarily conserved system that regulates cell fate decisions through ligand–receptor interactions between adjacent cells. Upon activation, the Notch intracellular domain translocates to the nucleus and binds to the transcription factor RBP-J to drive downstream gene expression. It controls key developmental processes – including lateral inhibition, stemness maintenance, and differentiation – from embryogenesis to tissue homeostasis. Dysregulation disrupts neural patterning, somite formation, and intestinal cell homeostasis [15].

4.1.2 Notch signaling pathway and myeloid cell differentiation

Notch signaling activation is closely associated with innate immunity and inflammatory responses, but its role in the differentiation of hematopoietic stem cells (HSCs) into myeloid cells remains controversial.

Some studies suggest that Notch activation maintains the self-renewal and stemness of hematopoietic progenitor and precursor cells while inhibiting their terminal differentiation into mature myeloid cells. For instance, overexpression of NICD1 reduces the formation of mature granulocytes, and Notch1 and Notch2 exhibit differential responses to colony-stimulating factors (G-CSF, GM-CSF). Overexpression of the Notch target gene *Hes1* or its ligands also blocks myeloid differentiation. However, other studies argue that Notch is required for myeloid maturation: inactivation of the γ -secretase complex (critical for Notch activation) leads to abnormal accumulation of granulocyte–macrophage progenitors, resembling chronic myelomonocytic leukemia (CMML). Additionally, somatic mutations in Notch-related genes (e.g., *Nicastrin*, *Notch2*) have been identified in CMML patients. The controversy may stem from the complexity of myeloid cell composition, the diversity of differentiation pathways, and the varying degrees of

Notch activation and ligand/receptor expression across different hematopoietic cells. Its regulatory role in myeloid differentiation may depend on the stage and intensity of activation, warranting further investigation.

4.1.3 Notch signaling pathway and macrophage activation/polarization

Notch signaling regulates macrophages through four key mechanisms. First, it controls inflammatory factor expression via RBP-J/Hes1, such as directly activating IL-6 and iNOS transcription. Notch1 inhibition reduces TNF- α during wound healing, while Notch blockade alleviates inflammation in lupus. Second, Notch modulates key transcriptional regulators. It upregulates SOCS3 to inhibit Stat3 and promote M1 polarization, impacting tumor suppression and BCG-induced inflammation. In liver fibrosis, RBP-J deficiency elevates CYLD to suppress NF- κ B. Notch also enhances TLR-JNK-IRF8 signaling to drive inflammatory responses. Third, Notch employs ncRNAs for epigenetic regulation. NICD1 activates miR-125a/99b to promote M1 polarization and reprogram TAMs, while miR-148a boosts macrophage microbicidal activity. Finally, Notch regulates metabolic reprogramming. It promotes Pdp1-mediated mitochondrial ROS release for M1 polarization and suppresses lactate metabolism to influence macrophage maturation. These mechanisms enable Notch to finely balance pro- and anti-inflammatory responses in immunity and disease. However, whether they exhibit context-dependent specificity or concurrent action requires further study, particularly through genome-wide profiling of Notch-RBP-J binding under diverse inflammatory conditions [16].

4.2 NF- κ B, JAK-STATs, and IRFs signaling in macrophage activation/polarization

4.2.1 NF- κ B, JAK-STATs, and IRFs signaling pathways

NF- κ B complexes consist of Rel proteins with RHD domains. The canonical p65/p50 heterodimer is sequestered by I- κ B until IKK-mediated phosphorylation triggers I- κ B degradation, enabling nuclear translocation and transcriptional activation. PRRs and TNF receptors initiate this pathway in innate immunity. The JAK/STAT pathway transduces cytokine signals through receptor-associated JAK kinases that phosphorylate STAT transcription factors. Phosphorylated STATs dimerize and regulate target gene expression. With four JAKs and six STATs exhibiting tissue-specific distributions, this pathway enables diverse cellular responses to environmental cues. IRFs regulate interferon and immune responses through conserved DNA-binding domains. While IRF3/7 mediate interferon production, IRF5 drives inflammation, and IRF4/8 control immune cell differentiation. Activation mechanisms vary: IRF3/5/7 require phosphorylation, whereas IRF1/2/4/8 are phosphorylation-independent. IRF9 cooperates with STATs for transcriptional activity [17].

4.2.2 Crosstalk of NF- κ B, JAK-STATs, and IRFs signaling in M1 macrophage polarization

In IFN γ /LPS-stimulated M1 macrophages, NF- κ B, IRFs, and STATs pathways are robustly activated. PAMPs like LPS trigger these pathways through TLR4's

MyD88/TRIF branches – MyD88 activates NF- κ B via IRAK4/TRAF6/IKK β , while TRIF activates IRF3 to induce IFN-I/STAT1 signaling. This enables macrophages to rapidly initiate inflammatory responses even without external IFN γ . The *Ifn β* gene exemplifies multi-factor synergy: basal expression depends on PU.1/IRF8, while LPS/viral infection recruits IRF3/7 and NF- κ B to its promoter, inducing chromatin remodeling for rapid transcriptional upregulation. AP1 family members may also contribute. NF- κ B and IRFs show remarkable cooperation – IRF3 binds RelA/p65's RHD domain, enabling co-activation at sites containing either factor's motif. This lowers activation thresholds and explains why many inflammatory genes lack dual binding sites. Similarly, IRF5 requires NF- κ B for DNA binding, and its knockout impairs M1 polarization. STATs extensively interact with IRFs: the STAT1/2/IRF9 complex promotes M1 polarization, while IRF1/7/8 cooperate with STAT1. Interestingly, STAT1 pre-marks inflammatory gene loci in resting macrophages, maintaining chromatin accessibility. Upon stimulation, recruited IRFs initiate transcription, while NF- κ B relieves RNA polymerase pausing – together enabling rapid inflammatory responses [18].

4.2.3 Crosstalk of NF- κ B, JAK-STATs, and IRFs signaling in M2 macrophage polarization

M2 polarization driven by IL-4/IL-13 depends critically on STAT6 activation, which directly induces M2 markers and anti-inflammatory factors. STAT6 also upregulates IRF4 through JMJD3-mediated H3K27me3 demethylation, forming a core regulatory axis with IRF4–PU.1 complexes initiating M2 programming. During M2 polarization, STAT6 and IRF4 cooperate with PPAR γ , C/EBP β , and KLF4 in a context-dependent manner. While STAT6 preferentially binds 4-nucleotide-spaced GAS motifs (vs. STAT1's 3-nucleotide preference), both factors can occupy overlapping genomic sites. STAT6 uniquely recruits HDAC3 to suppress inflammatory genes, explaining its anti-inflammatory effects. IRF4 exhibits complex regulatory interactions, forming ternary complexes with PU.1 and IRF8 at ISRE elements. However, the functional consequences of these interactions in macrophage polarization remain unclear, highlighting the need for further mechanistic studies on IRF4's context-dependent regulatory roles. The mutual inhibition between M1 and M2 polarization states involves epigenetic reprogramming, including H3K27ac/H3K27me3 modifications and chromatin remodeling. However, this reciprocal regulation appears asymmetric: while IFN γ globally suppresses IL-4-induced genes by erasing STAT6 binding across the genome, IL-4's inhibition of IFN γ -responsive genes is more selective [19]. This selectivity may reflect macrophages' need to preserve core inflammatory functions (e.g., antiviral responses) even in M2 states, suggesting context-dependent regulatory mechanisms that warrant further investigation.

5. Conclusion

This chapter provides an overview of the role and regulatory mechanisms of macrophage activation/polarization in inflammatory responses. Due to space limitations, many scientific questions are only briefly addressed. With recent

technological advances, single-cell sequencing data from myeloid cells/macrophages in various disease models have become increasingly available, expanding the conceptual framework of macrophage plasticity from a binary “polarization” model to a multidimensional “subpopulation” perspective. This progress allows for more precise identification of cellular subsets and molecular targets in strategies aimed at therapeutically targeting macrophages. Rapid developments in genetic engineering and materials science have significantly lowered the technical barriers to “reprogramming macrophages.” Mature applications, such as targeted drugs, nanomaterial delivery systems, adeno-associated viruses, and CRISPR–Cas9 systems, offer promising avenues for translating basic macrophage research into clinical interventions.

Several questions remain open for discussion. For instance, in the regulation of macrophage polarization, both epigenetic modifications and signaling pathways/transcription factors play crucial roles and interact extensively – but which mechanism is more fundamental? As multi-omics data (including transcriptomic, translational, metabolomic, and epigenomic profiles) from macrophages of different polarization states continue to accumulate, can we develop better analytical tools and computational platforms to integrate these datasets? Furthermore, can future research connect dynamic changes in chromatin three-dimensional structure to functional polarization, thereby providing a more comprehensive and high-resolution understanding of the underlying regulatory mechanisms? These questions and challenges will be addressed and refined in future studies.

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References

- [1] Lavin Y, Winter D, Blecher-Gonen R, et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell*. 2014;**159**(6):1312–1326. DOI: 10.1016/j.cell.2014.11.018
- [2] Okabe Y, Medzhitov R. Tissue-specific signals control reversible program of localization and functional polarization of macrophages. *Cell*. 2014;**157**(4):832–844. DOI: 10.1016/j.cell.2014.04.016
- [3] Murray PJ, Allen JE, Biswas SK, et al. Macrophage activation and polarization: Nomenclature and experimental guidelines. *Immunity*. 2014;**41**(1):14–20. DOI: 10.1016/j.immuni.2014.06.008
- [4] Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. *Nature Immunology*. 2015;**16**(4):343–353. DOI: 10.1038/ni.3123
- [5] Saha B, Kodys K, Szabo G. Hepatitis C virus-induced monocyte differentiation into polarized M2 macrophages promotes stellate cell activation via TGF- β . *Cellular and Molecular Gastroenterology and Hepatology*. 2016;**2**(3):302–316.e8. DOI: 10.1016/j.jcmgh.2015.12.005
- [6] Ghesquière B, Wong BW, Kuchnio A, Carmeliet P. Metabolism of stromal and immune cells in health and disease. *Nature*. 2014;**511**(7508):167–176. DOI: 10.1038/nature13312
- [7] Pyonteck SM, Akkari L, Schuhmacher AJ, et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. *Nature Medicine*. 2013;**19**(10):1264–1272. DOI: 10.1038/nm.3337
- [8] Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. *Cell*. 2011;**145**(3):341–355. DOI: 10.1016/j.cell.2011.04.005
- [9] Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. *Cell*. 2010;**141**(1):39–51. DOI: 10.1016/j.cell.2010.03.014
- [10] Lecoœur H, Prina E, Rosazza T, et al. Targeting macrophage histone H3 modification as a leishmania strategy to dampen the NF- κ B/NLRP3-mediated inflammatory response. *Cell Reports*. 2020;**30**(6):1870–1882.e4. DOI: 10.1016/j.celrep.2020.01.030
- [11] Lauterbach MA, Hanke JE, Serefidou M, et al. Toll-like receptor signaling rewires macrophage metabolism and promotes histone acetylation via ATP-Citrate lyase. *Immunity*. 2019;**51**(6):997–1011.e7. DOI: 10.1016/j.immuni.2019.11.009
- [12] Wang X, Cao Q, Yu L, Shi H, Xue B, Shi H. Epigenetic regulation of macrophage polarization and inflammation by DNA methylation in obesity. *JCI Insight*. 2016;**1**(19):e87748. DOI: 10.1172/jci.insight.87748
- [13] Liu Y, Olanrewaju YO, Zheng Y, et al. Structural basis for Klf4 recognition of methylated DNA. *Nucleic Acids Research*. 2014;**42**(8):4859–4867. DOI: 10.1093/nar/gku134
- [14] Nazari-Jahantigh M, Wei Y, Noels H, et al. MicroRNA-155 promotes atherosclerosis by repressing Bcl6 in macrophages. *Journal of Clinical*

Investigation. 2012;**122**(11):4190–4202.
DOI: 10.1172/JCI61716

[15] Hu X, Chung AY, Wu I, et al.
Integrated regulation of Toll-like
receptor responses by Notch and
interferon-gamma pathways.
Immunity. 2008;**29**(5):691–703.
DOI: 10.1016/j.immuni.2008.08.016

[16] Xu H, Zhu J, Smith S, et al. Notch-
RBP-J signaling regulates the
transcription factor IRF8 to promote
inflammatory macrophage polarization.
Nature Immunology. 2012;**13**(7):
642–650. DOI: 10.1038/ni.2304

[17] Honda K, Taniguchi T. IRFs:
Master regulators of signalling by Toll-
like receptors and cytosolic pattern-
recognition receptors. Nature Reviews
Immunology. 2006;**6**(9):644–658.
DOI: 10.1038/nri1900

[18] Chen X, Vinkemeier U, Zhao Y,
Jeruzalmi D, JE Jr D, Kuriyan J. Crystal
structure of a tyrosine phosphorylated
STAT-1 dimer bound to DNA. Cell.
1998;**93**(5):827–839. DOI: 10.1016/
s0092-8674(00)81443-9

[19] Kang K, Park SH, Chen J, et al.
Interferon- γ represses M2 gene
expression in human macrophages by
disassembling enhancers bound by the
transcription factor MAF. Immunity.
2017;**47**(2):235–250.e4. DOI: 10.1016/
j.immuni.2017.07.017

Perspective Chapter: Epigenetic Frontier – Decoding Host–Pathogen Dynamics in the Evolution of Parasitic and Microbial Diseases in an Animal Model

Falmata Kyari, Yasheruram Muhammad Shettima and Lawan Adamu

Abstract

The complex relationship between hosts and pathogens in parasitic and infectious microbial illnesses has traditionally been studied from the perspectives of genetics, immunology, and molecular biology. However, this paper has identified epigenetics as a crucial regulatory component that governs these processes. Epigenetic mechanisms such as DNA methylation, histone modification, and non-coding RNA activity play significant roles in regulating host defense, pathogen disease susceptibility, and the progression of infectious diseases. This paradigm shift highlights the epigenetic framework as a transformative structure for understanding the dynamic interaction of host and pathogen genomes in disease progression. This study examines epigenetic responses and adaptations in host and pathogen systems using animal models, which provide controlled environments and evolutionary significance. It explores how hosts modify gene expression patterns to generate effective immune responses, how pathogens exploit or regulate host epigenomes to facilitate infection and persistence, and how these interactions influence recurrence, resistance, and clinical outcomes. The use of animal models such as rats, zebrafish, and livestock enables high-quality epigenetic analysis and experimental manipulation to uncover causal links and phylogenetic pathways. Furthermore, the paper underscores the importance of epigenetic plasticity in pathogen adaptation and survival, investigating how infectious organisms manage their own epigenomes and modulate the expression of pathogenicity components. It identifies homologous epigenetic processes across species, uncovers epigenetic biomarkers with diagnostic or prognostic significance, and proposes therapeutic strategies targeting epigenetic regulators. It positions epigenetics as a central architect in the biological interplay between host immunity and microbial persistence.

Keywords: epigenetic landscape, nosogenesis, parasitic diseases, microbial diseases, animal model

1. Introduction

Infectious disorders caused by parasites and microbes pose an ongoing threat to global health, veterinary research, and biodiversity [1]. Conventional genetic and immunological paradigms for investigating interactions among hosts and pathogens have been tremendously valuable, but they typically overlook a more widely acknowledged and important stratum of biological regulation called epigenetics [2]. As researchers discover more about how epigenetic changes shape host responses to illnesses and pathogenic adaptability, an entirely novel field has emerged: understanding the constantly bidirectional coupling of host and pathogen epigenomes in the context of infectious disease progression (**Figure 1**).

Epigenetics refers to transmissible alterations in gene expression that do not affect the fundamental DNA sequence [3]. These alterations occur through mechanisms such as DNA methylation, histone modification, and non-coding RNAs (ncRNAs), which are heavily influenced by environmental factors, especially infections [4]. In host animal species, genetic reprogramming is a key evolutionary technique that enables fast and reversible gene regulation in response to parasite or microbial invasion [2]. It refines immune responses, regulates inflammation, and even produces memory-like states in innate immunity, thereby impacting disease genesis and resolution [5].

Pathogens, which include protozoan parasites, bacteria, and viruses, are not passive participants. Emerging research suggests that viruses might directly alter host epigenetic landscapes to overcome immune systems and establish persistent infections [6, 7]. Some pathogens utilize their own epigenetic machinery to

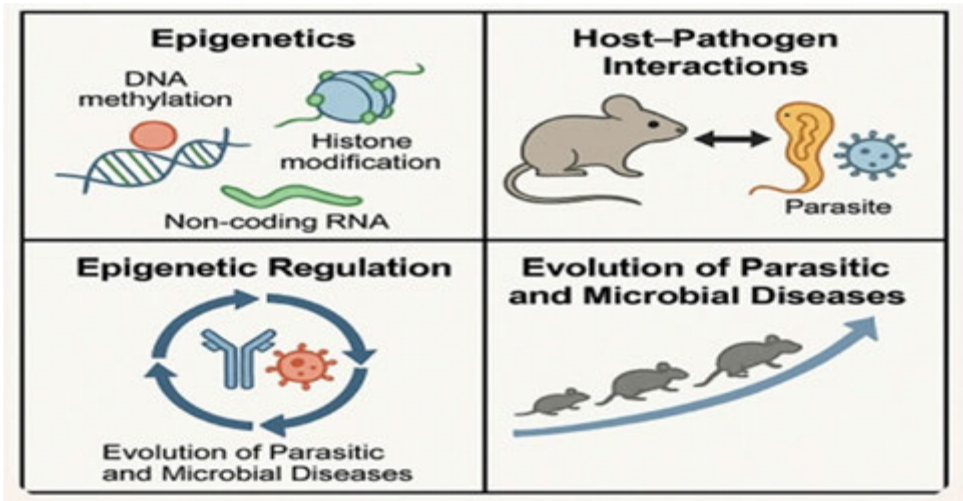


Figure 1. *Epigenetic Frontier: decoding host-pathogen dynamics in the evolution of parasitic and microbial diseases in an animal model. (Source: Copilot AI-generated image).*

modulate virulence gene expression, evade host detection, and adapt to changing host environments [8]. This trans-epigenetic relationship between host and pathogen epigenomes challenges our understanding of virulence and host adaptability.

Animal models provide valuable insights into this complex dynamic. Animal models, such as mice, zebrafish, cows, *Pan troglodytes*, *Dictyostelium discoideum*, *Schmidtea mediterranea*, rabbits, and sheep, allow for precise alteration and monitoring of epigenetic modifications under regulated circumstances [9, 10]. They provide frameworks for researching long-term alterations, detecting causal linkages, and evaluating medicines that target epigenetic circuits. Similarly, comparative epigenomic studies in animals can reveal genetically conserved processes and species-specific adjustments that influence vulnerability to illness and resilience [9].

This research into the epigenetic frontier not only broadens our molecular understanding of host–pathogen biology, but it also has translational potential. Epigenetic biomarkers may aid in diagnostics, while therapeutic techniques targeting epigenetic modifiers, such as histone deacetylase inhibitors or DNA methyltransferase blockers, may provide novel treatments for chronic infections with accompanying pathological conditions [11].

Finally, incorporating epigenetics as a critical component in infectious disease research enhances our understanding of how parasitic and microbial diseases arise, persist, and interact with hosts. Understanding the epigenetic language shared by species and biological kingdoms brings us closer to comprehending the mechanisms that regulate life, disease, and preservation.

1.1 Outline of the emergence of epigenetic discoveries in infectious diseases

Epigenetics refers to transmissible changes in gene expression that do not involve modifications to the DNA sequence [12, 13]. These changes influence how genes are turned on or off and are mediated by DNA methylation, histone modification, and ncRNAs [14]. These pathways act as a regulatory overlay above the genome, allowing for immunity and sensitivity to environmental stimuli such as infections [15, 16].

Usually, infectious diseases have been studied from a pathogen-centric standpoint, whereas epigenetics has shifted the focus to host–pathogen interactions, how environmental exposures affect host immunity, and the lingering biological imprint of infections [17]. This novel approach enables researchers to investigate why specific animals respond differently to infections and how viruses can alter host epigenetic circuitry.

DNA methylation is a critical epigenetic mechanism in infection that modifies gene expression in immune cells and plays a role in cytokine response and inflammation [15]. For example, hypomethylation of interferon genes occurs in viral infections such as influenza. Histone changes impact both chromatin structure and gene accessibility [18]. MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are ncRNAs that fine-tune immune signaling and regulate T-cell activation and antigen presentation [19]. Histones are also modified by pathogens like *Mycobacterium tuberculosis* to dampen immune responses. Viral infections frequently alter the regulation of these RNAs for replication purposes [20].

2. Conceptual framework of epigenetics and regulation in an animal model

Epigenetics refers to transmissible changes in gene expression that do not involve DNA sequence modifications. Pathogens in infectious diseases, such as parasites, bacteria, or viruses, can reprogram host gene expression using epigenetic pathways to evade immune responses, resulting in chronic infections and impacting disease severity [15]. Animal models (e.g. mice, rats, zebrafish) are essential for studying epigenetic responses to infection because they allow for controlled experimental conditions, enabling longitudinal studies of infection and immunity [21]. They also provide access to tissue-specific epigenetic profiling, which aids in the identification of therapeutic targets and biomarkers.

Microbial and parasite pathogens manipulate epigenetics, such as in *Helicobacter pylori* infections, altering DNA methylation in the gastric mucosa and causing cancer-like alterations [22]. *Mycobacterium tuberculosis* alters histone marks to impair host immunity. *Plasmodium falciparum* (malaria) modulates host miRNA expression to avoid immune detection [23] (**Table 1**). In contrast, *Theileria annulata* reprograms host macrophages through histone acetylation (**Table 1**), resulting in uncontrolled proliferation [24]. HIV and hepatitis viruses modify the host epigenome to survive and reproduce (**Table 1**). Furthermore, HBV's HBx protein affects host miRNA patterns, which influence viral load and liver disease [25].

In animal models, experimental approaches use chromatin immunoprecipitation (ChIP) to identify histone alterations at specific gene loci. Bisulfite sequencing reveals DNA methylation patterns [26]. RNA-seq and miRNA profiling indicate variations in ncRNA expression levels [27]. Epigenetic drug testing employs HDAC inhibitors such as vorinostat and belinostat, which show potential against parasite infections [28].

2.1 Epigenetic memory and plasticity

Epigenetic memory refers to the long-term retention of gene expression patterns due to epigenetic alterations (such as DNA methylation or histone changes), even after the initial stimulus (e.g. infection) is gone [29]. Epigenetic plasticity is the ability of cells to dynamically adjust gene expression in response to environmental signals, including infections [15]. Together, these systems enable hosts to adapt to infections, sometimes across generations, without affecting their DNA sequence. Animal models of infection, such as mice, zebrafish, and non-human primates, play an important role in studying these phenomena because they allow for controlled infection and re-infection

Mechanism	Role in infection	Example in animal models
DNA methylation	Silences immune genes or activates pathogen-friendly genes	HIV-infected mice show methylation of anti-viral genes
Histone modifications	Alters chromatin structure to regulate transcription	<i>Theileria</i> parasites induce histone acetylation to promote host cell survival
Non-coding RNAs	Regulate immune signaling and inflammation	miRNAs modulate cytokine expression in malaria-infected mice

Table 1.
Strategic epigenetic mechanisms in host–pathogen interactions.

experiments, facilitating the tracking of epigenetic changes over time and providing insights into trained immunity and long-term immune reprogramming [30].

Host–pathogen interactions are among the most flexible and unpredictable systems in the natural environment. To cope with the discriminatory restrictions enforced by their hosts, numerous parasitic and microbial diseases have acquired an exceptional level of phenotypic diversity in their life-history features [14, 31]. Pathogens can radically modify a host's phenotype, and the parasitized or infected phenotype may be passed down through generations [32]. Furthermore, co-adaptations between hosts and parasitic/microbial diseases frequently occur over relatively short evolutionary time scales, calling into question the exclusive involvement of genetic alterations (i.e. modification and/or recombination) as an underlying process [14, 33]. In such scenarios, epigenetic alterations could provide an additional source of effective, reversible, and easily obtainable phenotypic variance that might be influenced by both host and pathogen selection pressures [14]. Epigenetic mechanisms have been found to play a role and are broadly characterized as (1) pathogen-induced host changes and (2) pathogen plasticity.

2.1.1 Plasticity of pathogen

Pathogens' structural and evolutionary flexibility play a crucial role in their transmission and existence in the host. Complicated transitions that occur in response to a changing host environment necessitate quick and significant changes in gene expression profiles. *Plasmodium* malaria parasites have distinct hepatocytic and erythrocytic stages in vertebrate hosts such as primates. Conversely, these parasites possess a rich arsenal of histone variants, histone and chromatin-modifying enzymes, and RNA-mediated silencing pathways [14]. *Toxoplasma gondii* relies on histone acetylation to transition between replicative and non-replicative phases [14, 34]. *Trypanosoma brucei* constitutes the solitary apicomplexan where DNA methylation has been discovered, although the importance of these epigenetic changes in parasite cell cycle control continues (Table 5) to be unknown [14, 35]. DNA methylation has been linked to the yeast-hyphal transition in *Candida albicans*, a polymorphic yeast [14].

An additional remarkable feature of pathogen plasticity is the capacity to change the expression of genes associated with virulence pathways, allowing them to colonize, multiply, and/or disperse inside the host. *Plasmodium falciparum* shifts between different surface proteins throughout the erythrocytic stage to escape the host's immune system (antigenic variation). These surface proteins are generated by extremely polymorphic gene families, including *var*, *rif*, *stevor*, and *pfmc-2tm*. In the case of the *var* family, the parasite's capacity to express only one of the 60 genes that encode these proteins (named PfEMP1) is epigenetically regulated by histone modification [14, 36]. Furthermore, recent research has demonstrated that the parasite's epigenetic status is retained during multiple rounds of cell division [37]. Epigenetic regulation of virulence factors has been well documented in various microbial pathogens. Numerous microbial pathogens exhibit epigenetic regulation of virulence components. In *Entamoeba histolytica*, for instance, demethylation and histone methylation influence the expression of the amoebapore protein, the pathogen's cytotoxic protein [38]. DNA methylation is also an important virulence regulation mechanism in many pathogenic bacteria [14]. Lack of Dam (DNA adenine methyltransferase) methylation in *Salmonella enterica* leads to envelope instability, decreased motility, and poor invasion of the intestinal epithelium [14].

Alterations in the host caused by pathogens: Information is now available showing that histone changes and chromatin remodeling regulate gene expression, making them important avenues for pathogen modification during an infection [39]. The host's immune system is a prominent target. Epigenetic regulation of host defense genes has become a typical feature of pathogenic microbial and viral infections [14, 39]. Bacteria are an indicator of microbial epigenetic studies, and they provide multiple pioneering examples of infection-induced host gene remodeling [40]. A wide range of microbial triggers have been found that either imitate or inhibit the host cellular mechanisms, hence enabling the pathogen's life cycle [14]. Microbial-induced post-translational changes, such as acetylation, phosphorylation, and ubiquitylation of histones and chromatin-associated proteins, commonly target MAPK, IFN, and NF- κ B signaling pathways [14, 41]. *Mycobacterium tuberculosis* suppresses interferon- γ -induced immune gene expression in alveolar macrophages by histone acetylation [42], leading to persistent tuberculosis infections in some animal species. This method appears to be quite common among intracellular infections and is not limited to bacteria. Influenza viruses proceed a step further by bypassing the host's immunological systems. In a recent work, Gómez-Díaz et al. [2] described an influenza protein termed NS1 that has an amino-acid sequence (ARTK) that is extremely comparable to the host's histone tail.

2.2 Importance of parasites and microbial diseases in animal models

Animal research has, in past years, benefited biology and continues to do so. Animals have become tools for unlocking genetic secrets that underpin many aspects of existence (**Figure 2**). The accessibility of animal models that can mimic such sophisticated processes has greatly assisted research on microbial diseases and wide-ranging studies of the connections comprising parasite–host associations, much as it does in developmental biology [43].

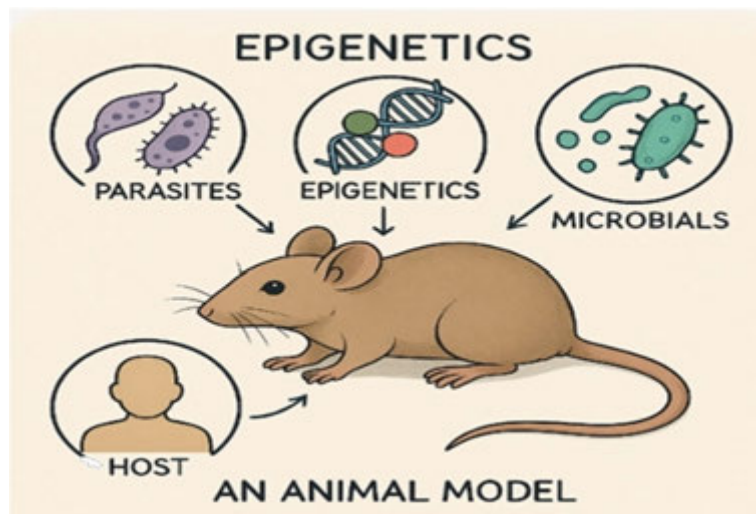


Figure 2. This diagram depicts the relationships between epigenetic variance, phenotypic variability, and interactions between hosts, parasites, and microbial diseases. (Source: Copilot AI-generated image).

The work of Edward Jenner, an English scientist, can be seen as a breakthrough moment. Milkmaids were thought to be immune to smallpox around the turn of the nineteenth century [43]. Jenner theorized that pus produced by cowpox blisters (a less severe illness) safeguarded milk workers from smallpox infection. A little time later, Jenner scraped a component of cowpox and inoculated it into a youngster's arm in a bid to render him unwell by means of cowpox while also testing its anti-smallpox properties. Jenner intentionally injected the virus into the boy's arm, preventing him from getting smallpox after he recovered completely from cowpox a week later. After the boy developed immunity, Jenner tested his theory on additional people. Similar to Pasteur, who created vaccines against rabies and chicken cholera a few years later, Jenner had transformed livestock, namely cows, into tools for medical advancement [43].

After this, the status of animals changed. They were once again merely targets of research, and like any tool, animals such as large animals, zebrafish, Dictyostelia, mice, rabbits, and flies underwent numerous rounds of refinement. Although large animals were used in the early days of experimental immunology, mouse models, as well as *Drosophila* and *Caenorhabditis elegans*, now dominate contemporary research in biology [43]. All too frequently, scientists regard these models as excellent mimics for biological research, as if altering a few genes could genuinely recreate the physiology of parasitic and microbial diseases in many species [43]. Experiments with mice, whether genetically engineered or not, are incredibly useful in mirroring the pathophysiology of most parasitic and microbial diseases.

Danio rerio (zebrafish) (see **Figure 3c**) is gaining popularity in biomedical research as a disease model for studying illnesses such as tuberculosis. Zebrafish are

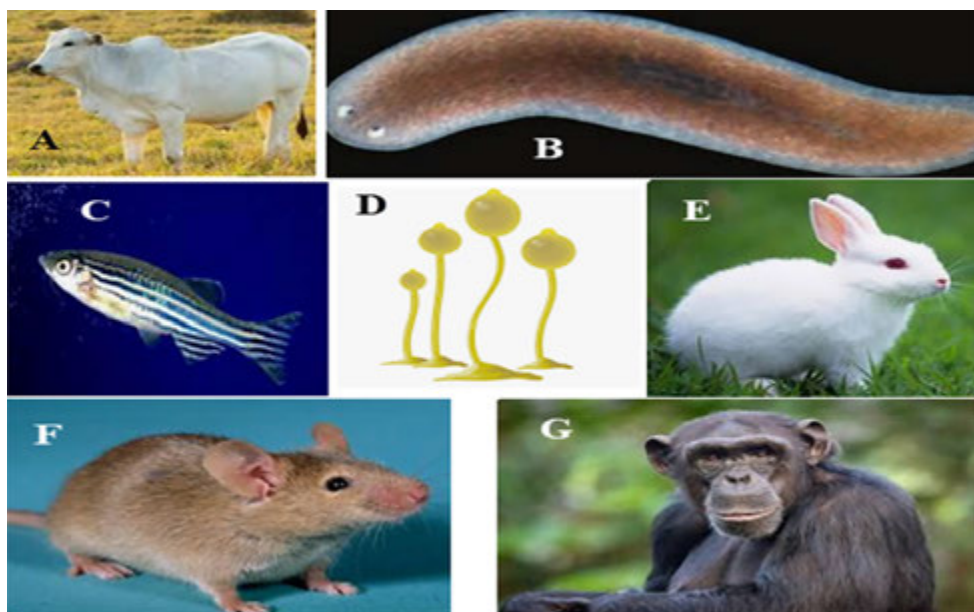


Figure 3. (a) *Bos taurus* or cow, (b) the soil-dwelling amoeba *dictyostelium discodinium*, (c) the tropical freshwater fish *danio rerio* (often known as zebrafish), (d) the freshwater flatworm *schmidtea mediterranea*, (e) *Oryctolagus cuniculus* or rabbit, (f) *Mus musculus* or mouse, (g) *Pan troglodytes*. (Source: Conti et al. [43], with some modifications).

inherently sensitive to tuberculosis initiated by *Mycobacterium marinum*.

Tuberculosis prevention involves both innate and adaptive immunity, just as it does in other animals. Davis et al. [44], in 2002, exploited the visual transparency of zebrafish embryos to carry out a real-time visualization of granuloma formation after *M. marinum* infection [44]. In this work, the researchers reported that granuloma formations appeared remarkably early in the course of illness, despite the presence of innate resistance. This finding was significant because prior studies had claimed that elements of adaptive immunity, particularly T lymphocytes, played a primary role in recruiting and stimulating macrophages to create *Mycobacterium* granuloma tissue [45].

The utilization of large animals as research models has led to major breakthroughs in the advancement of growth-oriented immunology studies, and cattle, horses, sheep, deer, and swine may be equally useful as or superior to mice for studying a variety of pathologies such as viral diarrhea, influenza, and tuberculosis. In addition to the evident benefits of their size (e.g. facilitating the collection of liquids with minimal surgical interference or sampling of tissues), it is worth mentioning that large animals have commonly evolved as outbred species over thousands of years; thus, their immune systems may have been influenced by exposure to a similar range of pathogenic agents. *Mycobacterium avium* (spp. paratuberculosis or MAP) causes Johne's disease in large animals (see **Figure 3a**), with the primary clinical signs being diarrhea and wasting, which often appear two or more years after the initial infection. Surprisingly, this research has shown that IL-23 contributes to the disease; thus, extending this research to cows within the clinical stage of infectious disease could imply that IL-23 likewise serves an essential function and is one of the variables that contribute to the breakdown of protective immunity.

The planarians' perspective: Planarians (see **Figure 3b**) are flatworms that are non-parasitic and inhabit freshwater. The academic community knows them best for their ability to regenerate practically indefinitely due to the abundance of neoblasts throughout their bodily tissues. Neoblasts are pluripotent somatic stem cells found in the parenchyma that can induce approximately 30–40 varieties of cells. Planarians have been widely utilized as animal models because they are inexpensive, small, and can reproduce simply by cutting their bodies — a trait that is mostly exploited in developmental biology research. Additionally, they do not raise ethical concerns [43, 46]. Planarians have recently emerged as a model for understanding stem cell biology, but they might also be beneficial for investigating other biological challenges. Over 20 years ago, Conti et al. [43] identified the distinctive function of “reticular cells” in mediating the initial stages of immune response. These high-mobility cells could distinguish extraneous material, such as bacteria, in as little as 8 hours after being introduced. Zhou et al. [47] found that the flatworm *Dugesia japonica* enhanced the production of a serine protease after consuming *E. coli* DH5a, a non-pathogenic laboratory strain of bacterium. As serine proteases may mediate immunological responses in mosquitoes, the authors suggested that this induction, which occurs when worms are exposed to bacteria, might represent the initial phase in a larger and unexplored host–pathogen interaction in planarians.

According to Abnave et al. [48], planarians have a strong immune response to pathogenic bacteria, including *C. elegans* and *D. melanogaster*. This response involves at least three genes: DUSP19 (Dual-Specificity Phosphatase Enzyme), MORN2 (Membrane Occupation and Recognition Nexus 2), and PAQR3. The study

found that MORN2 plays a role in LC3-associated phagocytosis (LAP) and is crucial for the elimination of bacterial infections in both macrophages and flatworms. A previous dataset reveals a significant interest in exploring planarian defense systems and identifying homologous immune components [48]. Investigators hypothesized that *Schmidtea mediterranea* and *D. japonica*, two species of planarians, could serve as useful models for studying schistosomiasis, a common parasitic disease caused by *S. mansoni*, a parasitic tapeworm with its own neoblast population [43, 49].

Mouse models, while valuable, may have limitations in biological processes [50, 51]. Utilizing animal models could potentially be a viable alternative, but financial and ethical concerns remain a barrier for many research institutions. Research on host–parasite illnesses using novel models, such as zebrafish, could lead to new avenues of inquiry. Using *D. japonica* flatworms or *S. mediterranea* as model organisms to investigate the composition of *S. mansoni* could aid in developing new treatments to combat schistosomiasis, which currently relies on just one medication. Cows are a good model for studying intestinal and uterine infections due to their production of lipopolysaccharides and pro-inflammatory cytokines. Although mice have long been considered the supreme and ideal model organism for practical purposes in genetic exploration, it is now evident that they do not represent the best genetic prototype due to inherent differences between humans and rodents. Developing and studying lesser-known species could provide valuable insights into intricate host–pathogen relationships.

3. Disease tolerance vs. resistance: An epigenetic perspective

Defining the concepts: Both strategies are necessary for survival, but they work via unique biological pathways, with epigenetics playing an important role in modifying both disease tolerance and resistance.

Epigenetic regulation is the molecular circuit of epigenetic processes that govern gene expression without altering the DNA sequence. These include histone modifications, DNA methylation, chromatin remodeling, and ncRNAs. These systems are both dynamic and reversible, allowing hosts to respond to infections in real time. Epigenetics in disease resistance (**Table 2**) signals activate immune genes through histone acetylation at the promoters of cytokines such as IFN- γ , IL-6, and TNF- α , resulting in increased transcription [52]. Similarly, training innate immunity through pathogen or vaccine exposure causes long-term epigenetic alterations in monocytes and macrophages. Furthermore, modulating the lineage commitment of DNA methylation patterns guides the development of T cells, B

Concept	Description
Disease resistance	The ability of a host to prevent or eliminate pathogens, thereby reducing pathogen burden.
Disease tolerance	The ability of a host to minimize the damage caused by pathogens or immune responses without necessarily reducing pathogen load.

Table 2.
The concept and description of disease tolerance and resistance.

cells, and NK cells. These alterations enable the host to detect and eliminate infections more efficiently.

Epigenetics in disease tolerance mechanisms (**Table 2**) focuses on limiting tissue damage and preserving homeostasis, which suppresses excessive inflammation by the epigenetic silencing of pro-inflammatory genes and prevents immunopathology. This includes encouraging tissue repair through histone changes, which activate genes involved in regeneration and detoxification [52]. Metabolic reprogramming is the epigenetic regulation of energy expenditure and nutrient use that aids in survival during illness [53]. For example, during bacterial infections, hosts may enter a hypometabolic state to preserve energy and decrease collateral damage. Resistance reduces pathogen load, but it might induce collateral damage through inflammation (**Table 2**). Tolerance reduces this damage, but it may allow parasitic and microbial diseases to persist (**Table 2**).

Epigenetic plasticity allows the host to balance these methods based on the pathogen type, tissue affected, host age and gender, and environmental factors [54]. Surprisingly, some infections use the host's epigenetic machinery to decrease resistance and enhance tolerance, thereby extending their survival. A case study with *Mycobacterium tuberculosis* shows that altering host histone methylation suppresses antigen presentation and oxidative stress responses while also promoting a tolerant state that allows for latent infection (**Table 2**).

Therapeutic implications include the ability of epigenetic medicines (such as HDAC inhibitors) to alter immunological responses in order to increase resistance or tolerance [55]. CRISPR-based epigenome editing allows for precise control over immune gene expression, whereas trained immunity-based vaccinations attempt to produce positive epigenetic reprogramming [56]. Understanding these pathways enables tailored therapy, particularly in infectious illnesses and autoimmune diseases.

4. Pathogen-induced epigenomic modulation

Pathogen-induced epigenomic modulation is the ability of infectious agents like bacteria, viruses, fungi, and parasites to change the host's epigenetic landscape [2]. These alterations can influence gene expression without altering the underlying DNA sequence, allowing infections to control host immunity, enhance their own survival, and occasionally lead to chronic disease. Epigenomic regulation refers to transmissible and transitory changes in gene expression caused by DNA methylation, which is the addition of methyl groups to cytosine bases, often decreasing gene expression [57]. It also includes histone modifications like acetylation, methylation, and phosphorylation, which alter chromatin structure and gene accessibility. Furthermore, ncRNAs play an equally important role in the epigenetic modulation of lncRNAs, miRNAs, and circular RNAs, all of which affect gene expression after transcription. Chromatin remodeling refers to structural changes in chromatin that affect transcription factor binding and gene transcription [57].

Pathogens have evolved sophisticated strategies to hijack host epigenetic machinery via DNA methylation alterations. For example, *Mycobacterium tuberculosis* secretes proteins such as Rv2966c that mimic host DNA methyltransferases, resulting in non-CpG methylation and immune-related gene silencing [58]. Similarly, *Helicobacter pylori* infection is related with hypermethylation of tumor suppressor genes, which contributes to gastric cancer [58]. *Listeria monocytogenes* and *Chlamydia*

Effect	Mechanism	Outcome
Immune evasion	Silencing of cytokine or antigen-presentation genes	Reduced host defense
Chronic infection	Epigenetic reprogramming of immune cells	Persistent inflammation
Oncogenesis	Methylation of tumor suppressor genes	Increased cancer risk
Latency/ reactivation	Histone modifications at viral promoters	Viral persistence or reactivation

Table 3.
Functional consequences of epigenetic modulation.

trachomatis can cause histone deacetylation by suppressing pro-inflammatory genes. *Fusobacterium nucleatum* exposure causes downregulation of chromatin remodeling, and histone modification genes particularly in endothelial tissues, which promotes inflammation and probably cancer progression [59]. The ncRNA regulation allows viruses like HIV and HBV to suppress antiviral responses or promote latency. In sepsis, ncRNAs influence inflammatory pathways like NF- κ B, JAK/STAT, and PI3K/AKT, impacting illness severity [4].

M. tuberculosis affects histone acetylation and DNA methylation in macrophages, inhibiting genes such as CIITA and IL17RA, which are required for antigen presentation and inflammation (Table 3). In HIV, DNA methylation of the BST2 gene inhibits its expression, reducing antiviral defense (Table 3). Demethylating drugs can restore expression while inhibiting viral replication. Fungal β -glucan affects chromatin accessibility in monocytes, boosting glutathione metabolism and immunological response [4].

Epigenetic medicines, such as histone deacetylase inhibitors (e.g. vorinostat) and DNA methyltransferase inhibitors (e.g. 5-azacytidine), are being investigated for infectious disorders. Epigenetic signature biomarkers, such as methylation of TLR2 or IL6R, may be used as diagnostic or prognostic tools. Understanding epigenetic regulation can help in vaccine development by informing strategies for improving immunological memory and vaccine effectiveness [60].

4.1 Infectious agents as epigenetic designers

Pathogens, which include viruses, bacteria, fungi, and parasites, are more than just invading organisms; they are epigenetic designers who alter host gene expression to facilitate their continued existence, procreation, and dissemination. This alteration does not change the host's DNA sequence but rather relies on epigenetic mechanisms such as ncRNA regulation, histone modification, and DNA methylation. To function as an epigenetic designer, a pathogen must be able to impact host gene expression through epigenetic mechanisms. In a similar vein, it must have the ability to evade immune recognition by thwarting or stimulating specific host genes. It must also be capable of restructuring cellular networks to boost tenacity, delay, or replication. In addition, it must cause long-term or transmissible changes in host cells, often persisting even after the infection is eradicated [61].

4.2 Virulence factors and host chromatin alteration

Pathogens have evolved an arsenal of virulence factor molecules that enable them to infect, survive, and thrive within host organisms (Table 4). One of the most

Mechanism	Virulence factor example	Effect on chromatin
Histone deacetylation	Listeriolysin O (LLO) from <i>Listeria monocytogenes</i>	Deacetylates H3/H4, represses pro-inflammatory genes
Histone methylation	RomA from <i>Legionella pneumophila</i>	Methylates H3K14, represses transcription globally
Histone demethylation	OspF from <i>Shigella flexneri</i>	Dephosphorylates MAPKs, blocks H3S10 phosphorylation
Chromatin remodeling	InlB from <i>Listeria</i>	Activates SIRT2, alters chromatin accessibility
DNA methylation	Rv1988 from <i>Mycobacterium tuberculosis</i>	Mimics methyltransferases, silences immune genes

Table 4.
Key mechanisms of virulence factors in altering host chromatin.

insidious strategies they employ is the modulation of host chromatin architecture (Table 4), which allows them to reprogram gene expression and evade immune responses [8]. This interplay between microbial effectors and host chromatin is a frontier of infection biology, revealing how deeply pathogens can infiltrate cellular control systems. Chromatin is the complex of DNA and histone proteins that packages the genome within the nucleus. Its structure determines gene accessibility; euchromatin is packed loosely and actively transcribed.

Meanwhile, heterochromatin is tightly packed and genetically silent. Chromatin state is regulated by epigenetic modifications, including histone acetylation, which promotes gene expression (Table 4). Histone methylation can activate or repress genes depending on the residue. DNA methylation typically represses gene transcription (Table 4), whereas chromatin remodeling causes ATP-dependent repositioning of nucleosomes [8].

Pathogens deploy nucleomodulin effector proteins that enter the host nucleus and directly manipulate chromatin (Table 4). These virulence factors mimic or hijack host enzymes to repress immune genes, promote pathogen survival, and induce cellular reprogramming (Table 4).

4.3 Comparative case studies: protozoa, helminths, bacteria, and viruses

Studying how different types of pathogens interact with the host and with one another provides important insights into disease progression, immune regulation, and treatment effects. This comparative approach investigates protozoa, helminths, bacteria, and viruses using case studies to illustrate their distinct and overlapping methods in pathogenesis (Table 6), immune evasion (Table 5), and co-infection dynamics [62]. The transmission of female Anopheles mosquitoes targets hepatocytes and erythrocytes, causing immunological regulation and antigenic variation via PfEMP1 proteins. It suppresses adaptive immunity, namely T-cell responses. Co-infection increases susceptibility to bacterial infections, such as *Salmonella* and *Mycobacterium tuberculosis*, and alters vaccination effectiveness by reducing immunological memory.

Schistosoma mansoni (Schistosomiasis) is transmitted through contact with contaminated freshwater, where it targets blood vessels in the intestines and liver,

Targeted host function	Pathogen benefit
Cytokine gene expression	Immune evasion
Apoptosis regulation	Prolonged host cell survival
Antigen presentation	Avoidance of T-cell detection
Cell cycle control	Enhanced replication niche
DNA repair pathways	Genomic instability, potential oncogenesis

Table 5.
Functional consequences of chromatin alterations.

Feature	Protozoa	Helminths	Bacteria	Viruses
Size	Microscopic (unicellular)	Macroscopic (multicellular)	Microscopic (unicellular)	Submicroscopic (acellular)
Immune response	Th1 suppression	Th2 dominance, Treg induction	Neutrophil/macrophage activation	CD4+ T-cell depletion
Immune modulation	Antigenic variation	Cytokine skewing	Immune evasion via biofilms, capsules	Latency, immune exhaustion
Co-infection role	Enhances bacterial/viral persistence	Dampens vaccine efficacy	Exploits immune suppression	Amplifies other infections
Treatment challenge	Drug resistance (e.g. <i>Plasmodium</i>)	Limited anthelmintic options	Antibiotic resistance	Antiviral resistance, latency

Table 6.
Comparative case studies: protozoa, helminths, bacteria, and viruses.

causing immunological modulation and Th2 division (IL-13, IL-5, IL-4). This increases regulatory T-cell (Treg) activity, reducing inflammation [62]. Co-infection lessens the severity of viral infections (e.g. hepatitis) while increasing bacterial and protozoal infections, and this may reduce vaccine efficacy by altering immunological responses. *Mycobacterium tuberculosis* is transmitted by airborne droplets and targets alveolar macrophages, triggering immunological modulation that inhibits phagosome-lysosome fusion and encourages granuloma formation to limit infection. The impact of co-infection could be exacerbated by HIV (virus) and *Plasmodium* (protozoan), and this could persist in immunosuppressed conditions caused by helminths (Table 6). HIV is transmitted through the bloodstream, sexual interactions, and vertical transmission, targeting CD4+ T cells and causing immunological modulation, resulting in progressive immunodeficiency, latency, and immune exhaustion [63]. Co-infection raises the risk of tuberculosis, malaria, and helminth infections while altering cytokine profiles, allowing other pathogens to survive.

5. Conclusion

As we approach the epigenetic frontier, the delicate dance between host and pathogen appears not as a static fight, but as a dynamic, evolving dialog inscribed in the language of chromatin and chemical markers. This study of the epigenetic

foundation of parasite and microbial diseases in animal models reveals an important truth: the genome is not fixed, but rather a script that is continually updated by environmental cues, pathogenic connections, and genetic limitations. Evaluating these molecular signals gives us not only a better understanding of disease recurrence and resistance, but also a powerful lens through which to view life's mutual evolution. The outcomes of these models offer insight into potential routes for precision medicine, personalized treatments, and predictive diagnostic tools, which could transform our approach to infectious disease in both animals and people. In this constantly shifting biological scenery, epigenetics serves as a navigational device. As we keep trekking through this domain, one thing becomes clear: the future of disease warfare lies not only in eradicating diseases, but also in recognizing the silent, reusable tracks they leave behind and how hosts adjust, oppose, and evolve in response.

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Conflict of interest

The authors declare no conflicts of interest.

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References

- [1] Froment A. Biodiversity and health: The place of parasitic and infectious diseases. *Biodiversity Change and Human Health: From Ecosystem Services to Spread of Disease*. 2012;**69**:211
- [2] Gómez-Díaz E, Jorda M, Peinado MA, Rivero A. Epigenetics of host–pathogen interactions: The road ahead and the road behind. *PLoS Pathogens*. 2012;**8**(11):e1003007
- [3] Gibney ER, Nolan CM. Epigenetics and gene expression. *Heredity*. 2010;**105**(1):4–13
- [4] Kulkarni S, Arumugam T, Chuturgoon A, An P, Ramsuran V. Editorial: Epigenetics of infectious diseases. *Frontiers in Immunology*. 2022;**13**:1054151
- [5] Planas R, Felber M, Vavassori S, Pachlopnik Schmid J. The hyperinflammatory spectrum: From defects in cytotoxicity to cytokine control. *Frontiers in Immunology*. 2023;**14**:1163316
- [6] Schator D, Gomez-Valero L, Buchrieser C, Rolando M. Patho-epigenetics: Histone deacetylases as targets of pathogens and therapeutics. *MicroLife*. 2021;**2**:uqab013
- [7] Valeria M, Daniela DA, Francesca M, Nicola P, Luigi A. Epigenetics and infectious disease: State-of-the-art and perspectives in new generation therapies. *OBM Genetics*. 2018;**2**(4):1–2
- [8] Denzer L, Schrotten H, Schwerk C. From gene to protein—How bacterial virulence factors manipulate host gene expression during infection. *International Journal of Molecular Sciences*. 2020;**21**(10):3730
- [9] Phillips NL, Roth TL. Animal models and their contribution to our understanding of the relationship between environments, epigenetic modifications, and behavior. *Genes*. 2019;**10**(1):47
- [10] Triantaphyllopoulos KA, Ikonopoulou I, Bannister AJ. Epigenetics and inheritance of phenotype variation in livestock. *Epigenetics and Chromatin*. 2016;**9**:1–8
- [11] Zhou Z, Rajasingh S, Barani B, Samanta S, Dawn B, Wang R, Rajasingh J. Therapy of infectious diseases using epigenetic approaches. *Epigenetics in Human Disease*. 2018;**1**:689–715
- [12] Jablonka E. Epigenetic epidemiology. *International Journal of Epidemiology*. 2004;**33**(5):929–935
- [13] Jablonka E. The evolutionary implications of epigenetic inheritance. *Interface Focus*. 2017;**7**(5):20160135
- [14] Bure IV, Nemtsova MV, Kuznetsova EB. Histone modifications and non-coding RNAs: Mutual epigenetic regulation and role in pathogenesis. *International Journal of Molecular Sciences*. 2022;**23**(10):5801
- [15] Zhang Q, Cao X. Epigenetic regulation of the innate immune response to infection. *Nature Reviews Immunology*. 2019;**19**(7):417–432
- [16] Zhang S, Meng Y, Zhou L, Qiu L, Wang H, Su D, Zhang B, Chan KM, Han J. Targeting epigenetic regulators for inflammation: Mechanisms and

intervention therapy. *MedComm*. 2022;**3**(4)

[17] He S, Qi Y. The microbiota, the malarial parasite, and the mice—a three-sided relationship. *Frontiers in Microbiology*. 2025;**16**:1615846

[18] Klemm SL, Shipony Z, Greenleaf WJ. Chromatin accessibility and the regulatory epigenome. *Nature Reviews Genetics*. 2019;**20**(4):207–220

[19] Zeni PF, Mraz M. LncRNAs in adaptive immunity: Role in physiological and pathological conditions. *RNA Biology*. 2021;**18**(5):619–632

[20] Fatima S, Kumari A, Agarwal M, Pahuja I, Yadav V, Dwivedi VP, Bhaskar A. Epigenetic code during mycobacterial infections: Therapeutic implications for tuberculosis. *The FEBS Journal*. 2022;**289**(14):4172–4191

[21] Sullivan C, Kim CH. Zebrafish as a model for infectious disease and immune function. *Fish & Shellfish Immunology*. 2008;**25**(4):341–350

[22] Kashyap D, Rele S, Bagde PH, Saini V, Chatterjee D, Jain AK, Pandey RK, Jha HC. Comprehensive insight into altered host cell-signaling cascades upon *Helicobacter pylori* and Epstein-Barr virus infections in cancer.. *Archives of Microbiology*. 2023;**205**(7):262

[23] Arumugam T, Dayaram I, Gokul A, Adimulam T, Ramsuran V. Advances in personalized epigenetics in infectious diseases. In *Personalized Epigenetics*, Academic Press; 2024:629–655

[24] Sakura T, Tajeri S, Rchiad Z, Ansari HR, Kaushik A, Mourier T, Pain A, Wassef M, Langsley G. Epigenetic landscape of the H3K27me3 mark in

macrophages transformed by *Theileria annulata*. *bioRxiv*. 2025;**6**:2025–01

[25] Sartorius K, An P, Winkler C, Chuturgoon A, Li X, Makarova J, Kramvis A. The epigenetic modulation of cancer and immune pathways in hepatitis b virus-associated hepatocellular carcinoma: The influence of HBx and miRNA dysregulation. *Frontiers in Immunology*. 2021;**12**:661204

[26] Statham AL, Robinson MD, Song JZ, Coolen MW, Stirzaker C, Clark SJ. Bisulfite sequencing of chromatin immunoprecipitated DNA (BisChIP-seq) directly informs methylation status of histone-modified DNA. *Genome Research*. 2012;**22**(6):1120–1127

[27] Kumar A, Deepika, Sharda S, Avasthi A. Recent advances in the treatment of parasitic diseases: Current status and future. *Natural Product Based Drug Discovery against Human Parasites: Opportunities and Challenges*. 2023;**28**:249–286

[28] Gaona-López C, Vazquez-Jimenez LK, Gonzalez-Gonzalez A, Delgado-Maldonado T, Ortiz-Pérez E, Nogueda-Torres B, Moreno-Rodríguez A, Vázquez K, Saavedra E, Rivera G. Advances in protozoan epigenetic targets and their inhibitors for the development of new potential drugs. *Pharmaceuticals (Basel, Switzerland)*. 2023;**16**(4):543

[29] Levenson JM, Sweatt JD. Epigenetic mechanisms in memory formation. *Nature Reviews, Neuroscience*. 2005;**6**(2):108–118

[30] Kaito C, Murakami K, Imai L, Furuta K. Animal infection models using non-mammals. *Microbiology & Immunology*. 2020;**64**(9):585–592

- [31] Reece SE, Ramiro RS, Nussey DH. SYNTHESIS: Plastic parasites: Sophisticated strategies for survival and reproduction? *Evolutionary Applications*. 2009;**2**(1):11–23
- [32] Poulin R, Thomas F. Epigenetic effects of infection on the phenotype of host offspring: Parasites reaching across host generations. *Oikos*. 2008;**117**(3):331–335
- [33] Rando OJ, Verstrepen KJ. Timescales of genetic and epigenetic inheritance. *Cell*. 2007;**128**(4):655–668
- [34] Bougdour A, Maubon D, Baldacci P, Ortet P, Bastien O, Bouillon A, Barale JC, Pelloux H, Ménard R, Hakimi MA. Drug inhibition of HDAC3 and epigenetic control of differentiation in Apicomplexa parasites. *Journal of Experimental Medicine*. 2009;**206**(4):953–966
- [35] Croken MM, Nardelli SC, Kim K. Chromatin modifications, epigenetics, and how protozoan parasites regulate their lives. *Trends in Parasitology*. 2012;**28**(5):202–213
- [36] Chookajorn T, Dzikowski R, Frank M, Li F, Jiواني AZ, Hartl DL, Deitsch KW. Epigenetic memory at malaria virulence genes. *Proceedings of the National Academy of Sciences*. 2007;**104**(3):899–902
- [37] Volz JC, Bártfai R, Petter M, Langer C, Josling GA, Tsuboi T, Schwach F, Baum J, Rayner JC, Stunnenberg HG, Duffy MF. PfSET10, a *Plasmodium falciparum* methyltransferase, maintains the active var gene in a poised state during parasite division. *Cell Host & Microbe*. 2012;**11**(1):7–18
- [38] Huguenin M, Bracha R, Chookajorn T, Mirelman D. Epigenetic transcriptional gene silencing in *Entamoeba histolytica*: Insight into histone and chromatin modifications. *Parasitology*. 2010;**137**(4):619–627
- [39] Hamon MA, Cossart P. Histone modifications and chromatin remodeling during bacterial infections. *Cell Host & Microbe*. 2008;**4**(2):100–109
- [40] Bhavsar AP, Guttman JA, Finlay BB. Manipulation of host-cell pathways by bacterial pathogens. *Nature*. 2007;**449**(7164)
- [41] Ribet D, Cossart P. Post-translational modifications in host cells during bacterial infection. *FEBS Letters*. 2010;**584**(13):2748–2758
- [42] Pennini ME, Pai RK, Schultz DC, Boom WH, Harding CV. Mycobacterium tuberculosis 19-kDa Lipoprotein Inhibits IFN- γ -Induced Chromatin Remodeling of MHC2TA by TLR2 and MAPK Signaling. *The Journal of Immunology*. 2006;**176**(7):4323–4330
- [43] Conti F, Abnave P, Ghigo E. Unconventional animal models: A booster for new advances in host–pathogen interactions. *Frontiers in Cellular & Infection Microbiology*. 2014;**4**:142.
- [44] Davis CD, Uthus EO. Dietary selenite and azadeoxycytidine treatments affect dimethylhydrazine-induced aberrant crypt formation in rat colon and DNA methylation in HT-29 cells. *The Journal of Nutrition*. 2002;**132**(2):292–297
- [45] Flynn JL, Chan J. Immunology of tuberculosis. *Annual Review of Immunology*. 2001;**19**(1):93–129

- [46] Alvarado AS. Stem cells and the Planarian *Schmidtea mediterranea*. *Comptes Rendus Biologies*. 2007;**330** (6-7)
- [47] Zhou L, Wu S, Liu D, Xu B, Zhang X, Zhao B. Characterization and expression analysis of a trypsin-like serine protease from planarian *Dugesia japonica*. *Molecular Biology Reports*. 2012;**39**:7041–7047
- [48] Abnave P, Mottola G, Gimenez G, Boucherit N, Trouplin V, Torre C, Conti F, Amara AB, Lepolard C, Djian B, Hamaoui D. Screening in planarians identifies MORN2 as a key component in LC3-associated phagocytosis and resistance to bacterial infection. *Cell Host & Microbe*. 2014;**16**(3):338–350
- [49] Collins III JJ, Newmark PA. It's no fluke: The planarian as a model for understanding schistosomes.. *PLoS Pathogens*. 2013;**9**(7)
- [50] Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L, Finnerty CC. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proceedings of the National Academy of Sciences*. 2013;**110**(9):3507–3512
- [51] Özdemir BC, Pentcheva-Hoang T, Carstens JL, Zheng X, Wu CC, Simpson TR, Laklai H, Sugimoto H, Kahlert C, Novitskiy SV, De Jesus-Acosta A. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. *Cancer Cell*. 2014;**25**(6):719–734
- [52] Surace AE, Hedrich CM. The role of epigenetics in autoimmune/ inflammatory disease. *Frontiers in Immunology*. 2019;**10**:1525
- [53] Abraham MJ, El Sherbini A, El-Diasty M, Askari S, Szwczuk MR. Restoring epigenetic reprogramming with diet and exercise to improve health-related metabolic diseases. *Biomolecules*. 2023;**13**(2):318
- [54] Laberge S, Akoum D, Włodarczyk P, Massé JD, Fournier D, Semlali A. The potential role of epigenetic modifications on different facets in the periodontal pathogenesis. *Genes*. 2023;**14**(6):1202
- [55] Hull EE, Montgomery MR, Leyva KJ. HDAC inhibitors as epigenetic regulators of the immune system: Impacts on cancer therapy and inflammatory diseases. *BioMed Research International*. 2016;**2016** (1):8797206
- [56] Farhang N, Brunger JM, Stover JD, Thakore PI, Lawrence B, Guilak F, Gersbach CA, Setton LA, Bowles RD. CRISPR-based epigenome editing of cytokine receptors for the promotion of cell survival and tissue deposition in inflammatory environments. *Tissue Engineering Part A*. 2017;**23**(15-16)
- [57] Lim DH, Maher ER. DNA methylation: A form of epigenetic control of gene expression. *The Obstetrician & Gynaecologist*. 2010;**12** (1):37–42
- [58] Fol M, Włodarczyk M, Druszczyńska M. Host epigenetics in intracellular pathogen infections. *International Journal of Molecular Sciences*. 2020;**21**(13):4573
- [59] Despins CA, Brown SD, Robinson AV, Mungall AJ, Allen-Vercoe E, Holt RA. Modulation of the host cell transcriptome and epigenome by *Fusobacterium nucleatum*. *MBio*. 2021;**12**(5):10–128

[60] Minea H, Singeap AM, Minea M, Juncu S, Muzica C, Sfarti CV, Girleanu I, Chiriac S, Miftode ID, Stanciu C, Trifan A. The Contribution of Genetic and Epigenetic Factors: An Emerging Concept in the Assessment and Prognosis of Inflammatory Bowel Diseases. *International Journal of Molecular Sciences*. 2024;**25**(15):8420

[61] Abdulraheem MI, Xiong Y, Moshood AY, Cadenas-Pliego G, Zhang H, Hu J. Mechanisms of plant epigenetic regulation in response to plant stress: Recent discoveries and implications. *Plants*. 2024;**13**(2):163

[62] Perera DJ, Koger-Pease C, Paulini K, Daoudi M, Ndao M. Beyond schistosomiasis: Unraveling co-infections and altered immunity. *Clinical Microbiology Reviews*. 2024;**37**(1):e00098–23

[63] Okoye AA, Picker LJ. CD4⁺ T-cell depletion in HIV infection: Mechanisms of immunological failure. *Immunological Reviews*. 2013;**254**(1):54–64

Section 2

Epigenetics and Carcinogenesis

Perspective Chapter: BPA-Mediated Epigenetic Disruption in Endometrial Cancer Progression

Danushri Srinivasan, Kishore Thirunavukarasu, Selvakumar Subramaniam and Thirunavukkarasu Velusamy

Abstract

Endocrine disruptors are chemicals that interfere with the hormone system of humans and animals. They mimic, block, and alter hormonal levels, leading to a wide range of health effects, even at extremely low doses. Bisphenol A (BPA) is a ubiquitous synthetic organic compound used primarily in the manufacturing of plastics and resins. It leaches into the body mostly through the diet, followed by exposure to air, water, and dust. Traces of BPA have even been detected in breast milk. Acting as a xenoestrogen, BPA interferes with hormone signalling and modulates the expression of critical tumour suppressor genes and oncogenes. This dysregulation is largely mediated through epigenetic alterations, ranging from aberrant DNA methylation, histone modification, and dysregulation of non-coding RNAs such as micro-RNAs (miRNAs) and long non-coding RNAs (lncRNAs). This chapter explores the molecular mechanisms by which BPA disrupts the endocrine–epigenetic interface, with a focus on its role in reshaping the chromatin microenvironment and modulating the RNA network that governs gene expression in endometrial cancer (EC) pathogenesis. It highlights the BPA-induced dysregulation of miRNAs and lncRNAs contributing to cancer initiation, progression, and immune evasion. Importantly, the chapter explores the emerging concept of transgenerational epigenetic inheritance, the role of BPA in tumour microenvironment modulation, and implications for diagnostic biomarkers and therapy. By integrating findings from toxicogenomic and multiomics approaches, as well as *in vitro* and *in vivo* modelling, this chapter aims to present a comprehensive landscape of how BPA contributes to EC risk and progression at the molecular and epigenetic levels.

Keywords: endocrine disruptors, Bisphenol A, endocrine–epigenetic interface, endometrial cancer, non-coding RNA

1. Introduction

Endometrial cancer (EC) is a recurrent gynecological malignancy among women, leading to about 2% of deaths in women globally [1]. It is one of the few cancers whose incidence has drastically increased mortality over the past decade [2]. The disproportionate increase in incidences of endometrial neoplasm can be attributed to susceptibility to estrogen and chemicals that mimic endogenous hormones [3]. Underlying factors such as biology, race, ethnicity, and social class deprivation often indirectly contribute to the increasing burden of EC [4].

Humans are actively exposed to chemicals that interfere with the endocrine system and its function. These chemicals mimic, block, and alter hormonal levels, leading to a range of health effects and are referred to as endocrine disruptors (EDs). These EDs, even at very low doses, intensively affect the endocrine–epigenetic interface [3]. Various EDs, such as dioxins, diethylstilbestrol (DES), Bisphenol A (BPA), and phthalates, trigger epigenetic alterations. Among all these EDs, BPA is categorized as one of the most largely synthesized and utilized chemicals worldwide, estimated to be higher than 8 billion pounds processed annually and 100 tons eliminated into the atmosphere [5]. BPA is a ubiquitous, nonsteroid ED belonging to the bisphenol class of compounds. It is a man-made organic compound used in the manufacturing of epoxy resins and plastic containers [6]. It enters the human body through air, water, food, dust, and transdermal routes. Traces of BPA have been found in 90% of urinalysis and even detected in breast milk [7]. It is bio-transformed in the liver by glucurono- and sulfono-conjugations. The metabolized free and conjugate forms of BPA are detected in high amounts in biological fluids like cord blood, plasma, urine, and amniotic fluids [8].

BPA, as a weak estrogen-like ED, often binds to the nuclear endocrine receptor α - and β -estrogen receptors (ERs) in the interior lining of the uterus [9]. This alters the molecular mechanism and expression of genes involved *in utero*-related epigenetic function. Due to this, estrogen-responsive organs are more prone to malignant neoplasms and experience adverse effects on several signalling pathways. The dysregulation is mediated by alterations in the chromatin environment and modulation of non-coding RNA [10]. The remodelling of genetic and epigenetic patterns often induces alterations in the reproductive system, leading to endometriosis, miscarriages, drop-in oogenesis, and follicle development [11].

BPA possesses a complex chemical structure with intrinsic cross-linking properties that make it heat resistant, allowing it to be used extensively worldwide [8]. Epigenetic activities such as DNA methylation, histone modification, and chromatin remodelling are essential for genome stability and integrity, but exposure to EDs fuels genome and chromatin remodelling [12]. These epigenetic re-programming processes are essential to encounter environmental distress and promote the developmental aspects of humans. Since the epigenetic information is transferred from parents to offspring along with genetic information, exposure to EDs leads to epigenetic dysregulation that affects later stages of life by increasing lesions and cancer risks with ageing [13].

The epi-genotoxic effects of health-disturbing compounds like BPA are of heightened concern, as industrialization and changes in lifestyle contribute to acute and chronic exposure, increasing the probability of carcinogenesis in

hormone-sensitive organs [14]. The primary scope of this chapter is to define the role of BPA in disrupting the endocrine–epigenetic interface, with a focus on its role in reshaping the non-coding RNA network that governs gene expression in EC initiation and progression. It also aims to present findings from toxicogenomic, multiomics approaches, and *in vivo/in vitro* modelling to analyse diagnostic markers and epigenetic therapy.

2. Global exposure and penetration of BPA in female population

The global prevalence of BPA exposure among women is alarming and raises significant concern over endocrine disruption and epigenetic effects, particularly in hormone-sensitive tissues like the endometrium. A US study conducted by the National Health and Nutrition Examination Survey (NHANES) shows that over 90% of the female population has detectable BPA levels in urine, with a median concentration of around 2 ng/ml [15]. In South Korea, over 76% of adults had detectable BPA levels [16]. A 2025 survey in Northern Mexico detected BPA in 98.5% of total urine samples from women [17]. Meanwhile, in China (2008–2019), the BPA levels in the urine of females averaged approximately 1.77 ng/ml, with coastal provinces showing BPA levels as high as 3.5 ng/ml [18]. ED biomonitoring from diverse regions indicates that women worldwide carry measurable BPA loads within a 120 ng/ml urine range [19].

The significant route of entry of BPA into the female system is through ingestion of food and drinks packed in BPA-lined cans and polycarbonate containers. Heating plastics by microwaving, dishwashing polycarbonate containers, or prolonged storage increases the leaching of BPA into the system due to material breakdown [20]. Dermal absorption, especially through thermal paper receipts, shows that the receipts may contain 0.8%–3% BPA by weight. Studies also show that, depending upon the friction and contact duration, the transfer of BPA to the skin may range between 1 and 30 µg [21]. Although inhalation is a lesser-known route, it contributes significantly to total exposure. BPA concentration in indoor environments ranges from 0.1 to 29 ng/m³ [22]. Therefore, these exposure routes contribute to the systemic circulation of BPA and bioaccumulation, posing a chronic threat to the reproductive health of women, highlighting the urgent need for regulatory intervention.

3. The endocrine–epigenetic interface

The endocrine axis governs the normal functioning of endocrine glands. It allows the release of hormones from the glands, which circulate in the system in response to incitement. The hormone binds to receptors on the target organ to elicit its function. Endogenous (reactive oxygen species [ROS]) and exogenous (chemical compounds, UV radiation) environmental agents induce plausible molecular effects, which determine the variability of the endocrine system. Environmental factors often contribute significantly to the variation in endocrine activity. This plasticity, in turn, impacts the regulation of hormonal action [23]. **Figure 1** provides a schematic overview of BPA exposure with ERs and the resulting molecular alterations implicated in EC pathogenesis.

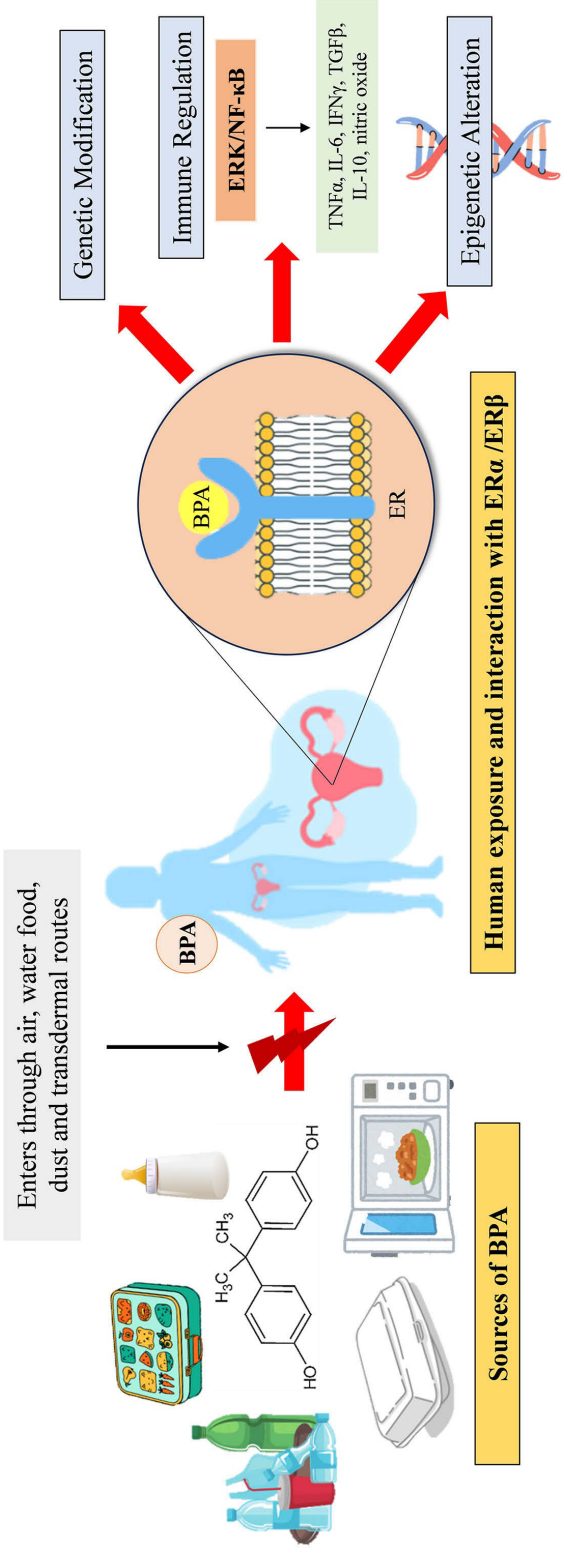


Figure 1. Overview of Bisphenol A (BPA) exposure sources, estrogen receptor interaction, and molecular consequences in endometrial cancer.

3.1 Hormonal signalling in endometrial tissue: role of estrogen and progesterone

The endometrium is a highly hormone-sensitive and dynamic tissue that plays a pivotal role in maintaining reproductive health and the menstrual cycle. It undergoes cyclical changes in response to two major steroid ovarian hormones, namely estrogen and progesterone. Estrogen, during the proliferative phase, initiates and promotes the endometrial epithelial and stromal cells via estrogen receptor α (ER α). In contrast, during the secretory phase, progesterone induces differentiation and inhibits estrogen-mediated proliferation through receptor isoforms progesterone receptor A (PRA) and progesterone receptor B (PRB). This allows for higher endometrial receptivity for embryo implantation [24].

Estrogen plays a central role through 17 β -estradiol during the proliferative phase by stimulating the proliferation of endometrial epithelial and stromal cells. It functions by binding to ERs (ER α and ER β), which act as ligand-activated transcription factors. Upon ligand binding, ERs translocate to the nucleus, where they bind to the estrogen response elements (EREs) in target gene promoters, driving the transcription of genes involved in cellular metabolism and growth [25]. Through membrane-associated ERs, estrogen activates the non-genomic signalling pathways, influencing kinase cascades such as MAPK/Erk and PI3k/Akt, further promoting mitogen activity [26].

Progesterone, in contrast, dominates the secretory phase and counteracts the proliferative effects of estrogen. Binding of progesterone to PRA and PRB promotes stromal decidualization, inhibits epithelial proliferation, and helps in the expression of genes involved in immune regulation and differentiation [27]. The cascades of signalling pathways modulate ER expression and its functioning by regulating the hormonal balance required for endometrial hemostasis and implantation. In cases of estrogen–progesterone disruption, either by endogenous imbalance or exogenous endocrine-disrupting chemicals like BPA, it escalates the imbalance and contributes substantially to uncontrolled endometrial proliferation, inflammation, neoplastic transformation, and carcinogenesis [28].

3.2 BPA as a xenoestrogen: Receptor binding and signal transduction

The structural similarity of BPA with 17 β -estradiol enables binding to both nuclear and membrane-bound ERs. BPA exhibits weak binding affinity to ER β compared to ER α . Still, it exerts potential biological activity even at a nanomolar concentration [29]. Additionally, BPA initiates rapid non-genomic signalling cascades by interacting with the G protein-coupled ER (also known as GPR30). This further alters intracellular calcium levels, activation of mitogen-activated protein kinase (MAPK), and various downstream transcription factors such as cAMP response element-binding protein (CREB) and activation protein-1 (AP-1) [30].

The receptor-based mimicry of BPA disrupts the transcriptional regulation of estrogen-responsive genes involved in cellular function. It interferes with the recruitment of co-regulatory complexes of ERs, shifting the balance from co-repressors to co-activators. These disruptions alter the gene expression patterns, which are responsible for the pathophysiological changes in endometrial tissues, including proliferation and resistance to apoptosis [5].

3.3 Crosstalk between hormone signalling and epigenetic regulation

The interplay between hormone signalling and epigenetic modification is an essential aspect of understanding the influence of EDs, such as BPA, on long-term gene expression. BPA binds to ERs, which upregulate EC-associated genes. It has been shown to initiate changes in biological processes involving miRNAs and lncRNAs. BPA induces permanent gene aberrations regulated diversely by ncRNAs. This, in turn, alters biochemical mechanisms and epigenetic programming [31]. Treatment with a low-dose of BPA leads to reduced methylation of CpG islands during maternal exposure. Mice treated with BPA showed increased cell multiplication, variation in tissue morphology, lower programmed cell death, and an altered rate of development. The duration of exposure, developmental stage, and concentration of BPA are sensitive factors contributing to epigenome alteration [32].

BPA, being sensitive to *in utero* hormone receptors, can have a significant impact on epigenomic and genomic molecular mechanisms. BPA reduces the expression levels of DNA methyltransferases (DNMTs), which are responsible for the addition of methyl groups to DNA. BPA downregulates the levels of DNMT1, DNMT3a, and DNMT3b by enhancing miRNA-29-mediated regulation [33]. The TET2 enzyme, responsible for demethylation processes, is disturbed by BPA, restricting its nuclear localization. Therefore, the balance between the methylation and demethylation patterns, which is essential for gene activation and expression, is eventually disturbed [34]. Furthermore, EDCs such as BPA have a significant effect on other important factors of epigenetic regulation, such as histone modification, and alter the epigenetic activities. The histone acetyltransferases (HATs) and histone deacetylases (HDACs) are downregulated by exposure to BPA; this, in turn, affects the chromatin structure and transcription. The changes in histone patterns and chromatin structure lead to heightened anomalies in later phases of life [35]. Moreover, the effect of BPA on chromatin loop structures and enhancer regions indicates that it affects border regulatory networks beyond promoters, causing an imbalance in the epigenetic profile.

4. BPA-induced epigenetic dysregulation

BPA interrupts the fundamental heritable composition of an organism. It impacts the epigenetic mechanisms, which are controlled by exposure to external factors. The cells carrying the genetic information are turned on or off by epigenetic modulation [36]. The epigenetic regulation impacted by BPA includes DNA methylation, histone modification, chromatin remodelling, and non-coding RNA that affect the regulation of gene transcription. BPA mimics the function of estrogen and interacts with specific ERs. This, in turn, results in impairment of *in utero* development and other reproductive complications. **Figure 2** depicts the epigenetic alterations induced by BPA and its transgenerational effect on EC development.

4.1 DNA methylation

In mammalian cells, exposure to BPA affects DNA methylation profiles, the most significant epigenetic mechanism in cancer biology. The mechanism of DNA methylation involves the addition of a methyl group to a cytosine base followed by

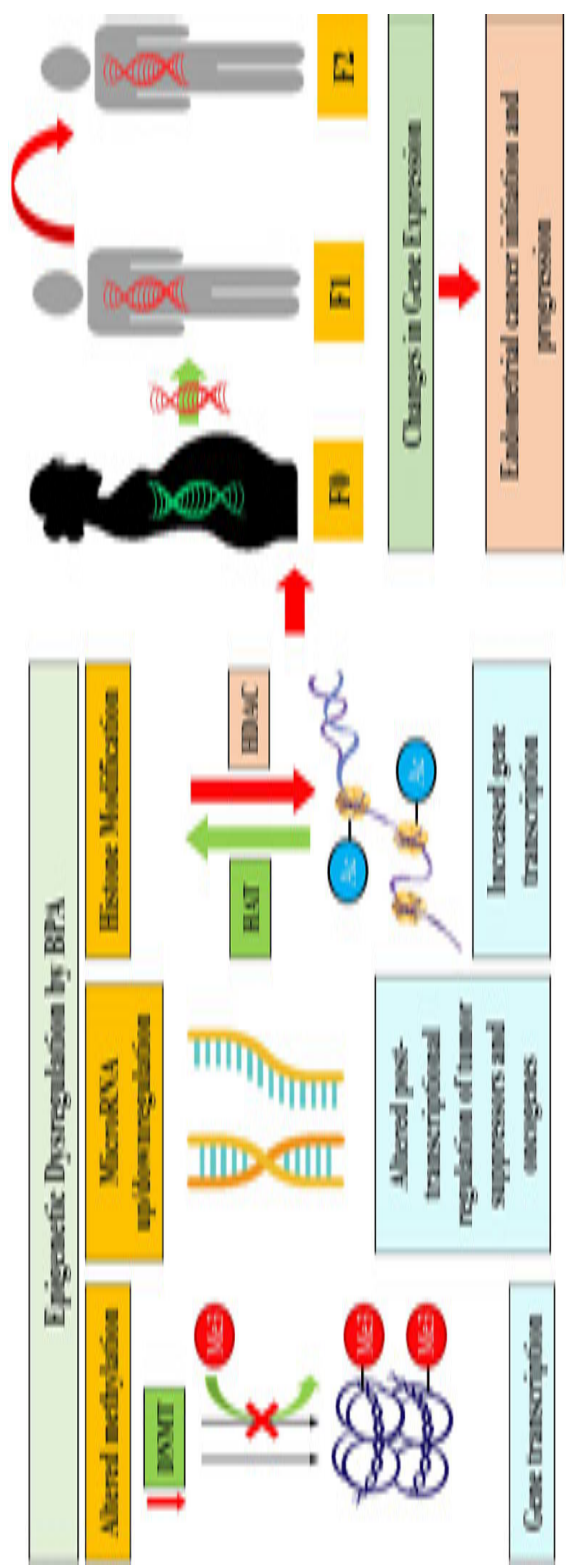


Figure 2.
BPA-induced epigenetic alterations and their heritable impact on endometrial cancer development.

a guanosine base, that is, CpG (cytosine–guanine dinucleotide phosphate) rich regions near the 5' coding site or the promoter [37]. These CpG regions contribute extensively to various levels of gene regulation and expression. DNA replication allows the conservation of new changes in the DNA. In both *in vivo* and *in vitro* models, the process of DNA methylation is regulated by DNMTs. It has been elucidated that BPA alters the DNA methylation pattern of cell signalling genes by decreasing the CpG methylation pattern upstream of the gene [38]. In the case of EC cells, the tumour suppressor genes such as *PTEN* and *p16INK4a* were shown to be hypermethylated, influenced by exposure to BPA [39].

Conversely, the global cause of DNA hypomethylation can be due to BPA exposure. It can lead to genomic instability, reactivation of transposable elements, and upregulation of oncogenes [40]. This ability to cause global hypomethylation and site-specific hypermethylation explains BPA's potent epigenetic disruptor ability to initiate early-stage tumorigenesis. To obtain a comprehensive overview of the effects of BPA on the endometrium, methylation profiling can be done by validating differentially methylated/expressed genes using microarray assays [41]. Specifically, DNA methylation arrays such as Illumina's 27k or 450k arrays can be utilized to identify DNA methylated regions and profile DNA methylation patterns [42].

4.2 Histone modifications

Histones are proteins involved in the winding up of DNA carrying genes into nucleosomes. Nucleosomes are primarily composed of four histone subunits: H2A, H2B, H3, and H4. The octamer of histones winds about 147 base pairs, wrapped around twice, to form a nucleosome core. The chromatin condensation pattern allows efficient packaging of genetic material into the nucleus. Histone modification disrupts the DNA conformation while, on the other hand, it tightens the histone–DNA interactions. During this conformation, DNA becomes sensitive to transcription factors, and this modification leads to alterations in the epigenetic mechanism. The dynamic compaction needs to be highly robust to allow remodelling of the chromatin structure depending on the situation. Thus, genetic stability and integrity are highly threatened due to exogenous molecules with genotoxic effects [43].

All histone modifications, such as methylation and acetylation, give rise to dynamic gene expression by altering chromatin accessibility. BPA is often observed to disturb the expression levels of HATs and HDACs, shifting the equilibrium more towards a condensed, transcriptionally repressive chromatin structure [44]. The increased function of HAT in BPA-exposed EC cells correlates with the hyperacetylation of histone H3 and H4 at tumour suppressor gene promoters. BPA enhances the histone methylation patterns associated with transcriptional activation while significantly reducing the repressive marks at key regulatory regions. This creates a chromatin environment that promotes tumour growth [45].

4.3 Chromatin remodelling

Chromatin structure remodelling is a dynamic process that governs the accessibility of DNA by modifying the position of nucleosomes. It helps in the regulation of various essential functions such as replication, transcription, and repair.

The genetic and epigenetic modifications are often associated with tumorigenesis pathways [46]. Upon exposure to BPA, the dysregulation of chromatin structure is influenced by the activity of ATP-dependent chromatin remodelling complexes. In EC progression models, BPA influenced remodelling through chromatin compaction, which results in the silencing of tumour suppressor genes and aberrant expression of oncogenes [47]. The variance in post-translational modification induced by epigenetic information can alter the overall chromatin structure.

Studies show that exposure to BPA enhances the involvement of BRG1 and BAF155, the key subunits of the ATP-dependent chromatin SWI/SNF complex. It regulates elements involved in proliferation genes such as MYC and CCND1. BPA exposure often facilitates transcriptional repression by reducing or displacing the occupancy of chromatin remodellers at the tumour suppressor loci. The addition of active chromatin marks such as H3K4me1 and H3K27ac can be confirmed by chromatin immunoprecipitation (ChIP) in BPA-treated EC cells. These data support the idea of the formation of a transcriptionally active landscape favouring neoplastic transformation mediated by BPA-induced chromatin remodelling [48].

Various pieces of evidence from human studies show that BPA exposure increases susceptibility in offspring, particularly with respect to hormone-sensitive cancers, which include endometrial hyperplasia and malignancy. Observations from the long-term impacts of BPA raise concerns about the necessity to re-evaluate regulatory guidelines and adopt a multigenerational approach to counteract the effects of BPA [49]. Neonatal exposure to BPA leads to estrogen hypersensitivity and uterine neoplasia in later phases of life, influenced by the epigenetic reprogramming of estrogen-responsive genes [50]. These genes may predispose progeny to aberrant endometrial proliferation and tumorigenesis, leading to multigenerational vulnerability.

4.4 Disruption of non-coding RNA networks

The non-coding RNA, especially miRNA and lncRNA, plays a vital role in the post-transcriptional regulation of chromatin modulation and gene expression. It has been observed that exposure to BPA disturbs the expression levels of key ncRNAs. The tumour suppressor miRNAs, miR-200c and miR-34a, are downregulated by BPA. These miRNAs are involved in cell cycle arrest, apoptosis, and epithelial–mesenchymal transition (EMT). Concurrently, the upregulation of miR-21 and miR-155 is responsible for targeting genes like *SOCS1* and *PTEN*, which in turn leads to the evasion of the immune system and enhanced proliferation [51].

BPA exposure similarly affects the expression of long non-coding RNA. It increases the expression of MALAT1 and HOTAIR, which are responsible for chromatin remodelling, invasion, and expansion in EC. The recruitment of PRC2 complex is mediated by HOTAIR, leading to the trimethylation of H3K27 at tumour suppressor loci. The oncogenic transcriptional program is sustained by interaction with chromatin-modifying complexes, and MALAT1-modulated splicing. The chromatin structure is significantly altered, along with generating a supporting landscape for the tumorigenic transcriptome [52].

5. Molecular pathways in BPA-driven EC progression

BPA has been reported to modulate genetic, epigenetic, and endocrine functions. It influences mitochondrial dysfunction and the cell's oncogenic signalling pathways.

The carcinogenesis mediated by BPA affects cell survival, proliferation, invasion, resistance, and apoptosis through various signalling pathways. Growing evidence reports that exposure to BPA triggers intrinsic signalling cascades, including Wnt, MAPK, NF- κ B, PI3K/AKT, apoptosis, and calcium homeostasis [53]. BPA, as a xenobiotic, can induce the production of ROS in endometrial cells and could play a vital role in escalating carcinogenic effects. **Figure 3** illustrates various signalling pathways induced by ROS, which, in turn, lead to the progression of EC.

5.1 ER-mediated signalling (genomic and epigenomic)

BPA exhibits estrogen-mimicking activity by binding completely to nuclear hormone receptors ER α and ER β , and functions as transcription factors. It disrupts the delicate balance between the hypothalamic–pituitary–gonadal (HPG) axis and steroid hormone levels. The local systemic endocrine homeostasis is highly essential to maintain the normal physiology of the uterus. BPA exposure leads to a state of estrogen dominance, where circulating estrogen activity increases without a proportional increase in progesterone, an essential hormone to counteract estrogen-mediated endometrial proliferation. Additionally, the suppression of the progesterone receptor (PR) exacerbates proliferative signals and impairs differentiation. Studies have shown that BPA exposure not only upregulates the biosynthesis of estrogen but also alters the feedback mechanism with the HPG axis. This hormonal shift towards low progesterone and high estrogen activity mimics the endocrine profile in women with unopposed estrogen exposure, a key etiological factor in type I EC [54].

The ERs undergo conformational changes upon BPA binding, which in turn leads to the dimerization and subsequent translocation of receptors into the nucleus [55]. Inside the nucleus, the BPA–ER complex gets attached to the promoter region of target genes of EREs and activates ligand-dependent transcription. As a result, this leads to over-expression of proliferation-associated genes, such as *cyclin D1* and *c-Myc*, both of which play a vital role in the G1–S transition of the cell cycle [5]. Simultaneously, BPA-induced ER signalling suppresses apoptotic regulators, including BAX, p21, and caspase, further promoting the balance in favour of survival and cell progression [56]. Such suppression compromises the apoptotic check, which eliminates malignant and damaged cell function. This further facilitates the accumulation of epigenetic and genetic aberrations, contributing to the early stages of tumorigenesis in hormone-sensitive tissues such as the endometrium.

BPA-driven estrogenic receptor signalling has profound implications from an epigenetic perspective. The activated ER complex acts as a scaffold for recruiting HAT, HMTs, and DNMTs to ERE-containing promoter regions. These enzymes, which act as the writers of epigenetic information, lead to chromatin remodelling, allowing an open and transcriptionally active chromatin state. Notably, histone modifications such as increased H3K9 acetylation and H3K4 trimethylation have been observed at the promoters of key encoding genes, including *EZH2* and *c-Myc*, following BPA exposure. The BPA-induced recruitment of DNMTs leads to hypermethylation of tumour suppressor genes such as *PTEN* and *CDKN2A*, effectively silencing their expression and allowing unopposed cell cycle progression [57]. These genomic and epigenomic changes exhibit inherent patterns that are not merely transient. The repetitive or chronic exposure of hormone-responsive tissues, such as the endometrium, to BPA induces an oncogenic phenotype through sustained estrogenic stimulation, epigenetic reprogramming, and apoptotic evasion.

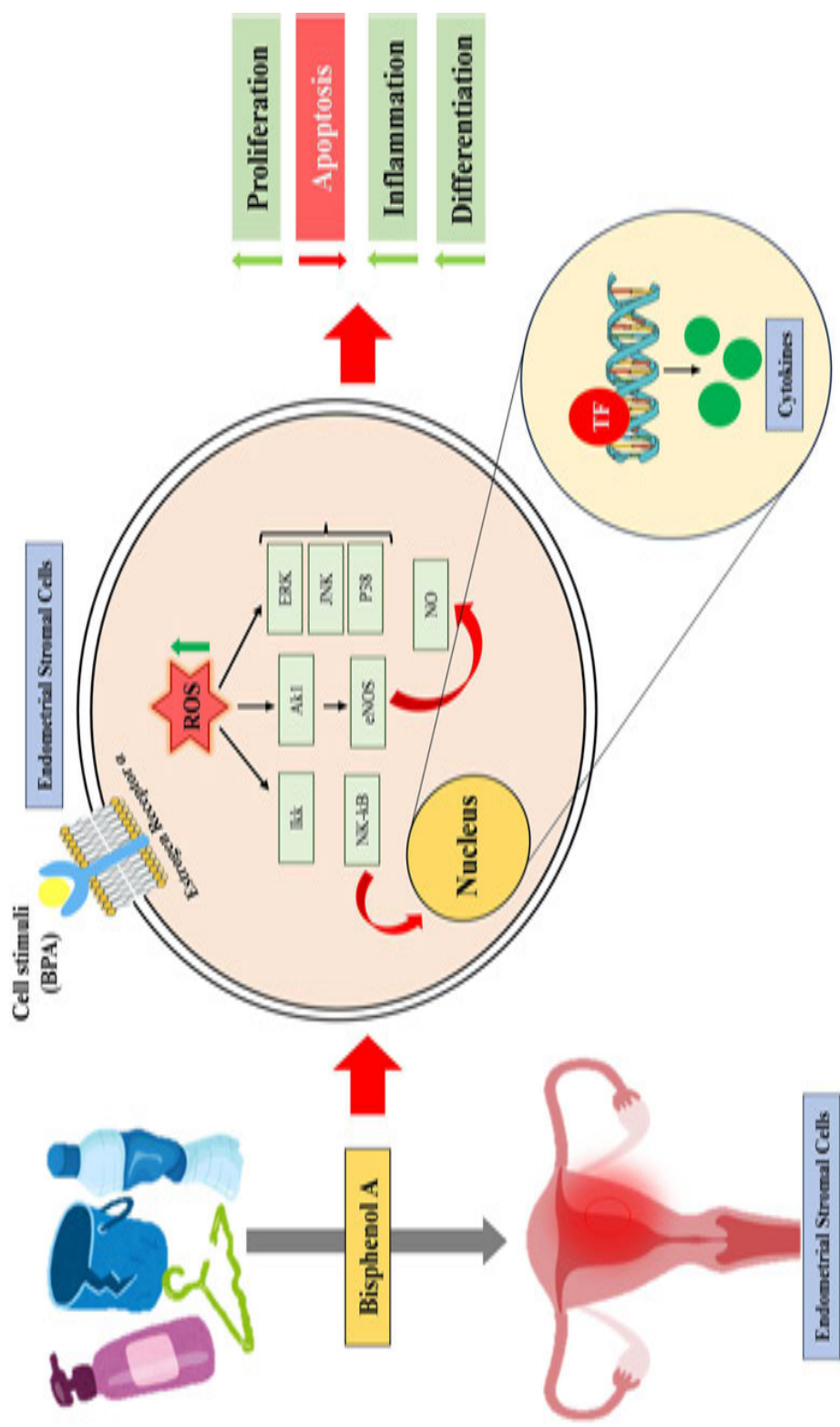


Figure 3.
Mechanistic illustration of BPA-mediated ROS-induced signaling alteration in endometrial stromal cells.

Risk of EC progression increases gradually with cumulative exposure to BPA. BPA is active at a very low dose (10 nM to 1 μ M), which significantly contributes to the migration of endometrial stromal cells. No exact BPA causative threshold guarantees cancer progression. *In vivo* human data are limited, and inter-individual variability, such as metabolism, diversity in traits, and hormonal status, affects the outcome [58].

5.2 ROS-mediated signalling and oxidative stress

The amount of oxidative stress induced by BPA in estrogen-responsive tissues, including the endometrium, results in the production of excessive ROS. Chronic BPA exposure triggers mitochondrial dysfunction, which interferes with the electron transport chain. This disruption causes electron leakage and the production of superoxide radicals. Moreover, BPA activates the NADPH oxidase enzymes on the plasma membrane, transferring electrons from NADH to molecular oxygen. This process generates superoxide and other ROS byproducts [59]. When ROS production exceeds the buffering capacity of endogenous antioxidants like glutathione, superoxide dismutase, and catalase, it leads to chronic oxidative stress within the endometrial stromal cells.

The accumulated ROS acts as a potential secondary messenger that activates redox-sensitive signalling pathways, notably NF- κ B, p38 MAPK, and c-Jun N-terminal kinase (JNK). These signalling cascades regulate a variety of genes related to inflammation, angiogenesis, cell proliferation, and cell survival. For example, the activation of NF- κ B by ROS increases the levels of pro-inflammatory cytokines such as interleukin-6 (IL-6), TNF- α , and COX-2, contributing to a chronic inflammatory microenvironment favouring tumour initiation and progression. Likewise, the phosphorylation of p38 MAPK and JNK often leads to enhanced expression of matrix metalloproteases (MMPs) and VEGF, which supports extracellular matrix degradation, angiogenesis, and invasive behavior of transformed endometrial cells [60].

Additionally, ROS directly influences the activity of endothelial nitric oxide synthase (eNOS). Under oxidative stress, eNOS becomes uncoupled, generating superoxide instead of nitric oxide (NO). This change reduces the availability of NO, an essential molecule for maintaining vascular homeostasis. It not only decreases NO levels but also promotes the formation of peroxynitrite (ONOO⁻), a highly reactive nitrogen species, which exacerbates oxidative damage to lipids, proteins, and DNA. The deterioration of vascular signalling and nitrosative stress disrupts the endometrial environment, impairing normal angiogenic signalling and promoting hypoxia, which is a known inducer of the tumour-promoting transcription factor HIF-1 α [61].

Importantly, ROS also directly induces DNA damage, including 8-oxo-guanine formation, strand breaks, and base modification, which, if not repaired, lead to mutations in oncogenes and tumour suppressors such as *TP53* and *PTEN*. BPA-mediated ROS can function as genotoxic agents, transforming normal endometrial cells into a neoplastic phenotype. Moreover, this oxidative stress affects epigenetic modifiers such as TET enzymes and histone demethylases, linking the redox imbalance to the epigenetic dysregulation of gene expression [62]. Altogether, ROS-mediated BPA leads to a pro-tumorigenic signalling network that encompasses inflammation, genomic instability, impaired vascular regulation, and transcriptional reprogramming, thereby playing a vital role in EC progression.

5.3 Inflammatory signalling via NF- κ B

The nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) is a central transcription factor mediating cancer progression through inflammation. In endometrial cells, BPA promotes the activation of the NF- κ B signalling pathway through an ER-mediated signalling cascade and oxidative stress. It increases ROS production, which acts as an upstream activator of I κ B kinase (IKK). IKK phosphorylates I κ B α , the inhibitory subunit bound to NF- κ B in the cytoplasm, leading to ubiquitination and subsequent degradation, resulting in the release of the NF- κ B p65/p50 heterodimer. This allows the translocation of the NF- κ B p65/p50 heterodimer into the nucleus, where it binds to specific DNA motifs and activates the transcription of pro-inflammatory and pro-survival genes [63].

The activation of the NF- κ B pathway promotes the release of various cytokines and inflammatory mediators such as IL-6, IL-8, TNF- α , COX-2, and iNOS. These together establish a chronic inflammatory microenvironment, contributing to a tumour-progressive niche. These cytokines, which are signalling molecules of the immune system, increase oxidative stress and promote angiogenesis. IL-6 and IL-8 remain key downstream targets of the NF- κ B pathway. Inflammation mediated by the persistent activation of the NF- κ B pathway not only serves as an initiator but also as a sustainer of EC. BPA-induced NF- κ B enhances the transcription of IL-6, which activates the Jak/STAT3 pathway. The STAT3 signalling upregulates the expression of cyclin D1, Bcl-XL, and VEGF, enhancing cell proliferation, migration, invasion, survival, and angiogenesis, thereby allowing the survival of mutated cells. The understanding of BPA-induced inflammatory activation through the NF- κ B/IL-6 axis remains limited but is considered a critically underexplored pathway contributing to EC progression [64].

5.4 Pi3k/AKT/mtor pathway activation

The rapid non-genomic signalling via the membrane-bound ERs is capable of activating G-protein-coupled receptors (GPCRs) and membrane-associated isoforms of ER α . These receptors initiate signalling cascades such as PI3K/AKT and MAPK/ERK, which lead to various downstream activations of mitogenic pathways and anti-apoptotic pathways. The activation of the PI3K/AKT pathway by BPA not only enhances glucose metabolism and cellular proliferation but also confers resistance to apoptosis by modulating the phosphorylation status of BAD, FOXO, and activating mTOR, a central regulator of cell growth and autophagy suppression [65].

In BPA-exposed cells, the G protein-coupled ER initiates the activation of phosphoinositide 3-kinase (PI3K) in the cell membrane. PI3K catalyses the conversion of PIP2 to PIP3, which, in turn, activates AKT by phosphorylation. The AKT pathway, upon activation, phosphorylates a set of downstream effectors, leading to the inactivation of BAD (a pro-apoptotic BCL-2 family member) and FOXO (a transcription factor). The phosphorylation of FOXO suppresses apoptotic and cell-cycle arrest genes and further activates mTORC1, an effective regulator of protein synthesis and inhibitor of autophagy. More importantly, anabolic processes such as ribosome biosynthesis and protein translation are promoted via phosphorylation [66].

In endometrial carcinogenesis, although direct evidence of BPA-induced PI3K/AKT activation is limited, *in vitro* studies demonstrate uncontrolled proliferation of cells upon BPA exposure. The enhanced proliferation is linked to the downregulation of *PTEN*, a negative regulator of PI3K signalling pathway. The sustained oncogenic signalling is amplified by the PI3K/AKT/mTOR axis with the loss of *PTEN*. Chronic exposure to BPA upregulates p-AKT and p-mTOR, increases glucose uptake, and induces resistance to apoptosis.

5.5 Epigenetic and post-transcriptional dysregulation

BPA is known to induce epigenetic modifications that parallel the onset and development of endometrial carcinogenesis upon exposure. BPA interferes with the process of controlling gene expression through alterations in the DNA sequence. BPA hyperactivates changes in DNA methylation, histone modification, and non-coding RNA profiles. One of the most critical alterations observed is significant changes in the DNA methylation pattern. BPA reduces the activity of DNMTs, specifically DNMT1 and DNMT3B, resulting in global hypomethylation of genomic DNA. This hypomethylation is associated with the activation of oncogenes such as *c-Myc* and *IGF2*, while at the same time, BPA causes hypermethylation of tumour suppressor genes like *PTEN* and *CDKN2A* [44].

BPA exposure increases the expression of HATs, such as P300/CBP, and reduces the activity of HDACs. This leads to an increase in acetylation at lysine residues on histones H3 and H4. Such changes relax the chromatin structure and promote the transcription of genes involved in cell proliferation, angiogenesis, and resistance to apoptosis, which favours a tumour-permissive state [67].

Beyond histone and DNA modification, BPA also affects non-coding RNAs at post-transcriptional levels. Several microRNAs important for maintaining cellular homeostasis and differentiation are disrupted by BPA exposure. It reduces the expression of members of the miR-200 family and miR-20a, which are vital for maintaining epithelial traits and regulating genes involved in cell cycle control and apoptosis. Their suppression leads to the upregulation of transcription factors such as ZEB1 and ZEB2, which inhibit E-cadherin and trigger EMT transition, a key event in EC invasion and metastasis. Additionally, BPA alters lncRNAs [68]. It upregulates oncogenic lncRNAs like HOTAIR and downregulates tumour-suppressive lncRNAs like MEG3, further promoting proliferation and invasive behavior in endometrial cells.

Overall, this multi-layered epigenetics and post-transcriptional modification by BPA shifts the transcriptional landscape of endometrial cells towards a pro-tumorigenic landscape. This disruption of tightly regulated gene expression pathways governing cell proliferation, apoptosis, and differentiation underscores BPA's role in the epigenetic re-programming of the endometrium and initiation of cancer, highlighting its potential as an environmental risk factor and a potent carcinogen.

6. Emerging concepts and broader implications of BPA

BPA's role in transgenerational epigenetic inheritance, modulation of the tumour microenvironment (TME), and epitranscriptomic modification – all of this suggests that BPA may exert persistent and multilayered effects contributing to cancer

initiation and progression. This dimension explains BPA biology and its broader implications in oncogenesis and regulatory toxicology.

6.1 Transgenerational epigenetic inheritance

The epigenetic modification inherited across generations tends to persist even in the absence of continued exposure. This is referred to as transgenerational epigenetic inheritance. BPA causes epigenetic mutations in the germ cells. It evades the epigenetic reprogramming during embryogenesis and gametogenesis, which leads to the transfer of altered phenotypes to the offspring [50].

The prenatal exposure to BPA in rodent models has helped in understanding the persistent changes in the DNA methylation pattern, histone modification, and non-coding RNA expression from one generation to another. It has been demonstrated that continued exposure to BPA during gestation can lead to altered gene expression patterns in F3 rats. This suggests an epigenetic mode of inheritance with altered methylation in imprinted genes and endocrine signal receptors [69].

6.2 Modulation of the TME and immune evasion

The genotoxicity effect of BPA plays a vital role in modulating the TME, which promotes tumour progression and immune evasion that are pivotal hallmarks of cancer progression. It further disrupts the immune surveillance mechanism by modifying cytokine signalling, macrophage polarization and the activity of T-cells in the endometrium. Additionally, it shifts the balance towards a pro-inflammatory and immunosuppressive TME [70].

Experimental models show that BPA exposure skews macrophages towards an M2-like phenotype, which is characterized by pro-tumoral and anti-inflammatory effects. The polarization is supported by angiogenesis, extracellular matrix remodelling and suppression of cytotoxic T lymphocyte response. The immune checkpoint molecules, such as PD-L1 in ER-positive cancer cells, are upregulated via ER-mediated transcriptional activation, allowing the tumour cells to evade T-cell-mediated killing [71]. It is reported that BPA increases IL-6 and VEGF secretion in endometrial stromal cells. Additionally, BPA impairs dendritic cell expression and major histocompatibility complex (MHC) class I expression, which are essential for effective antigen presentation. These pieces of evidence suggest that BPA acts as a tumour initiator and also orchestrates an immunosuppressive environment. Since evasion of immune response is a major escape point for cancer cells to initiate tumour formation, this context warrants deeper investigation into the endocrine disruption-immune axis in cancer biology [72].

6.3 Epigenetic modifications

The RNA biology advancements have unveiled the importance of epitranscriptomic modifications, such as N6-methyladenosine (m6A), in the regulation of post-transcriptional gene expression. Emerging patterns suggest that BPA alters the m6A methylation pattern, leading to dysregulated RNA stability, translation, and splicing. These mechanisms are closely associated with cancer development and progression [73].

BPA exposure modulates the expression of m6A writers (METTL3, METTL14), erasers (FTO, ALKBH5), and readers (YTHDF1/2), resulting in aberrant m6A

landscapes in mammary epithelial cells. The cellular transformation is fuelled by these modifications, which affects tumour suppressor genes and oncogenes. Another study shows that BPA-induced m6A changes alter the function of non-coding RNAs, which, in turn, lead to altered miRNA processing and circular RNA (circRNA) expression, indicating the impact of BPA on non-coding RNA transcriptomics [74].

Additionally, BPA is involved in mediating changes in the patterns of m6A expression. It affects the stability and processing of non-coding RNAs, including lncRNA and circRNA, which play a critical role in the regulatory network, therefore driving EC progression. These observations indicate that this mechanistic axis is an expanding field of research and further highlight an underexplored layer of BPA-mediated epigenetic control, which could have therapeutic implications [75]. Having established these molecular mechanisms of BPA-driven endometrial carcinogenesis, it is essential to explore how these mechanistic insights translate into clinical practice, especially in the context of diagnosis and targeted therapy.

7. Diagnostic and therapeutic opportunities in EC

The molecular heterogeneity and epigenetic complexity mostly observed in gynaecological malignancies, such as EC, are reported in developed countries, especially in the context of endocrine-disrupting exposure, such as BPA. Although early-stage EC is highly treatable, the advanced and recurrent forms often exhibit therapeutic resistance. Due to the associated complexity and heterogeneity of these cancers, there is a growing emphasis on molecular diagnosis and personalized treatment strategies to improve patient outcomes.

7.1 Diagnostic advances

BPA mimics itself as a hyper-estrogenic trigger, which influences abnormal cell proliferation in the endometrial lining. Given the hormone-responsive nature of endometrial tissue, early detection of EC in at-risk individuals, including those with chronic ED exposure, has become a critical clinical priority. Transvaginal ultrasonography (TVUS), a non-invasive assessment method, helps to evaluate endometrial thickness and is particularly useful in postmenopausal women with abnormal uterine bleeding. However, TVUS lacks specificity for malignant pathology and cannot distinguish between early carcinoma and hyperplasia [76]. Hence, the diagnosis of EC using an endometrial biopsy sample considered as the gold standard method. Nevertheless, the procedural discomfort of the sampling technique and its invasiveness remain as significant limitations [77].

To overcome these limitations, liquid biopsy-based diagnostics have gained traction. Non-invasive fluids such as urine, uterine fluid, blood, or cervico-vaginal fluid are major reservoirs of molecular biomarkers reflecting tumour-derived alterations. The screening of diagnostic biomarkers seems promising in the early diagnosis of EC. In blood, the genomic biomarkers, which include circulating miRNA with specificity and sensitivity of 87% and 84%, offer high diagnostic accuracy. The human epididymis protein 4 (HE4), a proteomic biomarker in serum, tends to predict malignancy with 100% specificity and 86.7% sensitivity. It is often used in conjunction with CA125 to improve predictive value [78]. Further meta-analyses and multiple studies have been performed to review its accuracy and performance. The

metabolomic study is becoming a useful tool in the screening of EC, as several hundred metabolites can be screened in a minimal volume of tissue or fluid sample. This approach is cost-effective, non-invasive, patient-friendly, inexpensive, and increasingly adopted for screening purposes. The urine-based DNA methylation assay using a three-marker combination (e.g., GHSR, ZSCAN12, SLC6A20) displays an AUC value of 0.95, reflecting high sensitivity and specificity in EC detection [79].

Furthermore, the MRI-based diagnosis is considered the clinical gold standard method for the visualization of deep myometrial invasion and advanced-stage endometriosis (FIGO stage 3 and 4), offering superior tissue contrast and staging capability compared to ultrasound [80]. With an in-depth understanding of proteomics, metabolomics, and genomic sequencing, diagnostic algorithms are shifting more towards multi-marker liquid biopsy panels that are less invasive but also represent the heterogeneity and dynamic progression of the tumour. These innovations hold relevance in the context of BPA-driven epigenetic and metabolic dysregulation.

Considering the success of the above diagnostic approaches in precisely detecting ECs both in early and advanced states, a tailor-made diagnostic panel incorporating the molecular targets influenced by BPA would be handy to use as a screening tool among the chronically BPA-exposed population. This would eventually help us achieve the mandate of predicting and diagnosing the disease at an early stage.

7.2 Therapeutic opportunities

The surgical intervention remains a cornerstone for early-stage EC as the main therapeutic option, followed by radiotherapy or platinum-based chemotherapy. However, molecular subtyping and identification of actionable mutations have transformed therapeutic approaches. Hormone therapy remains effective for low-grade, hormone receptor-positive ECs, including progestin or selective ER modulators [81]. Targeted therapies have shown promise in advanced or recurrent ECs. Inhibitors of PI3K/AKT/mTOR, immune checkpoint inhibitors (e.g., pembrolizumab for tumours high in microsatellite instability), and multi-kinase inhibitors are under clinical evaluation. DNMT inhibitors (e.g., decitabine), which are included in epigenetic therapy, and HDAC inhibitors (e.g., vorinostat) aim to reverse the aberrant gene silencing associated with BPA exposure and other epimutations. Non-coding RNA therapeutics targeting oncogenic miRNAs or restoring tumour-suppressive miRNAs are emerging as next-generation therapies and are currently being tested in preclinical models [82]. Therefore, by integrating molecular diagnostics with targeted epigenetic and immunotherapeutic interventions, a promising therapeutic modality could be developed as advanced precision medicine to treat ECs.

8. Experimental models and approaches to study BPA-driven EC and therapeutic screening

Experimental validation of the effects of BPA in endometrial carcinogenesis requires strong *in vivo*, *in vitro*, and multi-omics platforms. These models help analyse the molecular mechanism underlying BPA's estrogenic and epigenetic action in endometrial tissue and offer therapeutic screening of epigenetic drugs, pathway inhibitors, and hormone antagonists.

8.1 *In vitro* studies cell-based models

The *in vitro* system offers high-resolution insights into the molecular and cellular mechanisms of BPA-driven endometrial oncogenesis. The exposure of BPA to RL95-2 cells, a well-established endometrial adenocarcinoma line, significantly promoted the rate of migration ability and invasion efficiency. The ability for colony formation increased with increasing concentrations of BPA. It upregulated the expression of COX-2, a known inflammatory and tumorigenic mediator. RT-PCR analysis validation of RL95-2 cells exposed to BPA for two weeks, compared with controls, confirmed elevated COX-2 expression driven through the MAPK and EMT pathways, highlighting BPA's capacity to modulate key hallmarks of cancer such as cell proliferation, expansion, invasion, migration, and apoptosis [83].

BPA induced neoplasticity in HEC265 and Ishikawa cells, both representative of Grade I EC. These cells were used to analyse the nuclear translocation of estrogen-related receptor gamma (ERR γ). The nuclear and cytoplasmic fractions were extracted and studied for cell proliferation markers. In HEC265 cells, BPA affected the BPA/ERR γ signalling pathway, thereby inducing cell proliferation. While in the case of Ishikawa cells, cell proliferation was induced by BPA/ERR γ /EGF signalling axis, as BPA promoted an ERR-independent mechanism for cell proliferation by inducing EGF secretion, indicating cell-type-specific and receptor-dependent mechanisms for BPA-induced neoplastic effects [84]. These cell models are instrumental in screening pharmacological inhibitors targeting BPA-activated pathways such as MAPK, COX-2, or ER/ERR γ signalling, and are suitable for high-throughput drug sensitivity assays.

8.2 *In vivo* animal models

Rodent and non-human primate models provide strong experimental evidence for the estrogen-mimetic and tumour-promoting properties of BPA in the uterus. When 100 $\mu\text{g/kg/day}$ of BPA was chronically administered to CD-1 mice, a drastic increase in complex endometrial hyperplasia (CEH) was observed. Notably, when the mice reached 18 months of age, a sevenfold increase in the development of CEH was seen compared to control animals. Furthermore, evidence of leiomyoma was observed in all animals with each 10, 100, and 1,000 $\mu\text{g/kg/day}$ BPA dose [85].

The chronic exposure to BPA modulates uterine morphology and functions. It increases uterine abnormalities when observed in Sprague Dawley (SD) rats. The SD rats, which are often considered resistant strains to EDs in vaginal and uterine tissue, displayed marked uterine abnormalities following BPA exposure. The evaluation of BPA-exposed SD rats after four months revealed reduced uterine wall thickness and a twofold increase in the amount of condensed chromatin in the nucleus, implying neoplastic transformation [86].

Non-human primate models, such as rhesus macaques, when exposed to BPA during critical windows of uterine development, show enhanced epithelial cell proliferation, reinforcing the relevance of BPA's effects across species, including humans. These models reflect human-like hormonal physiology and are critical for translational studies on endometrial pathophysiology and ED toxicity [87].

8.3 Multi-omics and toxicological integration

With the advent of high-throughput platforms, multi-omics approaches are increasingly employed to understand BPA-induced endometrial carcinogenesis. Toxicogenomic studies have demonstrated BPA-induced deregulation of estrogen-responsive genes, inflammatory cytokines, and chromatin modifiers. Transcriptomic profiling of BPA-exposed endometrial cells reveals dysregulated gene networks associated with proliferation, immune modulation, and DNA repair, many of which overlap with known EC signatures [88].

DNA methylation arrays and RNA-seq have revealed differential expression of non-coding RNA, such as miR-21 and miR-200C, after BPA exposure. These epigenetic changes mirror *in vivo* findings and serve as mechanistic biomarkers for early transformation events. Integration of these datasets enables comprehensive mapping of BPA's multi-target action and offers systems for biomarker validation and therapeutic screening through *in silico* and *in vitro* experiments [89].

9. Advocacy

Given the detrimental impact caused by BPA on key physiological systems, particularly the reproductive system, transitioning to safer bio-based alternatives could be the main counteractive measure that can be advocated. However, it requires systemic changes across industries, informed public awareness, and supportive regulatory policies. The 'BPA-free' alternatives primarily involve glassware, stainless steel, and polymers such as polyacetic acid (PLA), derived from renewable starch sources [90]. Bisguaiacols, a safer and sustainable alternative to BPA, are produced from non-fossil residues, that is, from biomass origin. The production of bisguaiacols utilizes guaiacol (arenes) and para-propenylguaiacol (alkene). It is one of the widely available biopolymers derived from lignin [91]. These alternatives are considered safer, with good chemical resistance and heat stability. Developing them into viable and affordable products with their complete safety profile established is the need of the hour, and policy-driven impetus needs to be given to overcome the BPA-derived health hazards.

10. Conclusions and future perspectives in translational epigenetic research

The cumulative evidence highlighted in the chapter underscores the intricate connection between the ED BPA and epigenetic factors influencing the development of EC. BPA, acting primarily as a xenoestrogen, perturbs the estrogen and progesterone signalling pathways. It initiates widespread epigenetic alterations, ranging from aberrant DNA methylation, histone modification, dysregulation of non-coding RNAs, and emerging epitranscriptomic mechanisms such as m6A methylation. The transcriptome of endometrial cells and the chromatin landscape get reprogrammed, facilitating tumour initiation, progression, immune invasion, and therapeutic resistance.

Importantly, this chapter explored BPA's toxicology, specifically how dose-dependent exposure can induce inheritance and multilevel effects. It highlights BPA's role in modulating the TME and inducing transgenerational epigenetic inheritance. This helps to understand endometrial tumour biology and challenges traditional paradigms in toxicology. In light of these advancements in the understanding of endometrial tumour etiology, it is important to thoroughly understand epigenetic biomarkers such as miRNA, lncRNAs, and methylation patterns, and leverage them for early diagnosis and risk assessment of BPA-driven ECs.

In conclusion, it is clearly evident that BPA silently sculpts the epigenome and rewrites the trajectory of hormone-responsive cancers, such as EC, across the life span and even across generations. Decoding the endocrine–epigenetic interface in EC represents a step towards precision medicine and environmental health safeguards. Therefore, the grave danger of BPA-mediated cancer risk can be better tackled by using sustainable and safe alternatives in day-to-day life to avoid the biomagnification of epigenetic dysregulation and to maintain epigenetic equilibrium.

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Conflict of interest

The authors declare no conflict of interest.

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References

- [1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *International Journal of Cancer*. 2015;**136**(5). DOI: 10.1002/ijc.29210
- [2] Cote ML, Ruterbusch JJ, Olson SH, Lu K, Ali-Fehmi R. The growing burden of Endometrial Cancer: A major racial disparity affecting black women. *Cancer Epidemiology Biomarkers and Prevention*. 2015;**24**(9):1407–1415. DOI: 10.1158/1055-9965.EPI-15-0316
- [3] Foster WG. Environmental estrogens and endocrine disruption: Importance of comparative endocrinology. *Endocrinology*. 2008;**149**(9):4267–4268. DOI: 10.1210/en.2008-0736
- [4] Donkers H, Bekkers R, Massuger L, Galaal K. Systematic review on socioeconomic deprivation and survival in endometrial cancer. *Cancer Causes and Control*. 2019;**30**(9):1013–1022. DOI: 10.1007/s10552-019-01202-1
- [5] Rubin BS. Bisphenol A: An endocrine disruptor with widespread exposure and multiple effects. *The Journal of Steroid Biochemistry and Molecular Biology*. 2011;**127**(1–2):27–34. DOI: 10.1016/j.jsbmb.2011.05.002
- [6] Mountfort KA, Kelly J, Jickells SM, Castle L. Investigations into the potential degradation of polycarbonate baby bottles during sterilization with consequent release of Bisphenol A. *Food Additives and Contaminants*. 1997;**14**(6–7):737–740. DOI: 10.1080/02643759708839280
- [7] Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Ekong J, Needham LL. Urinary concentrations of Bisphenol A and 4-nonylphenol in a human reference population. *Environmental Health Perspectives*. 2005;**113**(4):391–395. DOI: 10.1289/ehp.7534
- [8] Fenichel P, Chevalier N, Brucker-Davis F. Bisphenol A: An endocrine and metabolic disruptor. *Annales d'Endocrinologie*. 2013;**74**(3):211–220. DOI: 10.1016/j.ando.2013.04.002
- [9] Sheehan DM. Activity of environmentally relevant low doses of endocrine disruptors and the bisphenol A controversy: Initial results confirmed. *Proceedings of the Society for Experimental Biology and Medicine*. 2000;**224**(2):57–60. DOI: 10.1046/j.1525-1373.2000.22401.x
- [10] Fan J, Zhang M, Wu H, Ye Z, Wang L. Estrogen promotes endometrial cancer development by modulating ZNF626, SLK, and RFWD3 gene expression and inducing immune inflammatory changes. *Biomedicines*. 2025;**13**(2):498. DOI: 10.3390/biomedicines13020498
- [11] Caserta D, Di Segni N, Mallozzi M, Giovanale V, Mantovani A, Marci R, et al. Bisphenol a and the female reproductive tract: An overview of recent laboratory evidence and epidemiological studies. *Reproductive Biology & Endocrinology*. 2014;**12**(1):37. DOI: 10.1186/1477-7827-12-37
- [12] Iso T, Watanabe T, Iwamoto T, Shimamoto A, Furuichi Y. DNA damage caused by Bisphenol A and estradiol through estrogenic activity. *Biological*

- and Pharmaceutical Bulletin. 2006;**29** (2):206–210. DOI: 10.1248/bpb.29.206
- [13] Li G, Chang H, Xia W, Mao Z, Li Y, Xu S. F0 maternal BPA exposure induced glucose intolerance of F2 generation through DNA methylation change in Gck. *Toxicology Letters*. 2014;**228**(3):192–199. DOI: 10.1016/j.toxlet.2014.04.012
- [14] Ferreira LL, Couto R, Oliveira PJ. Bisphenol A as epigenetic modulator: Setting the stage for carcinogenesis? *European Journal of Clinical Investigation*. 2015;**45**(s1):32–36. DOI: 10.1111/eci.12362
- [15] Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. Exposure of the U.S. population to Bisphenol A and 4-*tertiary* -Octylphenol: 2003–2004. *Environmental Health Perspectives*. 2008;**116**(1):39–44. DOI: 10.1289/ehp.10753
- [16] Palacio DA, Leiton LM, Urbano BF, Rivas BL. Tetracycline removal by polyelectrolyte copolymers in conjunction with ultrafiltration membranes through liquid-phase polymer-based retention. *Environmental Research*. 2020;**182**:109014. DOI: 10.1016/j.envres.2019.109014
- [17] Barajas B, Mérida-Ortega Á, López-Carrillo L, Cebrián ME. Urinary concentrations of total and free bisphenol A and daily intake estimation in northern Mexican women. *Environmental Monitoring and Assessment*. 2025;**197**(6):675. DOI: 10.1007/s10661-025-14074-7
- [18] Zhang W, Li Y, Wang T, Zhang X, Zhang J, Ji X, et al. Distribution and potential risk factors of bisphenol a in serum and urine among Chinese from 2004 to 2019. *Front Public Health*. 2024;**12**. DOI: 10.3389/fpubh.2024.1196248
- [19] Vandenberg LN, Chahoud I, Heindel JJ, Padmanabhan V, Paumgartten FJR, Schoenfelder G. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to Bisphenol A. *Environmental Health Perspectives*. 2010;**118**(8):1055–1070. DOI: 10.1289/ehp.0901716
- [20] Geens T, Aerts D, Berthot C, Bourguignon JP, Goeyens L, Lecomte P, et al. A review of dietary and non-dietary exposure to bisphenol-A. *Food and Chemical Toxicology*. 2012;**50** (10):3725–3740. DOI: 10.1016/j.fct.2012.07.059
- [21] Hormann AM, Vom Saal FS, Nagel SC, Stahlhut RW, Moyer CL, Ellersieck MR, et al. Holding thermal receipt paper and eating food after using hand sanitizer results in high serum bioactive and urine total levels of Bisphenol A (BPA). *PLoS One*. 2014;**9** (10):e110509. DOI: 10.1371/journal.pone.0110509
- [22] Vasiljevic T, Harner T. Bisphenol A and its analogues in outdoor and indoor air: Properties, sources and global levels. *Science of the Total Environment*. 2021;**789**:148013. DOI: 10.1016/j.scitotenv.2021.148013
- [23] Zhang X, Ho SM. Epigenetics meets endocrinology. *Journal of Molecular Endocrinology*. 2011;**46**(1):R11–32. DOI: 10.1677/jme-10-0053
- [24] Clarke CL, Sutherland RL. Progestin regulation of cellular proliferation*. *Endocrine Reviews*. 1990;**11**(2):266–301. DOI: 10.1210/edrv-11-2-266

- [25] McLachlan SM, Nagayama Y, Rapoport B. Insight into graves' hyperthyroidism from animal models. *Endocrine Reviews*. 2005;**26**(6):800–832. DOI: 10.1210/er.2004-0023
- [26] Fuentes N, Silveyra P. Estrogen receptor signaling mechanisms. *Advances in Protein Chemistry and Structural Biology*. 2019;135–170. DOI: 10.1016/bs.apcsb.2019.01.001
- [27] Gellersen B, Brosens I, Brosens J. Decidualization of the human Endometrium: Mechanisms, functions, and clinical perspectives. *Seminars in Reproductive Medicine*. 2007;**25**(6):445–453. DOI: 10.1055/s-2007-991042
- [28] Kim J, Chapman-Davis E. Role of progesterone in Endometrial Cancer. *Seminars in Reproductive Medicine*. 2010;**28**(01):081–90. DOI: 10.1055/s-0029-1242998
- [29] Jacob A, Raj R, Allison DB, Myint ZW. Androgen receptor signaling in prostate cancer and therapeutic strategies. *Cancers (Basel)*. 2021;**13**(21):5417. DOI: 10.3390/cancers13215417
- [30] Alonso-Magdalena P, Roperio AB, Soriano S, García-Arévalo M, Ripoll C, Fuentes E, et al. Bisphenol-A acts as a potent estrogen via non-classical estrogen triggered pathways. *Molecular and Cellular Endocrinology*. 2012;**355**(2):201–207. DOI: 10.1016/j.mce.2011.12.012
- [31] Rattan S, Flaws JA. The epigenetic impacts of endocrine disruptors on female reproduction across generations†. *Biology of Reproduction*. 2019;**101**(3):635–644. DOI: 10.1093/biolre/ioz081
- [32] Lite C, Raja GL, Juliet M, Sridhar VV, Subhashree KD, Kumar P, et al. In utero exposure to endocrine-disrupting chemicals, maternal factors and alterations in the epigenetic landscape underlying later-life health effects. *Environmental Toxicology and Pharmacology*. 2022;**89**:103779. DOI: 10.1016/j.etap.2021.103779
- [33] Tao H, Shi KH, Yang JJ, Li J. Epigenetic mechanisms in atrial fibrillation: New insights and future directions. *Trends in Cardiovascular Medicine*. 2016;**26**(4):306–318. DOI: 10.1016/j.tcm.2015.08.006
- [34] Desaulniers D, Vasseur P, Jacobs A, Aguila MC, Ertych N, Jacobs MN. Integration of epigenetic mechanisms into non-genotoxic carcinogenicity hazard assessment: Focus on DNA methylation and histone modifications. *International Journal of Molecular Sciences*. 2021;**22**(20):10969. DOI: 10.3390/ijms222010969
- [35] Adam AHB, de Haan LHJ, Estruch IM, Hooiveld GJEJ, Louisse J, Rietjens IMCM. Estrogen receptor alpha (ERα)–mediated coregulator binding and gene expression discriminates the toxic ERα agonist diethylstilbestrol (DES) from the endogenous ERα agonist 17β-estradiol (E2). *Cell Biology & Toxicology*. 2020;**36**(5):417–435. DOI: 10.1007/s10565-020-09516-6
- [36] Shahidehnia M. Epigenetic effects of endocrine disrupting chemicals. *Journal of Environmental and Analytical Toxicology*. 2016;**6**(4). DOI: 10.4172/2161-0525.1000381
- [37] Hsieh CL. Dynamics of DNA methylation pattern. *Current Opinion in Genetics & Development*. 2000;**10**

- (2):224–228. DOI: 10.1016/S0959-437X.(00)00064-2
- [38] Prins G, Birch L, Tang W, Ho S. Developmental estrogen exposures predispose to prostate carcinogenesis with aging☆. *Reproductive Toxicology*. 2007;**23**(3):374–382. DOI: 10.1016/j.reprotox.2006.10.001
- [39] Dolinoy DC, Huang D, Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. *Proceedings of the National Academy of Sciences*. 2007;**104**(32):13056–13061. DOI: 10.1073/pnas.0703739104
- [40] Yin L, Dai Y, Jiang X, Liu Y, Chen H, Han F, et al. Role of DNA methylation in bisphenol A exposed mouse spermatocyte. *Environmental Toxicology and Pharmacology*. 2016;**48**:265–271
- [41] Ho SM, Johnson A, Tarapore P, Janakiram V, Zhang X, Leung YK. Environmental epigenetics and its implication on disease risk and health outcomes. *ILAR Journal*. 2012;**53**(3–4):289–305. DOI: 10.1093/ilar.53.3-4.289
- [42] Nakachi Y, Ishii K, Bundo M, Masuda T, Iwamoto K. Use of the Illumina EPIC methylation array for epigenomic research in the crab-eating macaque (*Macaca fascicularis*). *Neuropsychopharmacology Reports*. 2020;**40**(4):423–426. DOI: 10.1002/npr2.12145
- [43] Papamichos-Chronakis M, Peterson CL. Chromatin and the genome integrity network. *Nature Reviews Genetics*. 2011;**12**(1):62–75. DOI: 10.1038/nrg1000
- [44] Singh S, Li SSL. Epigenetic Effects of Environmental Chemicals Bisphenol A and Phthalates. *International Journal of Molecular Sciences*. 2012;**13**(8):10143–10153. DOI: 10.3390/ijms130810143
- [45] Miranda Furtado CL, Dos Santos Luciano MC, Da Silva Santos R, Furtado GP, Moraes MO, Pessoa C. Epidrugs: Targeting epigenetic marks in cancer treatment. *Epigenetics*. 2019;**14**(12):1164–1176. DOI: 10.1080/15592294.2019.1640546
- [46] Handy DE, Castro R, Loscalzo J. Epigenetic modifications: Basic mechanisms and role in cardiovascular disease. *Circulation*. 2011;**123**(19):2145–2156. DOI: 10.1038/s41392-019-0095-0
- [47] Cheng Y, He C, Wang M, Ma X, Mo F, Yang S, et al. Targeting epigenetic regulators for cancer therapy: Mechanisms and advances in clinical trials. *Signal Transduction and Targeted Therapy*. 2019;**4**(1):62. DOI: 10.1161/CIRCULATIONAHA.110.956839
- [48] Senyildiz M, Karaman EF, Bas SS, Pirincci PA, Ozden S. Effects of BPA on global DNA methylation and global histone 3 lysine modifications in SH-SY5Y cells: An epigenetic mechanism linking the regulation of chromatin modifying genes. *Toxicology in Vitro*. 2017;**44**:313–321. DOI: 10.1016/j.tiv.2017.07.028
- [49] Newbold RR. Developmental exposure to endocrine-disrupting chemicals programs for reproductive tract alterations and obesity later in life. *The American Journal of Clinical Nutrition*. 2011;**94**(6):1939S–1942S. DOI: 10.3945/ajcn.110.001057

- [50] Bromer JG, Zhou Y, Taylor MB, Doherty L, Taylor HS. Bisphenol-A exposure *in utero* leads to epigenetic alterations in the developmental programming of uterine estrogen response. *The FASEB Journal*. 2010;**24**(7):2273–2280. DOI: 10.1096/fj.09-140533
- [51] Piergentili R, Zaami S, Cavaliere AF, Signore F, Scambia G, Mattei A, et al. non-coding RNAs as prognostic markers for endometrial cancer. *International Journal of Molecular Sciences*. 2021;**22**(6):3151. DOI: 10.3390/ijms22063151
- [52] Bhan A, Soleimani M, Mandal SS. Long non coding RNA and Cancer: A new paradigm. *Cancer Research*. 2017;**77**(15):3965–3981. DOI: 10.1158/0008-5472.CAN-16-2634
- [53] Adiga D, Nadeem Khan G, Eswaran S, Sriharikrishnaa S, Chakrabarty S, Rai PS, et al. Chapter 3. Bisphenol an associated signaling pathways in human diseases. 2022. p. 42–86. DOI: 10.1039/9781839166495-00042
- [54] Newbold R, Padillabanks E, Snyder R, Phillips T, Jefferson W. Developmental exposure to endocrine disruptors and the obesity epidemic. *Reproductive Toxicology*. 2007;**23**(3):290–296. DOI: 10.1016/j.reprotox.2006.12.010
- [55] Watters JW, Roberts CJ. Developing gene expression signatures of pathway deregulation in tumors. *Molecular Cancer Therapeutics*. 2006;**5**(10):2444–2449. DOI: 10.1158/1535-7163.MCT-06-0340
- [56] Suzukawa K, Weber TJ, Colburn NH. AP-1, NF-kappa-B, and ERK activation thresholds for promotion of neoplastic transformation in the mouse epidermal JB6 model. *Environmental Health Perspectives*. 2002;**110**(9):865–870. DOI: 10.1289/ehp.02110865
- [57] Camacho L, Basavarajappa MS, Chang CW, Han T, Kobets T, Koturbash I, et al. Effects of oral exposure to bisphenol A on gene expression and global genomic DNA methylation in the prostate, female mammary gland, and uterus of NCTR Sprague-Dawley rats. *Food and Chemical Toxicology*. 2015;**81**:92–103
- [58] Takemura H, Ma J, Sayama K, Terao Y, Zhu BT, Shimoi K. In vitro and in vivo estrogenic activity of chlorinated derivatives of Bisphenol A. *Toxicology*. 2005;**207**(2):215–221. DOI: 10.1016/j.tox.2004.09.015
- [59] Bindhumol V, Chitra KC, Mathur PP. Bisphenol A induces reactive oxygen species generation in the liver of male rats. *Toxicology*. 2003;**188**(2–3):117–124. DOI: 10.1016/S0300-483X(03)00056-8
- [60] Fikai S, Papait A, Magatti M, Masserdotti A, Gasik M, Silini AR, et al. Bisphenol-A disrupts mitochondrial functionality leading to senescence and apoptosis in human amniotic mesenchymal stromal cells. *Cell Death Discovery*. 2025;**11**(1):327. DOI: 10.1038/s41420-025-02620-8
- [61] Daiber A, Xia N, Steven S, Oelze M, Hanf A, Kröller-Schön S, et al. New therapeutic implications of endothelial Nitric Oxide Synthase (eNOS) function/dysfunction in cardiovascular disease. *International Journal of Molecular Sciences*. 2019;**20**(1):187. DOI: 10.3390/ijms20010187

- [62] Iqbal MJ, Kabeer A, Abbas Z, Siddiqui HA, Calina D, Sharifi-Rad J, et al. Interplay of oxidative stress, cellular communication and signaling pathways in cancer. *Cell Communication and Signaling*. 2024;22(1):7. DOI: 10.1186/s12964-023-01398-5 e32754. DOI: 10.1371/journal.pone.0032754
- [63] Cho YJ, Park SB, Park JW, Oh SR, Han M. Bisphenol A modulates inflammation and proliferation pathway in human endometrial stromal cells by inducing oxidative stress. *Reproductive Toxicology*. 2018;81:41–49. DOI: 10.1016/j.reprotox.2018.06.016
- [64] Karin M, Greten FR. NF- κ B: Linking inflammation and immunity to cancer development and progression. *Nature Reviews Immunology*. 2005;5(10):749–759. DOI: 10.1038/nri1703
- [65] Dvorak O, Ndukwe M, Slavickova M, Laco J, Spacek J. DNA methylation of selected tumor suppressor genes in endometrial hyperplasia. *Biomedical Papers*. 2024;168(1):68–73. DOI: 10.5507/bp.2022.053
- [66] Zhang M, Xu T, Tong D, Li S, Yu X, Liu B, et al. Research advances in endometriosis-related signaling pathways: A review. *Biomedicine & Pharmacotherapy*. 2023;164:114909. DOI: 10.1016/j.biopha.2023.114909
- [67] Susiarjo M, Sasson I, Mesaros C, Bartolomei MS. Bisphenol an exposure disrupts genomic imprinting in the mouse. *PLoS Genetics*. 2013;9(4): e1003401. DOI: 10.1371/journal.pgen.1003401
- [68] Tilghman SL, Bratton MR, Segar HC, Martin EC, Rhodes LV, Li M, et al. Endocrine disruptor regulation of microRNA expression in breast carcinoma cells. *PLoS One*. 2012;7(3): e32754. DOI: 10.1371/journal.pone.0032754
- [69] Manikkam M, Tracey R, Guerrero-Bosagna C, Skinner MK. Plastics derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. *PLoS One*. 2013;8(1): e55387. DOI: 10.1371/journal.pone.0055387
- [70] Huang R, Kang T, Chen S. The role of tumor-associated macrophages in tumor immune evasion. *Journal of Cancer Research and Clinical Oncology*. 2024;150(5):238. DOI: 10.1007/s00432-024-05777-4
- [71] Lu X, Li M, Wu C, Zhou C, Zhang J, Zhu Q, et al. Bisphenol A promotes macrophage proinflammatory subtype polarization via upregulation of IRF5 expression in vitro. *Toxicology in Vitro*. 2019;60:97–106
- [72] Ma LY, Zhai XY, Qiao YX, Zhang AP, Zhang N, Liu J, et al. Identification of a novel function of a component in the jasmonate signaling pathway for intensive pesticide degradation in rice and environment through an epigenetic mechanism. *Environmental Pollution*. 2021;268:115802. DOI: 10.1016/j.envpol.2020.115802
- [73] Yao Y, Liu P, Li Y, Wang W, Jia H, Bai Y, et al. Regulatory role of m6A epitranscriptomic modifications in normal development and congenital malformations during embryogenesis. *Biomedicine & Pharmacotherapy*. 2024;173:116171. DOI: 10.1016/j.biopha.2024.116171
- [74] Cao DQ, Tian F, Wang X, Zhang WY, Hao XD, Wang QH.

- Recovery of polymeric substances from excess sludge: Surfactant-enhanced ultrasonic extraction and properties analysis. *Chemosphere*. 2021;**283**:131181. DOI: 10.1016/j.chemosphere.2021.131181
- [75] Wen T, Li T, Xu Y, Zhang Y, Pan H, Wang Y. The role of m6A epigenetic modifications in tumor coding and non-coding RNA processing. *Cell Communication and Signaling*. 2023;**21**(1):355. DOI: 10.1186/s12964-023-01385-w
- [76] Cabrera S, de la Calle I, Baulies S, Gil-Moreno A, Colas E. Screening strategies to improve early diagnosis in Endometrial Cancer. *Journal of Clinical Medicine*. 2024;**13**(18):5445. DOI: 10.3390/jcm13185445
- [77] Van Den Bosch T, Verbakel JY, Valentin L, Wynants L, De Cock B, Pascual MA, et al. Typical ultrasound features of various endometrial pathologies described using International Endometrial Tumor Analysis (IETA) terminology in women with abnormal uterine bleeding. *Ultrasound in Obstetrics & Gynecology*. 2021;**57**(1):164–172. DOI: 10.1002/uog.22109
- [78] Dewan R, Dewan A, Hare S, Bhardwaj M, Mehrotra K. Diagnostic Performance of Serum Human Epididymis Protein 4 in Endometrial Carcinoma: A Pilot Study. *Journal of Clinical and Diagnostic Research : JCDR*. 2017;**11**(7):XC01–5. DOI: 10.7860/JCDR/2017/28926.10285
- [79] Troisi J, Mollo A, Lombardi M, Scala G, Richards SM, Symes SJK, et al. The metabolomic approach for the screening of Endometrial Cancer: Validation from a large cohort of women scheduled for Gynecological Surgery. *Biomolecules*. 2022;**12**(9). DOI: 10.3390/biom12091229
- [80] Kido A, Himoto Y, Moribata Y, Kurata Y, Nakamoto Y. MRI in the diagnosis of Endometriosis and related diseases. *Korean Journal of Radiology*. 2022;**23**(4):426–445. DOI: 10.3348/kjr.2021.0405
- [81] Corr B, Cosgrove C, Spinoso D, Guntupalli S. Endometrial cancer: Molecular classification and future treatments. *BMJ Medicine*. 2022;**1**(1):e000152. DOI: 10.1136/bmjmed-2022-000152
- [82] LoRusso PM. Inhibition of the PI3K/AKT/mTOR pathway in solid tumors. *Journal of Clinical Oncology*. 2016;**34**(31):3803–3815. DOI: 10.1200/JCO.2014.59.0018
- [83] Gulappa T, Menon B, Menon KMJ. Hypusination of eukaryotic initiation factor 5A via cAMP-PKA-ERK1/2 pathway is required for ligand-induced downregulation of LH receptor mRNA expression in the ovary. *Molecular and Cellular Endocrinology*. 2015;**413**:90–95. DOI: 10.1016/j.mce.2015.06.014
- [84] Klinbunga S, Petkorn S, Kittisenachai S, Phaonakrop N, Roytrakul S, Khamnamtong B, et al. Identification of reproduction-related proteins and characterization of proteasome alpha 3 and proteasome beta 6 cDNAs in testes of the giant tiger shrimp *Penaeus monodon*. *Molecular and Cellular Endocrinology*. 2012;**355**(1):143–152. DOI: 10.1016/j.mce.2012.02.005
- [85] Neff AM, Blanco SC, Flaws JA, Bagchi IC, Bagchi MK. Chronic exposure of mice to Bisphenol-A Alters Uterine fibroblast growth factor

signaling and leads to Aberrant Epithelial proliferation. *Endocrinology*. 2019;**160**(5):1234–1246. DOI: 10.1210/en.2018-00872

[86] Suvorov A, Waxman DJ. Early programing of uterine tissue by bisphenol A: Critical evaluation of evidence from animal exposure studies. *Reproductive Toxicology*. 2015;**57**: 59–72. DOI: 10.1210/en.2018-00872

[87] Tharp AP, Maffini MV, Hunt PA, VandeVoort CA, Sonnenschein C, Soto AM. Bisphenol A alters the development of the rhesus monkey mammary gland. *Proceedings of the National Academy of Sciences*. 2012;**109** (21):8190–8195. DOI: 10.1073/pnas.1120488109

[88] Chen M, Gong L, Qi X, Xing G, Luan Y, Wu Y, et al. Inhibition of renal NQO1 activity by dicoumarol suppresses nitroreduction of Aristolochic Acid I and Attenuates its Nephrotoxicity. *Toxicological Sciences*. 2011;**122**(2):288–296. DOI: 10.1093/toxsci/kfr138

[89] Sabry R, May DR, Favetta LA. The relationship between miR-21, DNA methylation, and bisphenol a in bovine COCs and granulosa cells. *Frontiers in Cell and Developmental Biology*. 2023;**11**:1294541. DOI: 10.3389/fcell.2023.1294541

[90] Malek NSA, Faizuwan M, Khusaimi Z, Bonnia NN, Rusop M, Asli NA. Preparation and characterization of biodegradable Polylactic Acid (PLA) Film for food packaging application: A review. *Journal of Physics: Conference Series*. 2021;**1892** (1):012037. DOI: 10.1088/1742-6596/1892/1/012037

[91] Rinaldi R, Jastrzebski R, Clough MT, Ralph J, Kennema M, Bruijnincx PCA, et al. Paving the way for lignin valorisation: recent advances in bioengineering, biorefining and catalysis. *Angewandte Chemie International Edition*. 2016;**55** (29):8164–8215. DOI: 10.1002/anie.201510351

Chapter 6

Unraveling the Involvement of Epigenetics in Acute Myeloid Leukemia (AML): Focusing on Epigenetic Treatments in AML

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Abstract

Acute myeloid leukemia (AML) is a complex hematologic cancer marked by both genetic and epigenetic abnormalities that lead to the expansion of immature myeloid cells and a block in their normal differentiation. Among the various molecular mechanisms implicated in its pathogenesis, epigenetic alterations have gained increasing attention for their crucial role in initiating and sustaining leukemic transformation. Mutations in key epigenetic regulators such as *DNMT3A*, *TET2*, *IDH1/2*, and *EZH2* have been frequently identified in AML, contributing to widespread disruptions in DNA methylation patterns, histone modification states, and chromatin structure. These changes profoundly influence gene expression programs by favoring malignant progression. Unlike permanent genetic mutations, epigenetic changes are potentially reversible, making them particularly appealing as therapeutic targets. Agents that modulate the epigenome, such as DNA hypomethylating drugs or other epi-drugs, have shown significant clinical activity, especially in older patients or those medically unsuitable for intensive chemotherapy. Additionally, novel compounds are being actively evaluated, both alone and in combination with existing therapies. Overall, the epigenetic landscape of AML offers valuable opportunities for innovative treatment approaches. Ongoing research is crucial to better understand resistance mechanisms, define predictive biomarkers, and refine therapeutic strategies aimed at improving clinical outcomes for AML patients.

Keywords: epigenetics, leukemia, DNA methylation, histone modifications, therapies

1. Introduction

Epigenetics is defined as the study of the functionally heritable gene expression in the genome, which does not include structural changes in the DNA sequence, resulting in phenotypic modulation without genotypic alteration [1]. It is mostly

influenced by environmental factors such as diet, stress, infections, or chemical exposure, which can modulate the expression of certain genes by determining “how” and “when” they should be expressed or silenced [2]. These factors can trigger epigenetic mechanisms responsible for the regulation of gene expression through the addition or removal of covalent chemical modifications, post-translational histone modifications (PTMs), or different RNA species such as miRNA (microRNA), siRNA (small interfering RNA), or lncRNA (long non-coding RNA) [3]. During these years, several epigenetic modifications have been identified, such as DNA methylation, histone modifications, and non-coding RNAs (ncRNAs), as the results of the action of different enzymatic classes. These enzymes are classified as *writers*, able to add chemical modifications on DNA, histones, and proteins; *readers*, which read signals through their structural domains; *erasers*, involved in the removal of chemical groups; and *remodelers*, which are proteins or protein complexes that remodel the chromatin structure, making it more or less accessible to the transcription apparatus [4, 5]. In this context, the epigenome, which describes the set of epigenetic changes in an individual, represents one of the best targets for studying the regulation of gene expression [6]. Although epigenetics regulates primary processes such as development, cell proliferation, and cell death in response to environmental stimuli, alterations in epigenetic mechanisms may be involved in the initiation and development of high-social-impact diseases, including cancer, neurodegenerative diseases, or autoimmune disorders [7, 8]. In this context, epigenetic mechanisms can influence factors involved in disease-related cellular pathways and/or in the modulation of disease-associated gene products (i.e., oncogene activation or oncosuppressor silencing) [9]. The role that epigenetics has in pathogenesis is due to the alteration of the normal balance of gene expression, which allows abnormal activation or silencing of critical genes, leading to disruptions in cellular function and contributing to the development and progression of diseases [10]. Furthermore, unlike genetic mutations, epigenetic changes are often reversible, offering potential innovative therapeutic targets for the treatment of several morbidities [10]. As a bridge between environment, genetics, and disease, epigenetics helps us understand why two individuals with the same DNA develop or do not develop a certain disease or exhibit different symptoms [11]. The study of epigenetics in pathogenesis broadens our understanding of the complex biological mechanisms underlying life and disease and opens up new perspectives for personalized medicine.

In this regard, acute myeloid leukemia (AML) remains a significant clinical challenge due to its heterogeneous nature and complex pathogenesis [12]. Like other hematological malignancies, AML is characterized not only by genetic mutations but also by epigenetic dysregulation of the main epigenetic regulators, such as *DNMT3A*, *TET2*, *IDH1/2*, and *EZH2* which are associated with disease initiation, progression, and therapy resistance [13]. Recent advances have underscored the critical role of epigenetic dysregulation in leukemogenesis, including aberrant DNA methylation, histone modifications, and chromatin remodeling [14]. These epigenetic alterations contribute to the disruption of normal hematopoietic differentiation by promoting malignant transformation and uncontrolled proliferation [15]. Comprehensive investigation into the epigenetic landscape of leukemia is imperative to unravel disease mechanisms and develop more effective and targeted epigenetic therapies [16]. This approach holds the potential to improve patient stratification and overcome resistance to conventional chemotherapeutics, ultimately enhancing

clinical outcomes [17]. In particular, in this book chapter, a deeper understanding of how epigenetics is involved in leukemogenic processes will be provided, in order to offer an integrated perspective on its role in AML and the therapeutic opportunities that epigenetic treatments may bring to patient outcomes.

2. Acute myeloid leukemia

AML is the predominant type of acute leukemia in adults and represents a complex array of hematopoietic malignancies that disrupt normal blood cell development [18]. The disease arises from the clonal transformation of hematopoietic stem cells (HSCs), causing the uncontrolled proliferation of immature myeloid precursors. This clonal expansion interferes with normal hematopoiesis, ultimately leading to bone marrow failure [19]. Consequently, patients often present with anemia, thrombocytopenia, and increased susceptibility to infections due to the impaired production of functional blood cells [20]. The five-year overall survival (OS) rate among individuals diagnosed with AML remains approximately 30%, with marked variability depending on the patient's age [18]. The current classification of AML is primarily based on the 2022 World Health Organization (WHO) classification and the 2022 International Consensus Classification (ICC). These classification schemes are essential tools for accurate diagnosis, prognostic assessment, and treatment selection in clinical practice. Both systems underscore the central role of recurrent genetic alterations in defining biologically and clinically distinct AML subtypes [21, 22]. Based on its etiology, AML can be broadly classified into three main subtypes: i) *de novo* AML (dn-AML), which arises without any preexisting hematologic disorder or identifiable exposure, representing the most common form; ii) secondary AML (s-AML), which develops as a progression from antecedent hematologic conditions, such as myelodysplastic syndromes (MDS) or myeloproliferative neoplasms (MPNs); and iii) therapy-related AML (t-AML), which develops as a result of prior cytotoxic chemotherapy, radiation therapy, or environmental toxins, and is typically associated with a worse prognosis [23–25]. Advances in the understanding of AML biology and the identification of its underlying genetic drivers have significantly improved the molecular characterization of the disease and contributed to the development of more targeted and effective therapeutic strategies [26].

2.1 The pathogenesis of AML

The pathogenesis of AML involves a multifaceted network of genetic and epigenetic disruptions. Key contributors include chromosomal abnormalities, such as translocations and inversions, and somatic mutations in pivotal genes for hematopoietic regulation, such as *TP53*, *FLT3*, *NPM1*, *IDH1/2*, *DNMT3A*, and *TET2*. Collectively, these molecular alterations trigger a pathogenic cascade that impairs normal differentiation, promotes unchecked proliferation, and leads to leukemic transformation. Most mutations in AML involve the DNA-binding domain of the tumor suppressor *TP53*, the gene most commonly mutated in human cancers. The human *TP53* gene, located on chromosome 17p13.1, encodes p53 protein, a tumor suppressor that regulates the expression of numerous target genes involved in metabolism, apoptosis, DNA repair, cellular stress response, and cell cycle [27]. p53 is

functionally inactivated either through direct mutations in the *TP53* gene or indirectly via alterations in genes encoding proteins that interact with the p53 pathway. *TP53*-mutated AML is often resistant to conventional chemotherapy and shows limited benefit from allogeneic hematopoietic stem cell transplantation [28]. FMS-related tyrosine kinase 3 (*FLT3*) mutations are considered among the most frequent genetic alterations in AML, occurring in approximately 30% of cases. These mutations cause constitutive activation of the *FLT3* receptor tyrosine kinase, driving uncontrolled proliferation of leukemic stem cells (LSCs) [29]. Historically, *FLT3* mutations have been correlated with adverse prognosis. However, the advent of targeted therapies, specifically *FLT3* inhibitors, has significantly improved clinical outcomes in patients harboring *FLT3*-mutated AML [30]. Nucleophosmin (*NPM1*) gene mutations frequently occur with *FLT3* mutations, with prognostic classification determined by the presence or absence of concurrent *FLT3*-ITD mutations [31]. *NPM1* mutations occur in approximately one-third of AML cases and are considered highly specific to the disease. They typically affect exon 12 and are often found in association with other mutations such as *FLT3*-ITD, *DNMT3A*, *TET2*, and *IDH1/2*. The *NPM1* gene encodes a nucleolar chaperone protein, and its mutations lead to its aberrant cytoplasmic localization, contributing to leukemogenesis. Due to their distinct molecular and clinicopathologic profile, *NPM1*-mutated AML is identified as a separate entity in both the 2022 International Consensus Classification (ICC) and the WHO 5th edition [31]. Mutations in isocitrate dehydrogenase 1 and 2 (*IDH1/2*) are observed in a subset of AML cases, with frequencies ranging from 6–16% for *IDH1* and 8–19% for *IDH2* [32]. Under physiological conditions, *IDH* enzymes convert isocitrate to α -ketoglutarate (α -KG), generating NADPH. Mutant *IDH* proteins instead produce the oncometabolite 2-hydroxyglutarate (2-HG), which accumulates intracellularly, altering epigenetic regulation and contributing to leukemogenesis [33–35]. The prognostic significance of *IDH1/2* mutations in AML remains unclear, as various studies have reported conflicting results regarding their impact on patient outcomes [34–36]. *KMT2A* (*MLL1*), located on chromosome 11q23, encodes a large DNA-binding protein essential for normal hematopoietic development. Rearrangements involving *KMT2A* are frequent chromosomal abnormalities in AML, especially prevalent in infant leukemia (70–80%) and observed in 5–10% of AML cases [37–39]. By binding to chromatin, these fusion proteins induce leukemic transformation of hematopoietic stem cells through modulation of key developmental genes such as *HOXA9* and its cofactor *MEIS1*. These fusions are also common in therapy-related AML following exposure to topoisomerase II inhibitors. The resulting fusion proteins alter gene regulation by binding to chromatin, driving leukemic transformation through dysregulation of key genes such as *HOXA9* and its cofactor *MEIS1*. This aberrant gene expression promotes leukemogenesis and impairs normal hematopoiesis [40]. Clinically, AML with *KMT2A* rearrangements generally carries a poorer prognosis compared to cytogenetically normal AML and may vary depending on the specific fusion partner involved [41, 42]. CD123, the alpha subunit of the interleukin-3 receptor (IL-3 R), belongs to the beta common cytokine receptor family. While its expression is low on normal hematopoietic stem cells (HSCs), it increases as cells differentiate along the myeloid and monocytic lineages [43]. Conversely, CD123 is expressed at higher levels on LSCs in AML than on their normal counterparts. Its elevated expression on AML blasts has been associated with higher blast counts at diagnosis and poorer treatment response [44, 45]. These characteristics highlight CD123 as a promising therapeutic target in AML, and several treatment strategies

aimed at this molecule are currently under investigation. Additionally, alterations in transcriptional networks and epigenetic regulatory mechanisms play a critical role in leukemogenesis, promoting impaired differentiation and uncontrolled proliferation of myeloid progenitors [28, 46, 47].

In the clinical management of AML, comprehensive genetic profiling at diagnosis is essential, not only for identifying high-risk patients with adverse prognostic markers but also for guiding therapeutic decisions in those classified within favorable-risk categories.

3. Epigenetic alterations in the landscape of AML

AML is increasingly recognized as a paradigm of epigenetically driven malignancy, in which both genetic mutations and metabolic perturbations converge to reprogram the epigenome, thereby enforcing leukemic cell identity and self-renewal while blocking differentiation [48]. In AML, multiple players in the epigenetic regulatory machinery are disrupted, including DNA methylation, histone post-translational modifications, chromatin remodeling complexes, three-dimensional genome architecture, and RNA modifications (epitranscriptomics). These perturbations frequently arise from mutations in enzymes that act as writers, erasers, readers, or remodelers of the epigenetic marks, and they cooperate with oncogenic drivers to maintain leukemic stem cell programs [49]. DNA methylation is one of the most extensively altered epigenetic pathways in AML.

3.1 Alterations in DNA methylation

Mutations in DNMT3A, a *de novo* DNA methyltransferase, are found in ~ 25% of patients, particularly those with intermediate-risk cytogenetics [50]. The most frequent hotspot mutation, R882H, disrupts the tetramerization interface of DNMT3A, leading to dominant-negative effects and locus-specific hypomethylation. This epigenetic remodeling facilitates aberrant self-renewal of hematopoietic stem and progenitor cells by interfering with differentiation signals. Experimental models show that DNMT3A R882 mutations enforce a block in lineage commitment by deregulating enhancers and promoters involved in myeloid differentiation [51]. Interestingly, non-R882 DNMT3A variants often destabilize the protein, leading to proteasomal degradation and a global decrease in DNMT3A abundance. Although these destabilizing mutants show less focal hypomethylation compared to R882 variants, they generate higher intratumoral methylation heterogeneity and transcriptional alteration, which may provide clonal fitness advantages under selective pressures [52]. Functionally, both classes of DNMT3A mutations confer a competitive edge to hematopoietic stem cells (HSCs), facilitate clonal hematopoiesis, and often precede the acquisition of cooperating mutations such as FLT3-ITD and NPM1c, which drive full leukemic transformation [53]. Another critical regulator of DNA methylation altered in AML is TET2, encoding a dioxygenase that catalyzes the stepwise oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine, and 5-carboxycytosine, biochemical intermediates which belong to the active DNA demethylation process. TET2 mutations, found in 10–20% of AML cases, especially in older adults and secondary AML, lead to an adverse prognosis due to the reduced 5hmC levels and

accumulation of aberrant DNA hypermethylation at enhancers and lineage-specifying regulatory elements [54]. Loss of TET2 function compromises enhancer accessibility and perturbs the transcriptional activation of genes required for myeloid differentiation [55]. Beyond its canonical role in DNA demethylation, TET2 was recently shown to participate in RNA-mediated chromatin regulation. Specifically, TET2 oxidizes 5-methylcytosine on chromatin-associated RNA (m5C), interfering with the binding of methyl-RNA readers such as MBD6. In the absence of TET2, persistent m5C on RNA promotes MBD6 recruitment and accumulation of histone H2A ubiquitination at Lys119 (H2AK119ub), reinforcing repressive chromatin configurations. Thus, TET2 loss not only disrupts DNA methylation dynamics but also alters histone modification landscapes in an RNA-dependent manner [56]. Converging on the TET2 pathway are neomorphic mutations in the metabolic enzymes IDH1 and IDH2. These mutations, present in ~15–20% of AML patients, introduce a novel enzymatic activity that reduces α -ketoglutarate to 2-hydroxyglutarate, a competitive inhibitor of α -ketoglutarate-dependent dioxygenases. By suppressing TET2 activity and certain Jumonji-domain histone demethylases (Jmj-KDMs), 2-hydroxyglutarate accumulation leads to global DNA hypermethylation and impaired chromatin remodeling [57]. IDH1/2-mutant AML cells exhibit a characteristic DNA methylation signature, including hypermethylation of differentiation-promoting gene promoters and a transcriptional program biased toward stemness and self-renewal. Notably, IDH and TET2 mutations are mutually exclusive, consistent with their functional convergence [58]. Interestingly, IDH-like epigenetic reprogramming can also arise in IDH-wild-type AML through metabolic rewiring. For example, upregulation of BCAT1, a branched-chain amino acid transaminase that reduces intracellular α -KG levels, mimics the effects of mutant IDH by inhibiting TET2 activity, inducing a similar hypermethylation phenotype, and promoting a stem-like transcriptional state [59].

3.2 Alterations in histone methylation

AML subtypes characterized by rearrangements involving the MLL (KMT2A) gene produce oncogenic fusion proteins (e.g., MLL-AF4, MLL-AF9, MLL-ENL), which subsequently drive aberrant histone modifications at specific gene loci. These fusion proteins result from chromosomal translocations that fuse the N-terminal portion of MLL, which retains DNA-binding and chromatin-targeting capacity, with various transcriptional co-activators [60]. These MLL fusion proteins lack the SET domain, which normally mediates the activating methylation of histone H3 at lysine 4 (H3K4me) on MLL target genes, resulting in the loss of this modification [61]. Furthermore, these fusion proteins aberrantly recruit the histone methyltransferase DOT1L to the HOXA9 and MEIS1 loci, causing abnormal deposition of H3K79 methylation (H3K79me), which sustains the transcription of genes involved in self-renewal [62]. This mislocalization relies on the recognition of H3K36 dimethylation (H3K36me2) by lens epithelium-derived growth factor (LEDGF), which interacts with MLL fusion proteins through the scaffold protein Menin, encoded by MEN1. This interaction facilitates the chromatin tethering of DOT1L, reinforcing the aberrant HOX gene expression and leukemogenic transcriptional programs. Accordingly, inhibition of H3K79 methylation or blockade of the Menin–MLL interaction disrupts this epigenetic axis, promoting differentiation of leukemic cells and providing

a rational basis for emerging targeted therapies [63]. Independently from MLL rearrangements, point mutations in ENL (MLLT1), a component of the super elongation complex, further amplify aberrant H3K79 dimethylation (H3K79me₂) at *HOXA* and *MEIS1* loci. These mutations promote the formation of phase-separated transcriptional condensates at these loci, enhancing transcriptional elongation and further exacerbating epigenetic dysregulation and active transcription [64]. Several epigenetic regulators involved in AML exert their function by recognizing specific histone modifications, thereby influencing gene expression programs that drive leukemic transformation. For example, the chromatin reader SGF29, a component of the SAGA complex, binds to the activating marks H3K4me_{2/3} and promotes the transcription of oncogenic drivers. Loss of SGF29 impairs leukemic self-renewal, highlighting its critical role as a reader of active chromatin states [65]. Another frequently mutated epigenetic factor in AML is ASXL1, a scaffold protein that interacts with both Polycomb (PRC2) and trithorax group complexes. ASXL1 mutations disrupt H3K27 trimethylation (H3K27me₃), destabilize PRC2, and simultaneously enhance H2AK119 deubiquitination via persistent interaction with the deubiquitinase BAP1. These alterations lead to chromatin de-repression at developmental gene loci, including the HOX cluster, ultimately promoting leukemogenesis. EZH2, the catalytic subunit of PRC2, plays a dual, context-dependent role in AML. Loss-of-function mutations in EZH2 reduce H3K27me₃ levels and lead to the derepression of genes involved in leukemic initiation [66]. Conversely, in established AML, EZH2 contributes to disease maintenance by repressing pro-differentiation genes. This biphasic role complicates the therapeutic targeting of EZH2 and suggests that stage-specific intervention strategies may be required. Furthermore, mutations in UTX (KDM6A), an H3K27 demethylase, disrupt enhancer landscapes and chromatin accessibility in a non-catalytic fashion. In mouse models, UTX loss affects enhancer-bound transcription factors such as GATA2 and ETS-family proteins through altered H3K27ac and loss of H3K4me₁, thereby reshaping the regulatory architecture of leukemia-initiating cells [67].

3.3 Alterations in histone acetylation

Histone acetylation plays a pivotal role in the pathogenesis of AML, not only by modulating chromatin accessibility but also by functionally altering specific histone residues. These modifications promote transcriptional activation, enhance oncogene expression, disrupt normal differentiation programs, and support the aberrant proliferation and survival of leukemic cells. Acetylation of lysine residues, such as H3K27ac and H3K9ac, generally facilitates transcriptional activation by loosening chromatin structure and recruiting bromodomain-containing proteins like BRD4 [68]. Two histone acetyltransferases, KAT6A (MOZ) and KAT2A (GCN5), have emerged as critical regulators of transcriptional programs that sustain leukemic identity. Both belong to families of acetyltransferases that deposit H3K9ac marks at gene promoters, thereby influencing gene expression dynamics. KAT6A, a member of the MYST acetyltransferase family, has been identified as a key factor in maintaining the myeloid differentiation block characteristic of AML through regulation of genes such as PU.1, IRF8, and various ETS factors [69, 70]. It mediates H3K9 acetylation at promoter regions, which is subsequently recognized by the acetyl-lysine reader ENL. ENL recruitment leads to the assembly of transcriptional elongation complexes, enhancing the expression of MYC and supporting the associated oncogenic program. This

cooperative interaction between KAT6A and ENL exemplifies how epigenetic crosstalk reinforces the leukemic transcriptional state [71]. Similarly, KAT2A-dependent H3K9 acetylation occurs at the promoters of a defined subset of genes involved in essential biosynthetic and metabolic pathways. These include Rps7 and Rps15, coding for ribosomal subunits, and Eif4e and Eif3s2, which are critical for translation initiation. KAT2A also acetylates promoters of mitochondrial regulators such as Lonp1 and Parl, linking histone acetylation to mitochondrial function. The presence of H3K9ac at these loci facilitates stable transcription by promoting high-frequency bursting and consistent transcription factor recruitment, including MYC and GABPA. In the absence of KAT2A, these promoters exhibit a marked loss of H3K9ac, leading to reduced transcriptional burst frequency and increased expression variability [72]. H3K27ac also plays a key role in maintaining the leukemic phenotype by driving processes such as impaired differentiation, uncontrolled cell proliferation, and resistance to apoptosis. This acetylation mark is recognized by BRD9, a bromodomain-containing subunit of the non-canonical BAF (ncBAF) chromatin remodeling complex. BRD9 binding to H3K27ac-enriched regions helps maintain open chromatin at both promoters and enhancers, ensuring the transcription of genes critical for leukemic cell identity and proliferation. Notably, targets of this regulation include oncogenes such as MYC, KIT, and BCL2, as well as AML-specific enhancer elements like the BENC super-enhancer cluster [73].

3.4 Alterations in RNA modifications

RNA modifications, collectively termed epitranscriptomics, represent a novel and critical layer of AML epigenetic deregulation [74]. The most abundant internal mRNA modification, N6-methyladenosine (m6A), is installed by the METTL3/METTL14 complex and removed by FTO or ALKBH5. METTL3 is overexpressed in AML and localizes to promoter regions of active genes, depositing m6A within coding sequences to enhance translation efficiency and alleviate ribosome stalling [75]. Targets include BCL2, MYC, and SP1, which are essential for LSC maintenance. Meanwhile, YTHDF2, a cytoplasmic m6A reader, promotes the degradation of mRNAs encoding pro-differentiation or pro-apoptotic proteins. Together, METTL3 and YTHDF2 coordinate a post-transcriptional program that sustains LSCs. Inhibition of METTL3 selectively impairs leukemic, but not normal hematopoietic, cells and has entered clinical development [76]. Furthermore, recent profiling of 2'-O-methylation on rRNA revealed dynamic methylation signatures associated with stemness. Overexpression of fibrillarin (FBL), the 2'-O-methyltransferase, reprograms rRNA methylation and enhances LSC properties, identifying translational control as an epigenetic vulnerability [77].

3.5 Alterations in ncRNA regulation

In AML, dysregulation of miRNAs, a class of regulatory non-coding RNAs, has emerged as a crucial epigenetic mechanism contributing to leukemogenesis. miRNAs typically function post-transcriptionally to fine-tune gene expression, and their aberrant expression can disrupt key signaling pathways in AML. Several tumor suppressor miRNAs are downregulated in AML, such as miR-29b, whose decreased expression correlates with DNMT3A mutation and leads to increased DNA methylation and transcriptional repression of differentiation genes [78].

Similarly, miR-34a, downregulated in patients with poor prognosis, inhibits PD-L1 and DHAC2, modulating immune evasion and leukemic stem cell survival [79]. The miR-9 downregulation observed in AML enhances CXCR4 expression, fostering increased cell migration and chemoresistance [80]. Epigenetic silencing mechanisms, such as DNA hypermethylation, are implicated in the suppression of miR-9 and miR-34a, particularly in cases with EVI1 overexpression [81]. On the other hand, some miRNAs act as oncogenes when upregulated. For instance, miR-155, frequently overexpressed in AML, downregulates SHIP1, thereby activating the PI3K/AKT pathway and promoting proliferation [82]. Similarly, miR-126 is overexpressed in inv(16) AML subtypes, enhancing leukemic cell survival, while miR-21 represses the tumor suppressor KLF5, facilitating leukemogenesis [83, 84]. Additionally, the miR-17-5p-JAK1 axis is involved in modulating apoptosis and cell migration [85]. Transcriptional regulation also plays a role, as in the lncRNA XIST/miR-142-5p/PFKP pathway, where lncRNA XIST suppresses miR-142-5p, resulting in enhanced glycolytic activity and leukemic cell proliferation [86]. Several of these miRNAs, including miR-20a-5p, miR-103a-2-5p, and miR-455-3p, regulate apoptosis, autophagy, and cell cycle checkpoints via interactions with key targets such as PPP6C, LILRB3, and UBN2, respectively [87]. The diversity of miRNA-mediated regulatory networks underscores their dual role as either oncomiRs or tumor suppressors in AML pathobiology, depending on context and molecular subtype.

In summary, AML exemplifies an epigenetically driven malignancy, where genetic mutations and metabolic alterations synergize to reprogram the epigenome and reinforce leukemic identity. Central to this reprogramming are disruptions in DNA methylation and demethylation (e.g., DNMT3A, TET2, IDH1/2), histone modifications (e.g., H3K9ac, H3K27me3, H3K79me2), chromatin remodeling complexes, and non-coding RNA pathways. These alterations converge on common downstream effects: impaired differentiation, enhanced self-renewal, transcriptional dysregulation, and resistance to apoptosis. Furthermore, the integration of RNA modifications and miRNA networks adds a critical layer of post-transcriptional regulation that sustains leukemic stem cell function (**Table 1**, **Figure 1**). Collectively, these insights highlight the multifaceted epigenetic vulnerabilities in AML and provide a strong rationale for the development of targeted epigenetic therapies.

4. Epigenetic therapies in AML

One of the main hallmarks of AML is epigenetic dysregulation involving translocations and/or epi-mutations of molecular players that block myeloid cell differentiation, leading to the accumulation of undifferentiated myeloid cells and self-renewal [88]. In this context, epigenetic therapies designed to modulate or reverse the epigenetic changes are gaining growing recognition for personalized treatments [16].

4.1 DNA methyltransferase inhibitors (DNMTi)

DNMTi, such as azacitidine and decitabine, are the main epigenetic agents used for therapy, especially in elderly patients or those who are resistant to

Epigenetic process	Principal regulators	Mechanistic consequence	References
DNA methylation	DNMT3A	Dominant-negative effect (R882), global hypomethylation, enhancer deregulation, transcriptional noise, clonal fitness.	[50, 51]
DNA demethylation	TET2	Reduced 5hmC, hypermethylation at enhancers, impaired differentiation, altered histone modifications via RNA pathways.	[54, 55]
Metabolite-dependent DNA/histone demethylation	IDH1/2, BCAT1	2HG accumulation, inhibition of TET2/Jmj-KDMs, DNA hypermethylation, stem-like transcriptional program.	[55, 58, 59]
Histone methylation	MLL fusions, DOT1L, ENL, SGF29, ASXL1, EZH2, UTX	Misplaced H3K79me2 loss of H3K4me3 and H3K27me3, deregulated HOX gene expression, and altered chromatin structure.	[61–67]
Histone acetylation	KAT6A, KAT2A, BRD9	Enhanced H3K9ac/H3K27ac, stabilized transcription, MYC activation, mitochondrial gene regulation.	[68, 71–73]
RNA modifications (Epitranscriptomics)	METTL3, YTHDF2, FBL	Enhanced translation, post-transcriptional regulation of LSC genes, and altered rRNA methylation.	[75–77]
Non-coding RNAs (miRNAs, lncRNAs)	miR-29b, miR-34a, miR-9, miR-155, miR-126, miR-21, miR-17-5p, XIST	Disrupted differentiation, immune evasion, chemoresistance, altered apoptosis, and glycolysis.	[78, 80–85, 87]

Table 1.
Main epigenetic modifications and biological consequences in AML.

intensive chemotherapy [89]. These drugs act by reducing DNA methylation levels, promoting the expression of genes involved in cell growth control and apoptosis, thus eliminating leukaemic stem cells [90]. To date, the main drugs used to reduce DNA methylation for treating AML are nucleoside analogues, such as azacitidine and decitabine, which integrate into the DNA (or mostly RNA in the case of azacitidine) of cells, irreversibly binding to DNA methyltransferases (DNMTs) and causing a general reduction in the methylation status [90]. Azacitidine and decitabine have been extensively studied and were the first internationally approved epigenetic drugs for the treatment of myelodysplastic syndromes, as well as AML with low blast counts, even though their use has been extended in combination with Venetoclax (ABT-199) for elderly patients unsuitable for aggressive chemotherapies [89]. Associated with DNMTi, Venetoclax, which inhibits the anti-apoptotic protein BCL-2 [91], promotes a metabolic reprogramming that reduces oxidative phosphorylation and specifically initiates apoptosis in leukemic cells [16]. Furthermore, DNA hypomethylating agents possess both direct and indirect immunomodulatory effects on tumor cells by enhancing the reactivation of immune-related genes, such as testicular cancer antigens and those involved in interferon signaling pathways, including the major histocompatibility complex class I, and increasing the immune response [92, 93].

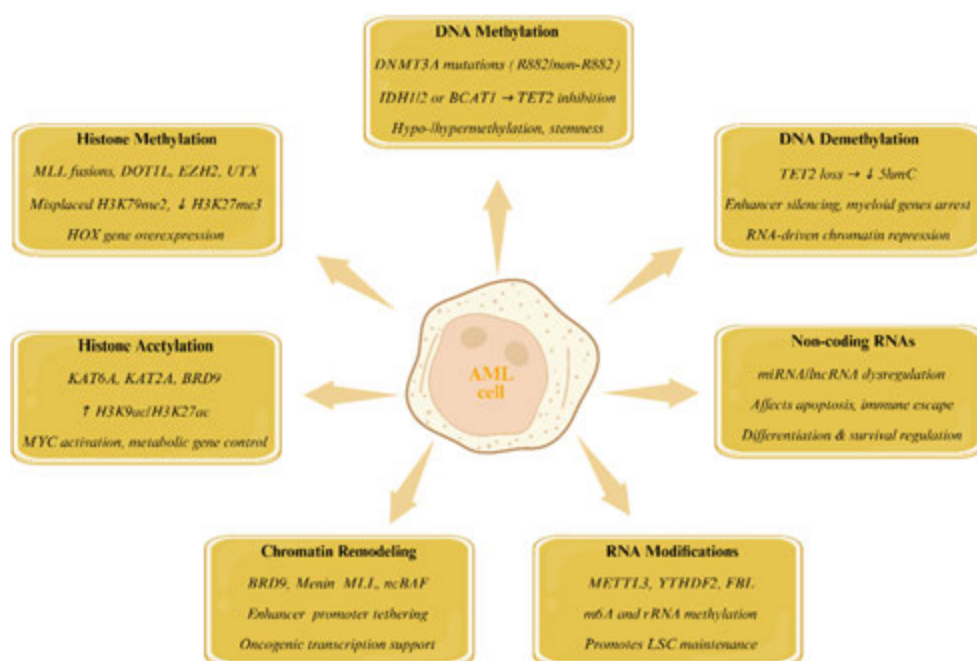


Figure 1.
Schematic representation of the main epigenetic modifications in AML.

4.2 IDH inhibitors

About 20% of patients with AML have mutations in IDH genes, but only a fraction of these respond to IDH inhibitor drugs [94]. The use of these molecular inhibitors, such as enasidenib, that is, AG-221 (for IDH2) and ivosidenib (for IDH1), reduces 2-hydroxyglutarate levels, reverses epigenetic hypermethylation, and removes the blockade of cell differentiation [95–97]. These drugs have achieved durable remissions in 20–30% of patients with relapsed or refractory AML with IDH mutations, leading to their approval by the FDA [90]. However, resistance to these therapies represents a significant challenge. The presence of concomitant mutations in NRAS or a high mutational load can cause primary resistance to enasidenib, suggesting that effective treatment also requires the elimination of tumor clones not carrying IDH mutations in AML [98]. Furthermore, the selective pressure exerted by these inhibitors may favor the emergence of resistant clones, for instance, by isoform switching between IDH1 and IDH2 mutations or by clonal evolution independent of further IDH mutations [90]. It has been reported that IDH2 inhibitor AG-221 acts on AML cells with IDH2 mutation and patient samples, showing a partial recovery of altered myeloid transcription factor activity [99]. Moreover, excessive methylation of HLA class I complex genes was detected after the treatment, which reduces their expression and promotes the activation of natural killer (NK) cells, thus increasing the immune system's ability to recognize and destroy leukaemic cells [99]. In patients who do not respond to treatment with IDH inhibitors, this hypermethylation persists, suggesting a mechanism of resistance [99].

4.3 Histone methylation-based therapies

Studies have also revealed that DOT1L, a unique histone methyl transferase (HMT) that specifically catalyzes the methylation of H3K79 [100], drives carcinogenesis through MLL fusion proteins [101]. Accordingly, the DOT1L inhibitor EPZ-5676 is also under investigation for other AML subtypes, including those harboring partial tandem duplications in the MLL-PTD region [16]. Up to now, GSK126 and EPZ-6438 (tazemetostat) are in clinical trials for hematological malignancies, including lymphomas [102]. Recently, attention has focused on molecules targeting specific epigenetic regulators implicated in AML, such as inhibitors of EZH2, a methyltransferase involved in gene repression via the PRC2 complex [103], and inhibitors of the menin protein, which interacts with MLL gene fusions typical of certain AML subtypes [104]. Some studies have demonstrated that PRC2 functions alongside MLL alterations to promote leukemia progression [105]. Based on these data, the dual EZH1/EZH2 inhibitor UNC1999 demonstrated efficacy in suppressing AML growth in MLL rearrangement models by reactivating target genes silenced by PRC2 [106]. In this context, lysine demethylase KDM1A (LSD1) leads to the removal of mono and di-methyl groups from histone H3 lysine 4 (H3K4), leading to transcriptional repression [107]. Inhibition or loss of LSD1 function has been shown to promote differentiation in AML cells, particularly in MLL-rearranged and other genetically defined subtypes [108]. Pharmacological agents targeting LSD1, such as tranylcypromine (TCP), have demonstrated the ability to induce myeloid gene expression and exhibit enhanced anti-leukemic activity in association with all-trans retinoic acid (ATRA) [109]. More recently, novel inhibitors NCD25 and NCD38 were found to suppress leukemic proliferation and induce differentiation, with NCD38 specifically reactivating enhancer regions, including super-enhancers, that regulate hematopoietic gene expression and are aberrantly silenced in AML [110].

4.4 HDAC inhibitors

A further group of compounds include histone deacetylase inhibitors (HDACi), which modify chromatin structure by increasing histone acetylation and altering the gene expression profile; however, their use as monotherapy has been limited, suggesting the need to use them in combination with other agents [90, 111]. Specifically, it has been reported that valproic acid (VPA), a short-chain fatty acid that promotes hyperacetylation of histones H3 and H4 and inhibits class I HDACs [112], exerts multiple effects on leukemic blasts, including induction of cell cycle arrest, promotion of apoptosis, and modulation of DNA repair mechanisms [113]. Beyond VPA, various HDACi (i.e., romidepsin, mocetinostat, entinostat) have undergone phase I/II evaluation for acute myeloid leukemia treatment, with enhanced efficacy observed primarily in combination with established anti-leukemic agents [114–116]. Panobinostat, which is FDA-approved for multiple myeloma [117], is currently being evaluated in clinical trials for AML. However, as a single agent, it demonstrates modest anti-leukemic efficacy in myeloid malignancies [118–120], whereas preclinical data indicate cytotoxicity when combined with other epigenetic or chemotherapeutic agents [121]. Nevertheless, recent trials combining panobinostat with intensive chemotherapy failed to improve clinical outcomes and resulted in increased toxicity [120].

4.5 BET inhibitors

Inhibitors of bromodomain and extraterminal domain (BET) proteins, which interfere with chromatin remodeling and transcriptional regulation, are emerging as potential therapeutic candidates for AML therapy [122]. Other agents commonly used for treating AML also include FLT3 inhibitors, lenalidomide, and antibody-drug conjugates [123]. BET inhibitors also play an important role in treating AML since they reduce the expression of immune checkpoint molecules such as PD-L1, thus promoting better immune surveillance against tumors [90]. Moreover, the small-molecule inhibitors JQ1 and I-BET151 have demonstrated potent efficacy in inducing cell cycle arrest and apoptosis in leukemic cells both in vitro and in vivo [124]. These agents exert their effects by displacing BRD4, thereby inhibiting RNA polymerase II-dependent transcriptional elongation of critical oncogenes, including c-MYC, BCL2, and CDK6 [125, 126]. Similarly, OTX015, another BET inhibitor, has shown the ability to induce apoptosis in several leukemic cell lines [127]. Currently, several clinical trials are underway to evaluate the efficacy of BET inhibitors; for example, the molecule RO6870810 (TEN-010), a derivative with higher stability than JQ1, has been in a phase I clinical trial for the treatment of AML and myelodysplastic syndromes (MDS) (NCT02308761). In addition, a phase II study is examining the inhibitor CPI-0610 combined with ruxolitinib in patients affected by myelofibrosis (NCT02158858). In parallel, GSK525762, a BRD4 inhibitor, has entered clinical trials for the treatment of relapsed or refractory hematological malignancies [128]. In 2018, BI-894999, a BET inhibitor characterized by a distinct chemical structure compared to other inhibitors, was identified and showed superior efficacy in suppressing the growth of AML-derived primary cells and xenograft models [129]. Interestingly, the combination with a CDK9 inhibitor, a key component of the transcriptional elongation complex, significantly amplified the antitumor activity of BI-894999 [129]. In a mouse model of AML with *MLL-AF9;Nras(G12D)* translocation, resistance to these inhibitors was associated with the remodeling of chromatin, which triggers the WNT signaling pathway [130]. A distinctive feature of these therapies is the lack of an immediate cytotoxic effect; the maximum benefit generally occurs only after several months, reflecting the involvement of the immune system [90].

4.6 miRNA-based therapies

An alternative strategy to target epigenetic dysregulation in AML involves miRNA-based therapies, which include the administration of synthetic miRNA mimics. These are double-stranded oligonucleotides delivered through viral or synthetic vectors, capable of restoring tumor-suppressor miRNA function, or antagomiRs, which are able to inhibit endogenous miRNA activity [131]. In this context, the therapeutic delivery of miR-29b, frequently dysregulated in AML blasts, via nanoparticle-based systems, has been shown to suppress leukemic cell proliferation and prolong survival in AML xenograft mouse models [132]. Reconstitution of miR-29 family members also resulted in reduced AML, accompanied by decreased splenomegaly, diminished neoplastic infiltration, and enhanced apoptotic activity in the spleen [132]. In addition, the miR-22 mimic, delivered by G7 poly(amidoamine) dendrimer nanoparticles, led to increased survival in MV4-11 xenotransplantation mouse models, as well as increased

survival in primary mouse AML (MLL-AF9 and AE9a) transplant models [133]. Another example of miRNA-based therapy is provided by the miR-181a mimic, delivered by transferrin-conjugated anionic lipid-based nanoparticles, which was able to decrease cell proliferation and colony formation in several leukemia cell lines, such as OCI-AML3, KG1a and MV4-11, as well as induce apoptosis in primary AML blasts [134]. On the other hand, *in vivo* models showed increased survival and reduced splenomegaly in NSG mice xenografted with MV4-11 cells [134]. Overexpression of miR-126 has been shown to contribute to the long-term maintenance and self-renewal of AML cells [135]. Therapeutic targeting of miR-126 by nanoparticles containing antagomiR-126 was shown to decrease long-term colony formation and self-renewal capacity *in vitro* and increase survival in NSG mice engrafted with human AML primary blasts and the MLL FLT3-ITD mouse model [135]. Similarly, miR-21/miR-196b antagomiRs, administered via implanted osmotic pumps, decreased colony formation in HOXA9, Nup98-HOXA9, or MLL-AF9-transduced mouse Lin⁻ bone marrow cells and increased survival in combination with induction chemotherapy in the MLL-AF9 xenograft model [136, 137].

4.7 Radiotherapy and other epi-treatments

Radiotherapy is also used for AML treatment, mainly as an adjunct to bone marrow or hematopoietic stem cell transplantation [138]. This treatment serves to eliminate diseased cells and create space for healthy cells, thus improving the chances of a successful transplant [139]. However, radiotherapy can cause significant side effects, including damage to healthy tissue and an increased risk of developing secondary forms of leukemia [140]. Treatment-associated acute myeloid leukemia (t-AML), which occurs as a late consequence of chemotherapy (ct-AML), radiotherapy (rt-AML), or immunosuppressive therapies, has been shown to be characterized by a general reduction in methylation in non-coding regions of DNA due to radiation exposure, together with an increase in methylation in promoters of disease-specific genes [141]. Validation by pyrosequencing techniques on additional samples confirmed high methylation in the promoters of the MEST and GATA5 genes in rt-AML, in contrast to other AML types and control subjects [141]. In summary, epigenetic therapies for AML are constantly evolving, and the integration of different epigenetic-based therapeutic strategies, especially in combination with conventional treatments, represents a promising approach to improve therapeutic efficacy and limit adverse effects for patient's outcomes. Genetic alterations involving MLL, such as translocations with various partner genes and MLL partial tandem duplications (MLL-PTD), are strongly associated with poor clinical outcomes in AML patients [142]. In response to this therapeutic challenge, a range of small-molecule inhibitors targeting MLL complexes have been developed, and among them, MM-401 has shown promising efficacy by specifically blocking the MLL-WDR5 interaction, halting myeloid cell proliferation, and inducing differentiation of leukemic cells without affecting normal hematopoietic stem and progenitor cells [143]. Furthermore, high-throughput screening has led to the identification of potent and selective compounds such as MIV-6 R, MI-463, and MI-503, which have demonstrated robust anti-leukemic activity in MLL-rearranged leukemia models both *in vitro* and *in vivo* [144] (Table 2, Figure 2).

Epidrug	Molecular targets	Mechanism of action	References
Azacitidine/ decitabine	DNMTs	Reduces DNA methylation, promotes the expression of tumor suppressor genes.	[89]
Venetoclax (ABT-199)	BCL-2	Induces metabolic rewiring and selective apoptosis in leukemic cells.	[91]
Enasidenib (AG-221)	IDH2	Reduces 2-HG, reverses hypermethylation, and differentiation block.	[95]
Ivosidenib	IDH1	Reduces 2-HG, reverses hypermethylation, and differentiation block.	[96]
MM-401	MLL-WDR5	Blocks MLL-WDR5 interaction, halts proliferation, and induces differentiation.	[143]
EPZ-5676	DOT1L	Inhibits H3K79 methylation, targets MLL fusion proteins.	[16, 101]
UNC1999	EZH1/EZH2	Suppresses AML growth by reactivating PRC2-silenced genes.	[106]
Tranylcypromine (TCP)	LSD1 (KDM1A)	Promotes differentiation of blasts, enhanced with ATRA.	[109]
NCD38	LSD1	Reactivates super-enhancers and induces differentiation of blasts.	[110]
Valproic acid	HDACs (Class I)	Histone hyperacetylation induces apoptosis and DNA repair.	[145]
Romidepsin	HDACs	HDAC inhibition, evaluated in combination therapies.	[114]
Panobinostat	HDACs	Limited monotherapy effect; cytotoxic in combinations.	[120]
JQ1	BRD4	Blocks BRD4, suppresses c-MYC, BCL2, and CDK6, and induces apoptosis.	[120, 125]
I-BET151	BRD4	Promotes apoptosis in MLL-rearranged AML.	[124, 125]
OTX015	BRD4	Induces apoptosis in leukemic cell lines.	[127]
RO6870810 (TEN-010)	BRD4	BET inhibitor tested in refractory AML.	NCT02308761 (Phase I)
CPI-0610	BRD4	BET inhibitors with ruxolitinib in myelofibrosis.	NCT02158858 (Phase II)
GSK525762	BRD4	Early clinical trials for relapsed/refractory AML.	[143]
BI-894999	BRD4	Suppresses AML growth; enhanced with CDK9 inhibitor.	[129]
miR29-b mimic	DNMT3A, DNMT3B, DNMT1, SP1, CDK6, FLT3, KIT	Reconstitution of miR-29 family activity suppresses leukemic cell proliferation and prolongs survival in AML xenograft mouse models.	[132]
miR-22 mimic	CRTC1, FLT3, MYCBP	Increase survival in MV4-11 xenotransplantation mouse models; increase survival in primary mouse AML transplant models.	[133, 137]
miR-181a mimic	KRAS, NRAS, MAPK1	Decrease cell proliferation as well as colony-forming in leukemic cell lines and AML blasts; increased survival and reduced splenomegaly in vivo.	[134, 137]

Epidrug	Molecular targets	Mechanism of action	References
miR-126 antagomiR	MMP7, CHD7, JAG1	Decrease long-term colony formation and self-renewal capacity in vitro; increased survival in AML mouse models.	[135]
miR-21/196b antagomiRs	N/A	Decreased colony formation in vitro; increased survival in combination with chemotherapy in the MLL-AF9 xenograft model.	[136, 137]

Table 2.
Summary of the current epigenetic therapies used for treating AML.

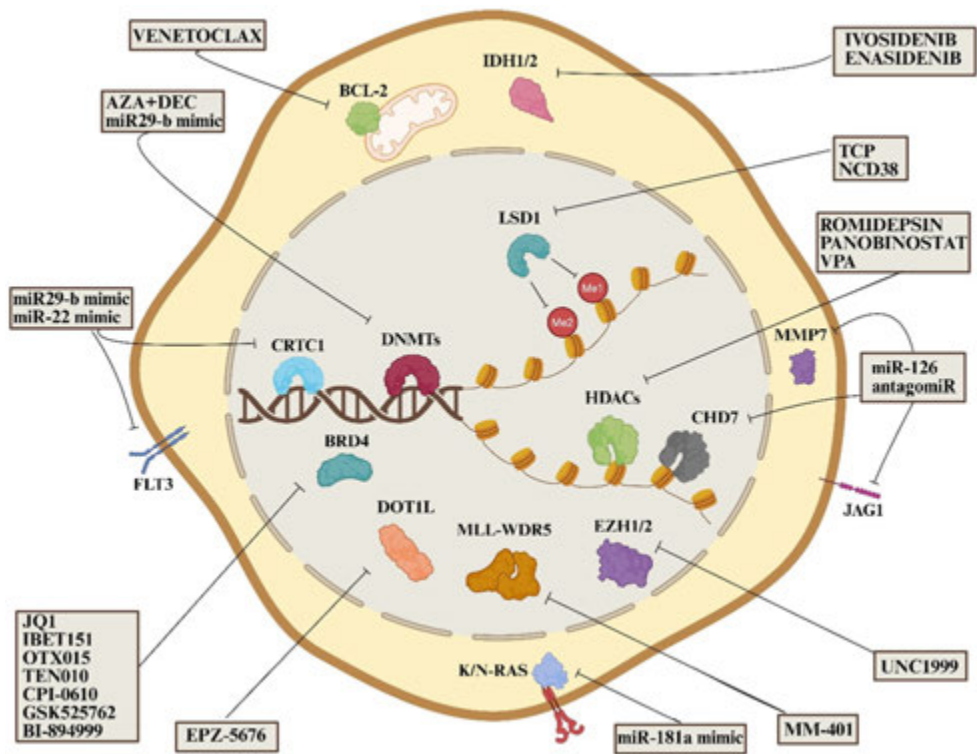


Figure 2.
Representation of the main epi-drugs and their targets used for treating AML.

5. Conclusion(s)

Epigenetic dysregulation plays a pivotal role in the pathogenesis of AML. In recent years, great strides have been made to understand how epigenetic modifications are involved in the initiation and development of AML and how these epi-changes can be modulated to improve patients' prognosis. Epigenetic changes are often early events in leukemic evolution and may cooperate with additional genetic lesions to drive disease progression [146]. Specifically, alterations in DNA methylation, histone modifications, chromatin remodeling, and RNA-based regulatory mechanisms contribute to leukemic initiation, maintenance, as well as

therapy resistance [147]. In contrast to permanent genetic mutations, epigenetic alterations can potentially be reversed, presenting a distinct therapeutic opportunity for treating AML patients. In this regard, the use of epi-drugs capable of modulating and/or restoring the expression of genes profoundly dysregulated in AML offers a unique therapeutic strategy by limiting the adverse effects of common therapies such as chemotherapy or radiotherapy [148]. The ability of *epi-drugs* to modulate chromatin states, reactivate silenced tumor suppressor genes, and enhance immune recognition highlights the therapeutic versatility of epigenetic approaches. In particular, these agents, used as monotherapy or in combination with major conventional therapies, are able to restore the expression of genes implicated in the main deregulated processes involved in the development of AML, such as cell differentiation, cell death, and evasion of the immune response, leading to a clinical improvement for the patients. As our understanding of AML's epigenetic complexity deepens, integrating epigenetic therapies into personalized treatment regimens holds great promise to improve patient outcomes and overcome current therapeutic limitations. Understanding how epigenetic drugs, alone or in combination with other agents, can restore the expression of certain genes by modulating chromatin accessibility and inducing physiological processes during AML could help to better define the molecular processes underlying the onset of leukemia and potentially overcome resistance to therapy, thereby improving AML patient outcomes.

Acronyms and abbreviations

PTMs	Post-translational histone modifications
miRNA	microRNA
siRNA	Small-interfering RNA
lncRNA	Long non-coding RNA
ncRNAs	Non-coding RNAs
AML	Acute myeloid leukemia
DNMT3A	DNA methyltransferase 3A
TET2	Ten-eleven translocation methylcytosine dioxygenase 2
IDH1/2	Isocitrate dehydrogenase 1 and 2
EZH2	Enhancer of zeste homolog 2
WHO	World Health Organization
ICC	International Consensus Classification
dn-AML	De novo acute myeloid leukemia
s-AML	Secondary acute myeloid leukemia
t-AML	Therapy-related acute myeloid leukemia
HSCs	Hematopoietic stem cells
OS	Overall survival
MDS	Myelodysplastic syndrome
MPNs	Myeloproliferative neoplasms
TP53	Tumor protein p53
FLT3	FMS-related tyrosine kinase 3
LSCs	Leukemic stem cells
NPM1	Nucleophosmin 1
ITD	Internal tandem duplication
KMT2A	Lysine methyltransferase 2A

HOXA9	Homeobox A9
MEIS	Meis homeobox
CD123	Alpha subunit of interleukin-3 receptor
IL-3R	Interleukin-3 receptor
α -KG	Alpha-ketoglutarate
2-HG	2-hydroxyglutarate
HSPCs	Hematopoietic stem and progenitor cells
NPM1c	Nucleophosmin 1 mutant
5mC	5-methylcytosine
5hmC	5-hydroxymethylcytosine
m5C	5-methylcytosine (RNA modification)
MBD6	Methyl-CpG binding domain protein
H2AK119ub	Histone H2A lysine 119 ubiquitination
Jmj-KDMs	Jumonji-domain containing histone demethylases
BCAT1	Branched-chain amino acid transaminase 1
MLL	Mixed lineage leukemia
DOT1L	Disruptor of telomeric silencing 1-like
LEDGF	Lens epithelium-derived growth factor
MEN1	Multiple endocrine neoplasia type 1
ENL	Eleven-nineteen leukemia protein
DHAC2	Dihydroorotate dehydrogenase
CXCR4	C-X-C chemokine receptor type 4
SHIP1	Src homology 2 domain-containing inositol 5'-phosphatase 1
PI3K/AKT	Phosphoinositide 3-kinase/AKT kinase signaling pathway
KLF5	Kruppel-like factor 5
JAK1	Janus kinase 1
XIST	X-inactive specific transcript
PFKP	Phosphofructokinase platelet
PPP6C	Protein phosphatase 6 catalytic subunit
LILRB	Leukocyte immunoglobulin-like receptor B3
UBN2	Ubinuclein 2
HDACi	Histone deacetylase inhibitors
BET	Bromodomain and Extra terminal domain proteins
DNMTi	DNA methyltransferase inhibitors
FDA	Food and Drug Administration
NRAS	Neuroblastoma RAS viral oncogene homolog
HLA	Human leukocyte antigen
PRC2	Polycolomb repressive complex 2
KDM6A	Lysine demethylase 6A
H3K27ac	Histone H3 lysine 27 acetylation
H3K9ac	Histone H3 lysine 9 acetylation
BRD4	Bromodomain containing protein 4
KAT6A	Lysine acetyltransferase 6A
KAT2A	Lysine acetyltransferase 2A
MYC	MYC-protoncogene
GABPA	GA-binding protein transcription factor alpha subunit
BRD9	Bromodomain-containing protein 9
ncBAF	Non-canonical BRG1/BRM-associated factor (chromatin-remodeling complex)

BCL-2	B-cell lymphoma 2
BENC	Bivalent enhancer cluster
m6A	N6-methyladenosine (RNA modification)
METTL3	Methyltransferase-like 3
METTL14	Methyltransferase-like 14
FTO	Fat mass and obesity-associated protein
ALKBH5	AlkB homolog 5
YTHDF	YTH N6-methyladenosine RNA-binding protein 2
FBL	Fibrillarin (2'-O-methyltransferase)
PD-L1	Programmed death-ligand 1
NK	Natural killer
MLL-PTD	MLL partial tandem duplication
WDR5	WD repeat domain 5
HMT	Histone methyltransferase
KDM1A/LSD1	Lysine demethylase 1
TCP	Tranylcypromine
VPA	Valproic acid
CDK6	Cyclin-dependent kinase 6
WNT	Wingless-related integration site
t-aml	Treatment-associated AML
ct-aml	Chemotherapy-associated AML
rt-aml	Radiotherapy-associated AML
miR-29b	MicroRNA-29b
miR-22	MicroRNA-22
miR-181a	MicroRNA-181a
miR-126	MicroRNA-126
miR-21	MicroRNA-21
miR-196b	MicroRNA-196b

Author contributions

Vincenzo Carafa (VC) and Lucia Altucci (LA) supervised the general concept of this review. Antonio Beato (AB), Gregorio Favale (GF), and Daniela Carannante (DC) reviewed the main literature and wrote the manuscript. All authors contributed to the editing process. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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References

- [1] Zhang L, Lu Q, Chang C. Epigenetics in health and disease. *Advances in Experimental Medicine and Biology*. 2020;**1253**:3–55. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/32445090>
- [2] Klibaner-Schiff E, Simonin EM, Akdis CA, Cheong A, Johnson MM, Karagas MR, et al. Environmental exposures influence multigenerational epigenetic transmission. *Clinical Epigenetics*. 2024;**16**(1):145. DOI: 10.1186/s13148-024-01762-3
- [3] Jurkowska RZ. Role of epigenetic mechanisms in the pathogenesis of chronic respiratory diseases and response to inhaled exposures: From basic concepts to clinical applications. *Pharmacology & Therapeutics*. 2024;**264**:108732. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0163725824001529>
- [4] Gillette TG, Hill JA. Readers, writers, and erasers: Chromatin as the whiteboard of heart disease. *Circulation Research*. 2015;**116**(7):1245–1253. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/25814685>
- [5] Tyagi M, Imam N, Verma K, Patel AK. Chromatin remodelers: We are the drivers!! *Nucleus*. 2016;**7**(4):388–404. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/27429206>
- [6] Ling C, Rönn T. Epigenetics in human obesity and type 2 diabetes. *Cell Metabolism*. 2019;**29**(5):1028–1044. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/30982733>
- [7] Zhou S, Liu J, Wan A, Zhang Y, Qi X. Epigenetic regulation of diverse cell death modalities in cancer: A focus on pyroptosis, ferroptosis, cuproptosis, and disulfidptosis. *Journal of Hematology & Oncology*. 2024;**17**(1):22. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/38654314>
- [8] Milicic L, Porter T, Vacher M, Laws SM. Utility of DNA methylation as a biomarker in aging and Alzheimer's disease. *Journal of Alzheimer's Disease Reports*. 2023;**7**(1):475–503. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/37313495>
- [9] Casari G, Romaldi B, Scirè A, Minnelli C, Marzioni D, Ferretti G, et al. Epigenetic properties of compounds contained in functional foods against cancer. *Biomolecules*. 2024;**15**(1):15. Available from: <https://www.mdpi.com/2218-273X/15/1/15>
- [10] Farsetti A, Illi B, Gaetano C. How epigenetics impacts on human diseases. *European Journal of Internal Medicine*. 2023;**114**:15–22. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0953620523001930>
- [11] Castillo-Fernandez JE, Spector TD, Bell JT. Epigenetics of discordant monozygotic twins: Implications for disease. *Genome Medicine*. 2014;**6**(7):60. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/25484923>
- [12] Romer-Seibert JS, Meyer SE. Genetic heterogeneity and clonal evolution in acute myeloid leukemia. *Current Opinion in Hematology*. 2021;**28**(1):64–70. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/33186150>
- [13] Conway O'Brien E, Prideaux S, Chevassut T. The epigenetic landscape

- of acute myeloid leukemia. *Advances in Hematology*. 2014;**2014**:103175. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/24778653>
- [14] Schrezenmeier J, Huntly BJP. Epigenetic dysregulation in acute myeloid leukemia. *Seminars in Hematology*. 2025;**62**:177–186. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0037196325000289>
- [15] Goyama S, Kitamura T. Epigenetics in normal and malignant hematopoiesis: An overview and update 2017. *Cancer Science*. 2017;**108**(4):553–562. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28100030>
- [16] Gambacorta V, Gnani D, Vago L, Di Micco R. Epigenetic therapies for acute myeloid leukemia and their immune-related effects. *Frontiers in Cell and Developmental Biology*. 2019;**7**:207. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/31681756>
- [17] Bukhari SNA. Emerging nanotherapeutic approaches to overcome drug resistance in cancers with update on clinical trials. *Pharmaceutics*. 2022;**14**(4). Available from: <https://www.ncbi.nlm.nih.gov/pubmed/35456698>
- [18] Shimony S, Stahl M, Stone RM. Acute myeloid leukemia: 2023 update on diagnosis, risk-stratification, and management. *American Journal of Hematology*. 2023;**98**(3):502–526. DOI: 10.1002/ajh.26822
- [19] Debnath A, Nath S. Prognosis and treatment in acute myeloid leukemia: A comprehensive review. *Egyptian Journal of Medical Human Genetics*. 2024;**25**(1):91. Available from: <https://jmhg.springeropen.com/articles/10.1186/s43042-024-00563-w>
- [20] Wachter F, Pikman Y. Pathophysiology of acute myeloid leukemia. *Acta Haematologica*. 2024;**147**(2):229–246. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/38228114>
- [21] Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka H-M, et al. International consensus classification of myeloid neoplasms and acute leukemias: Integrating morphologic, clinical, and genomic data. *Blood*. 2022;**140**(11):1200–1228. Available from: <https://ashpublications.org/blood/article/140/11/1200/485730/International-Consensus-Classification-of-Myeloid>
- [22] Venier LM, Patriquin CJ, Warkentin TE. Acute thrombocytopenia suggesting thrombotic microangiopathy. *American Journal of Hematology*. 2022;**97**(5):659–665. DOI: 10.1002/ajh.26515
- [23] Goel H, Rahul E, Gupta I, Chopra A, Ranjan A, Gupta AK, et al. Molecular and genomic landscapes in secondary & therapy related acute myeloid leukemia. *American Journal of Blood Research*. 2021;**11**(5):472–497. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/34824881>
- [24] Kirtonia A, Pandya G, Sethi G, Pandey AK, Das BC, Garg M. A comprehensive review of genetic alterations and molecular targeted therapies for the implementation of personalized medicine in acute myeloid leukemia. *Journal of Molecular Medicine*. 2020;**98**(8):1069–1091. DOI: 10.1007/s00109-020-01944-5
- [25] Khwaja A, Bjorkholm M, Gale RE, Levine RL, Jordan CT, Ehninger G, et al. Acute myeloid leukaemia. *Nature Reviews Disease Primers*. 2016;**2**

- (1):16010. Available from: <https://www.nature.com/articles/nrdp201610>
- [26] Estey E, Hasserjian RP, Döhner H. Distinguishing AML from MDS: A fixed blast percentage may no longer be optimal. *Blood*. 2022;**139**(3):323–332. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/34111285>
- [27] Finlay CA, Hinds PW, Levine AJ. The p53 proto-oncogene can act as a suppressor of transformation. *Cell*. 1989;**57**(7):1083–1093. Available from: <https://linkinghub.elsevier.com/retrieve/pii/0092867489900457>
- [28] Shin D-Y. TP53 mutation in acute myeloid leukemia: An old foe revisited. *Cancers (Basel)*. 2023;**15**(19):4816. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/37835510>
- [29] Daver N, Schlenk RF, Russell NH, Levis MJ. Targeting FLT3 mutations in AML: Review of current knowledge and evidence. *Leukemia*. 2019;**33**(2):299–312. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/30651634>
- [30] Alotaibi AS, Yilmaz M, Kanagal-Shamanna R, Loghavi S, Kadia TM, DiNardo CD, et al. Patterns of resistance differ in patients with acute myeloid leukemia treated with type I versus type II FLT3 inhibitors. *Blood Cancer Discovery*. 2021;**2**(2):125–134. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/33681815>
- [31] Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;**111**(4):424–447. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/27895058>
- [32] Im AP, Sehgal AR, Carroll MP, Smith BD, Tefferi A, Johnson DE, et al. DNMT3A and IDH mutations in acute myeloid leukemia and other myeloid malignancies: Associations with prognosis and potential treatment strategies. *Leukemia*. 2014;**28**(9):1774–1783. Available from: <https://www.nature.com/articles/leu2014124>
- [33] Ward PS, Lu C, Cross JR, Abdel-Wahab O, Levine RL, Schwartz GK, et al. The potential for isocitrate dehydrogenase mutations to produce 2-hydroxyglutarate depends on allele specificity and subcellular compartmentalization. *Journal of Biological Chemistry*. 2013;**288**(6):3804–3815. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0021925820463410>
- [34] Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Collier HA, et al. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting α -ketoglutarate to 2-hydroxyglutarate. *Cancer Cell*. 2010;**17**(3):225–234. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S153561081000036X>
- [35] Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell*. 2010;**18**(6):553–567. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1535610810004836>
- [36] Chan SM, Thomas D, Corces-Zimmerman MR, Xavy S, Rastogi S, Hong W-J, et al. Isocitrate dehydrogenase 1 and 2 mutations induce BCL-2 dependence in acute myeloid leukemia. *Nature Medicine*. 2015;**21**

(2):178–184. Available from: <https://www.nature.com/articles/nm.3788>

[37] La P, Desmond A, Hou Z, Silva AC, Schnepf RW, Hua X. Tumor suppressor menin: The essential role of nuclear localization signal domains in coordinating gene expression. *Oncogene*. 2006;**25**(25):3537–3546. Available from: <https://www.nature.com/articles/1209400>

[38] Krivtsov AV, Armstrong SA. MLL translocations, histone modifications and leukaemia stem-cell development. *Nature Reviews Cancer*. 2007;**7**(11):823–833. Available from: <https://www.nature.com/articles/nrc2253>

[39] Meyer C, Kowarz E, Hofmann J, Fischer T, El AS, Teigler-Schlegel A, et al. New insights to the MLL recombinome including 8 novel partner genes ACTN4, C2CD3, DCP1A, FLNA, LAMC3, LOC100128568, NRIP3, and TNRC18. *Blood*. 2008;**112**(11):2047–2047. Available from: <https://ashpublications.org/blood/article/112/11/2047/57641/New-Insights-to-the-MLL-Recombinome-Including-8>

[40] Sun Y, Zhou B, Mao F, Xu J, Miao H, Zou Z, et al. HOXA9 reprograms the enhancer landscape to promote leukemogenesis. *Cancer Cell*. 2018;**34**(4):643–658.e5. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1535610818303787>

[41] Issa GC, Zarka J, Sasaki K, Qiao W, Pak D, Ning J, et al. Predictors of outcomes in adults with acute myeloid leukemia and KMT2A rearrangements. *Blood Cancer Journal*. 2021;**11**(9):162. Available from: <https://www.nature.com/articles/s41408-021-00557-6>

[42] Falini B. NPM1-mutated acute myeloid leukemia: New pathogenetic

and therapeutic insights and open questions. *American Journal of Hematology*. 2023;**98**(9):1452–1464. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/37317978>

[43] Testa U, Pelosi E, Frankel A. CD 123 is a membrane biomarker and a therapeutic target in hematologic malignancies. *Biomarker Research*. 2014;**2**(1):4. Available from: <https://biomarkerres.biomedcentral.com/articles/10.1186/2050-7771-2-4>

[44] Jordan C, Upchurch D, Szilvassy S, Guzman M, Howard D, Pettigrew A, et al. The interleukin-3 receptor alpha chain is a unique marker for human acute myelogenous leukemia stem cells. *Leukemia*. 2000;**14**(10):1777–1784. Available from: <https://www.nature.com/articles/2401903>

[45] Testa U, Riccioni R, Militi S, Coccia E, Stellacci E, Samoggia P, et al. Elevated expression of IL-3R α in acute myelogenous leukemia is associated with enhanced blast proliferation, increased cellularity, and poor prognosis. *Blood*. 2002;**100**(8):2980–2988. Available from: [https://ashpublications.org/blood/article/100/8/2980/106412/Elevated-expression-of-IL3R \$\alpha\$ -in-acute-myelogenous](https://ashpublications.org/blood/article/100/8/2980/106412/Elevated-expression-of-IL3R%26alpha-in-acute-myelogenous)

[46] Wysota M, Konopleva M, Mitchell S. Novel therapeutic targets in Acute Myeloid Leukemia (AML). *Current Oncology Reports*. 2024;**26**(4):409–420. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/38502417>

[47] Snaith O, Poveda-Rogers C, Laczko D, Yang G, Morrisette JJD. Cytogenetics and genomics of acute myeloid leukemia. *Best Practice & Research Clinical Haematology*. 2024;**37**(1):101533. Available from:

<https://www.ncbi.nlm.nih.gov/pubmed/38490763>

[48] Liu X-L, Liu H-Q, Li J, Mao C-Y, He J-T, Zhao X. Role of epigenetic in leukemia: From mechanism to therapy. *Chemico-Biological Interactions*. 2020;**317**:108963. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/31978391>

[49] Goldman SL, Hassan C, Khunte M, Soldatenko A, Jong Y, Afshinnkoo E, et al. Epigenetic modifications in acute myeloid leukemia: Prognosis, treatment, and heterogeneity. *Frontiers in Genetics*. 2019;**10**:133. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/30881380>

[50] Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, et al. DNMT3A mutations in acute myeloid leukemia. *New England Journal of Medicine*. 2010;**363**(25):2424–2433. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/21067377>

[51] Russler-Germain DA, Spencer DH, Young MA, Lamprecht TL, Miller CA, Fulton R, et al. The R882H DNMT3A mutation associated with AML dominantly inhibits wild-type DNMT3A by blocking its ability to form active tetramers. *Cancer Cell*. 2014;**25**(4): 442–454. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/24656771>

[52] Potluri LB, Dhillon V, Aguilar J, Nanja Reddy S, Padmanabhan D, Dyson G, et al. Differential impact of domain stratified non-R882 DNMT3A mutations in myeloid neoplasms. *Blood*. 2023;**142**(Supplement 1):1853–1853. Available from: <https://ashpublications.org/blood/article/142/Supplement1/1853/504888/Differential-Impact-of-Domain-Stratified-Non-R882>

[53] Tavakoli P, Bywater M, Straube J, Ling V, Cooper E, Haldar R, et al. Dnmt3a mutation promotes expansion of a quiescent myeloid progenitor population in Npm1c-Flt3ITD acute myeloid leukemia. *Blood*. 2024;**144** (Supplement 1):1347–1347. Available from: <https://ashpublications.org/blood/article/144/Supplement1/1347/531869/Dnmt3a-Mutation-Promotes-Expansion-of-a-Quiescent>

[54] Pan X, Chang Y, Ruan G, Zhou S, Jiang H, Jiang Q, et al. TET2 mutations contribute to adverse prognosis in acute myeloid leukemia (AML): Results from a comprehensive analysis of 502 AML cases and the beat AML public database. *Clinical and Experimental Medicine*. 2024;**24**(1):35. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/38349460>

[55] Li Y, Xue M, Deng X, Dong L, Nguyen LXT, Ren L, et al. TET2-mediated mRNA demethylation regulates leukemia stem cell homing and self-renewal. *Cell Stem Cell*. 2023;**30** (8):1072–1090.e10. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/37541212>

[56] Zou Z, Dou X, Li Y, Zhang Z, Wang J, Gao B, et al. RNA m5C oxidation by TET2 regulates chromatin state and leukaemogenesis. *Nature*. 2024;**634**(8035):986–994. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/39358506>

[57] Ko M, Huang Y, Jankowska AM, Pape UJ, Tahiliani M, Bandukwala HS, et al. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. *Nature*. 2010;**468** (7325):839–843. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/21057493>

- [58] Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell*. 2010;**18**(6):553–567. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/21130701>
- [59] Raffel S, Falcone M, Kneisel N, Hansson J, Wang W, Lutz C, et al. BCAT1 restricts α KG levels in AML stem cells leading to IDHmut-like DNA hypermethylation. *Nature*. 2017;**551**(7680):384–388. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/29144447>
- [60] Wong N-HM, Cwe S. Novel therapeutic strategies for MLL-rearranged leukemias. *Biochimica Et Biophysica Acta Gene Regulatory Mechanisms*. 2020;**1863**(9):194584. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/32534041>
- [61] Konoplev S, Wang X, Tang G, Li S, Wang W, Xu J, et al. Comprehensive immunophenotypic study of acute myeloid leukemia with KMT2A (MLL) rearrangement in adults: A single-institution experience. *Cytometry Part B: Clinical Cytometry*. 2022;**102**(2):123–133. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/34964255>
- [62] Chen C-W, Armstrong SA. Targeting DOT1L and HOX gene expression in MLL-rearranged leukemia and beyond. *Experimental Hematology*. 2015;**43**(8):673–684. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/26118503>
- [63] Yokoyama A, Cleary ML. Menin critically links MLL proteins with LEDGF on cancer-associated target genes. *Cancer Cell*. 2008;**14**(1):36–46. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/18598942>
- [64] Liu Y, Li Q, Song L, Gong C, Tang S, Budinich KA, et al. Condensate-promoting ENL mutation drives tumorigenesis in vivo through dynamic regulation of histone modifications and gene expression. *Cancer Discovery*. 2024;**14**(8):1522–1546. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/38655899>
- [65] Barbosa K, Deshpande A, Perales M, Xiang P, Murad R, Pramod AB, et al. Transcriptional control of leukemogenesis by the chromatin reader SGF29. *Blood*. 2024;**143**(8):697–712. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/38048593>
- [66] Abdel-Wahab O, Adli M, LaFave LM, Gao J, Hricik T, Shih AH, et al. ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. *Cancer Cell*. 2012;**22**(2):180–193. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/22897849>
- [67] Gozdecka M, Meduri E, Mazan M, Tzelepis K, Dudek M, Knights AJ, et al. UTX-mediated enhancer and chromatin remodeling suppresses myeloid leukemogenesis through noncatalytic inverse regulation of ETS and GATA programs. *Nature Genetics*. 2018;**50**(6):883–894. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/29736013>
- [68] Jiang Y, Hu T, Wang T, Shi X, Kitano A, Eagle K, et al. AMP-activated protein kinase links acetyl-CoA homeostasis to BRD4 recruitment in acute myeloid leukemia. *Blood*. 2019;**134**(24):2183–2194. Available

from: <https://www.ncbi.nlm.nih.gov/pubmed/31697807>

[69] Sheridan M, Maqbool MA, Largeot A, Clayfield L, Xu J, Moncaut N, et al. The small inhibitor WM-1119 effectively targets KAT6A-rearranged AML, but not KMT2A-rearranged AML, despite shared KAT6 genetic dependency. *Journal of Hematology & Oncology*. 2024;**17**(1):91. DOI: 10.1186/s13045-024-01610-0

[70] Salarpour F, Goudarzipour K, Mohammadi MH. Transcription factors LEF1, PU.1 and IRF8 have decreased expression levels in majority of de novo acute myeloid leukemia patients. *World Family Medicine Journal/Middle East Journal of Family Medicine*. 2018;**16**(2):207–217. Available from: <https://plattform.almanhal.com/CrossRef/Preview/?ID=2-117372>

[71] Yan F, Li J, Milosevic J, Petroni R, Liu S, Shi Z, et al. KAT6A and ENL form an epigenetic transcriptional control module to drive critical leukemogenic gene-expression programs. *Cancer Discovery*. 2022;**12**(3):792–811. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/34853079>

[72] Domingues AF, Kulkarni R, Giotopoulos G, Gupta S, Vinnenberg L, Arede L, et al. Loss of Kat2a enhances transcriptional noise and depletes acute myeloid leukemia stem-like cells. *Elife*. 2020;**9**. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/31985402>

[73] Klein DC, Lardo SM, Hainer SJ. The ncBAF complex regulates transcription in AML through H3K27ac sensing by BRD9. *Cancer Research Communications*. 2024;**4**(1):237–252. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/38126767>

[74] Pauli C, Kienhöfer M, Göllner S, Müller-Tidow C. Epitranscriptomic modifications in acute myeloid leukemia: M 6 A and 2'-O-methylation as targets for novel therapeutic strategies. *Biological Chemistry*. 2021;**402**(12):1531–1546. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/34634841>

[75] Li M, Ye J, Xia Y, Li M, Li G, Hu X, et al. METTL3 mediates chemoresistance by enhancing AML homing and engraftment via ITGA4. *Leukemia*. 2022;**36**(11):2586–2595. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/36266324>

[76] Sang L, Wu X, Yan T, Naren D, Liu X, Zheng X, et al. The m 6 A RNA methyltransferase METTL3/METTL14 promotes leukemogenesis through the mdm2/p53 pathway in acute myeloid leukemia. *Journal of Cancer*. 2022;**13**(3):1019–1030. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/35154467>

[77] Luo H, Kharas MG. Dotting out AML by targeting fibrillarin. *Cancer Research*. 2024;**84**(17):2759–2760. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/38924716>

[78] Garzon R, Liu S, Fabbri M, Liu Z, Heaphy CEA, Callegari E, et al. MicroRNA-29b induces global DNA hypomethylation and tumor suppressor gene reexpression in acute myeloid leukemia by targeting directly DNMT3A and 3B and indirectly DNMT1. *Blood*. 2009;**113**(25):6411–6418. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/19211935>

[79] Wang X, Li J, Dong K, Lin F, Long M, Ouyang Y, et al. Tumor suppressor miR-34a targets PD-L1 and functions as a potential

immunotherapeutic target in acute myeloid leukemia. *Cellular Signalling*. 2015;**27**(3):443–452. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/25499621>

[80] Zhu B, Xi X, Liu Q, Cheng Y, Yang H. MiR-9 functions as a tumor suppressor in acute myeloid leukemia by targeting CX chemokine receptor 4. *American Journal of Translational Research*. 2019;**11**(6):3384–3397. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/31312352>

[81] Senyuk V, Zhang Y, Liu Y, Ming M, Premanand K, Zhou L, et al. Critical role of miR-9 in myelopoiesis and EVI1-induced leukemogenesis. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;**110**(14):5594–5599. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/23509296>

[82] Xue H, Hua L-M, Guo M, Luo J-M. SHIP1 is targeted by miR-155 in acute myeloid leukemia. *Oncology Reports*. 2014;**32**(5):2253–2259. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/25175984>

[83] Zhang L, Nguyen LXT, Chen Y-C, Wu D, Cook GJ, Hoang DH, et al. Targeting miR-126 in inv(16) acute myeloid leukemia inhibits leukemia development and leukemia stem cell maintenance. *Nature Communications*. 2021;**12**(1):6154. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/34686664>

[84] Li C, Yan H, Yin J, Ma J, Liao A, Yang S, et al. MicroRNA-21 promotes proliferation in acute myeloid leukemia by targeting krüppel-like factor 5. *Oncology Letters*. 2019;**18**(3):3367–3372. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/31452816>

[85] Liu M, Yu B, Tian Y, Li F. Regulatory function and mechanism research for m6A modification WTAP via SUCLG2-AS1- miR-17-5p-JAK1 axis in AML. *BMC Cancer*. 2024;**24**(1):98. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/38233760>

[86] Jiang Z, Liu T, Wang Y, Li J, Guo L. Effect of lncRNA XIST on acute myeloid leukemia cells via miR-142-5p-PFKP axis. *Hematology*. 2024;**29**(1):2306444. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/38305210>

[87] Wiśnik A, Jarych D, Krawiec K, Strzałka P, Potocka N, Czemerska M, et al. Role of microRNAs in acute myeloid leukemia. *Genes (Basel)*. 2025;**16**(4):446. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/40282406>

[88] Fennell KA, Bell CC, Dawson MA. Epigenetic therapies in acute myeloid leukemia: Where to from here? *Blood*. 2019;**134**(22):1891–1901. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/31697822>

[89] Short NJ, Kantarjian H. Hypomethylating agents for the treatment of myelodysplastic syndromes and acute myeloid leukemia: Past discoveries and future directions. *American Journal of Hematology*. 2022;**97**(12):1616–1626. DOI: 10.1002/ajh.26667

[90] Fennell KA, Bell CC, Dawson MA. Epigenetic therapies in acute myeloid leukemia: Where to from here? *Blood*. 2019;**134**(22):1891–1901. Available from: <https://ashpublications.org/blood/article/134/22/1891/387149/Epigenetic-therapies-in-acute-myeloid-leukemia>

[91] Scheffold A, Jebaraj BMC, Stilgenbauer S. Venetoclax: Targeting

BCL2 in hematological cancers. Recent Results in Cancer Research. 2018;**212**:215–242. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/30069633>

[92] Almstedt M, Blagitko-Dorfs N, Duque-Afonso J, Karbach J, Pfeifer D, Jäger E, et al. The DNA demethylating agent 5-aza-2'-deoxycytidine induces expression of NY-ESO-1 and other cancer/testis antigens in myeloid leukemia cells. *Leukemia Research*. 2010;**34**(7):899–905. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/20381863>

[93] Daver N, Boddu P, Garcia-Manero G, Yadav SS, Sharma P, Allison J, et al. Hypomethylating agents in combination with immune checkpoint inhibitors in acute myeloid leukemia and myelodysplastic syndromes. *Leukemia*. 2018;**32**(5):1094–1105. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/29487386>

[94] Byun JM, Yoo S-J, Kim H-J, Ahn J-S, Koh Y, Jang JH, et al. IDH1/2 mutations in acute myeloid leukemia. *Blood Research*. 2022;**57**(1):13–19. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/35197370>

[95] Kernytzsky A, Wang F, Hansen E, Schalm S, Straley K, Gliser C, et al. IDH2 mutation-induced histone and DNA hypermethylation is progressively reversed by small-molecule inhibition. *Blood*. 2015;**125**(2):296–303. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/25398940>

[96] Stein EM, DiNardo CD, Pollyea DA, Fathi AT, Roboz GJ, Altman JK, et al. Enasidenib in mutant IDH2 relapsed or refractory acute myeloid leukemia. *Blood*. 2017;**130**(6):722–731. Available

from: <https://www.ncbi.nlm.nih.gov/pubmed/28588020>

[97] Wang F, Travins J, DeLaBarre B, Penard-Lacronique V, Schalm S, Hansen E, et al. Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. *Science*. 2013;**340**(6132):622–626. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/23558173>

[98] Amatangelo MD, Quek L, Shih A, Stein EM, Roshal M, David MD, et al. Enasidenib induces acute myeloid leukemia cell differentiation to promote clinical response. *Blood*. 2017;**130**(6):732–741. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28588019>

[99] Palau A, Segerberg F, Lidschreiber M, Lidschreiber K, Naughton AJ, Needhamsen M, et al. Perturbed epigenetic transcriptional regulation in AML with IDH mutations causes increased susceptibility to NK cells. *Leukemia*. 2023;**37**(9):1830–1841. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/37495775>

[100] Shields EM. The registered nurse consultant to the intermediary care facility. Some gerontological perspectives: An overview. *ANA Publ*. 1977;**GE-6**:41–173. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/245261>

[101] Nguyen AT, Taranova O, He J, Zhang Y. DOT1L, the H3K79 methyltransferase, is required for MLL-AF9-mediated leukemogenesis. *Blood*. 2011;**117**(25):6912–6922. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/21521783>

[102] Bissierier M, Wajapeyee N. Mechanisms of resistance to EZH2 inhibitors in diffuse large B-cell

- lymphomas. *Blood*. 2018;**131**(19):2125–2137. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/29572378>
- [103] Duan R, Du W, Guo W. EZH2: A novel target for cancer treatment. *Journal of Hematology & Oncology*. 2020;**13**(1):104. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/32723346>
- [104] Fang J, Zhang J, Zhu L, Xin X, Hu H. The epigenetic role of EZH2 in acute myeloid leukemia. *PeerJournal*. 2024;**12**:e18656. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/39655332>
- [105] Xu B, Konze KD, Jin J, Wang GG. Targeting EZH2 and PRC2 dependence as novel anticancer therapy. *Experimental Hematology*. 2015;**43**(8):698–712. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/26027790>
- [106] Xu B, On DM, Ma A, Parton T, Konze KD, Pattenden SG, et al. Selective inhibition of EZH2 and EZH1 enzymatic activity by a small molecule suppresses MLL-rearranged leukemia. *Blood*. 2015;**125**(2):346–357. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/25395428>
- [107] Martinez-Gamero C, Malla S, Aguilo F. LSD1: Expanding Functions in Stem Cells and Differentiation. *Cells*. 2021;**10**(11):3252. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/34831474>
- [108] Cusan M, Cai SF, Mohammad HP, Krivtsov A, Chramiec A, Loizou E, et al. LSD1 inhibition exerts its antileukemic effect by recommissioning PU.1- and C/EBP α -dependent enhancers in AML. *Blood*. 2018;**131**(15):1730–1742. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/29453291>
- [109] Tayari MM, Dos SHG, Kwon D, Bradley TJ, Thomassen A, Chen C, et al. Clinical responsiveness to all-trans retinoic acid is potentiated by LSD1 inhibition and associated with a quiescent transcriptome in myeloid malignancies. *Clinical Cancer Research*. 2021;**27**(7):1893–1903. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/33495312>
- [110] Sugino N, Kawahara M, Tatsumi G, Kanai A, Matsui H, Yamamoto R, et al. A novel LSD1 inhibitor NCD38 ameliorates MDS-related leukemia with complex karyotype by attenuating leukemia programs via activating super-enhancers. *Leukemia*. 2017;**31**(11):2303–2314. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28210006>
- [111] José-Enériz E S, Gimenez-Camino N, Agirre X, Prosper F. HDAC inhibitors in acute myeloid leukemia. *Cancers (Basel)*. 2019;**11**(11). Available from: <https://www.ncbi.nlm.nih.gov/pubmed/31739588>
- [112] Göttlicher M, Minucci S, Zhu P, Krämer OH, Schimpf A, Giavara S, et al. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *The EMBO Journal*. 2001;**20**(24):6969–6978. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/11742974>
- [113] Ma ZE, Song BJ, Hallam TG. The threshold of survival for systems in a fluctuating environment. *Bulletin of Mathematical Biology*. 1989;**51**(3):311–323. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/2730966>
- [114] Garcia-Manero G, Tambaro FP, Bekele NB, Yang H, Ravandi F, Jabbour E, et al. Phase II trial of vorinostat with idarubicin and

cytarabine for patients with newly diagnosed acute myelogenous leukemia or myelodysplastic syndrome. *Journal of Clinical Oncology*. 2012;**30**(18):2204–2210. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/22585696>

[115] Kirschbaum M, Gojo I, Goldberg SL, Bredeson C, Kujawski LA, Yang A, et al. A phase 1 clinical trial of vorinostat in combination with decitabine in patients with acute myeloid leukaemia or myelodysplastic syndrome. *British Journal of Haematology*. 2014;**167**(2):185–193. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/25040094>

[116] Gojo I, Tan M, Fang H-B, Sadowska M, Lapidus R, Baer MR, et al. Translational phase I trial of vorinostat (suberoylanilide hydroxamic acid) combined with cytarabine and etoposide in patients with relapsed, refractory, or high-risk acute myeloid leukemia. *Clinical Cancer Research*. 2013;**19**(7):1838–1851. Available from: <https://aacrjournals.org/clincancerres/article/19/7/1838/284501/Translational-Phase-I-Trial-of-Vorinostat>

[117] Raedler LA. Farydak (panobinostat): First HDAC inhibitor approved for patients with relapsed multiple myeloma. *American Health & Drug Benefits*. 2016;**9**(Spec Feature):84–87. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/27668050>

[118] Giles F, Fischer T, Cortes J, Garcia-Manero G, Beck J, Ravandi F, et al. A phase I study of intravenous LBH589, a novel cinnamic hydroxamic acid analogue histone deacetylase inhibitor, in patients with refractory hematologic malignancies. *Clinical Cancer Research*. 2006;**12**(15):4628–

4635. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/16899611>

[119] DeAngelo DJ, Spencer A, Bhalla KN, Prince HM, Fischer T, Kindler T, et al. Phase Ia/II, two-arm, open-label, dose-escalation study of oral panobinostat administered via two dosing schedules in patients with advanced hematologic malignancies. *Leukemia*. 2013;**27**(8):1628–1636. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/23385375>

[120] Schlenk RF, Krauter J, Raffoux E, Kreuzer K-A, Schaich M, Noens L, et al. Panobinostat monotherapy and combination therapy in patients with acute myeloid leukemia: Results from two clinical trials. *Haematologica*. 2018;**103**(1):e25–8. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/29051280>

[121] Fiskus W, Sharma S, Shah B, Portier BP, Devaraj SGT, Liu K, et al. Highly effective combination of LSD1 (KDM1A) antagonist and pan-histone deacetylase inhibitor against human AML cells. *Leukemia*. 2014;**28**(11):2155–2164. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/24699304>

[122] Wang Z-Q, Zhang Z-C, Wu -Y-Y, Pi Y-N, Lou S-H, Liu T-B, et al. Bromodomain and extraterminal (BET) proteins: Biological functions, diseases, and targeted therapy. *Signal Transduction and Targeted Therapy*. 2023;**8**(1):420. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/37926722>

[123] Gardin C, Dombret H. Hypomethylating agents as a therapy for AML. *Current Hematologic Malignancy Reports*. 2017;**12**(1):1–10. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28286907>

- [124] Ma T, Chen Y, Yi Z-G, Li Y-H, Bai J, Li L-J, et al. BET in hematologic tumors: Immunity, pathogenesis, clinical trials and drug combinations. *Genes & Diseases*. 2023;**10**(6):2306–2319. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/37554207>
- [125] Dawson MA, Prinjha RK, Dittmann A, Giotopoulos G, Bantscheff M, Chan W-I, et al. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. *Nature*. 2011;**478**(7370):529–533. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/21964340>
- [126] Zuber J, Shi J, Wang E, Rappaport AR, Herrmann H, Sison EA, et al. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. *Nature*. 2011;**478**(7370):524–528. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/21814200>
- [127] Coudé -M-M, Braun T, Berrou J, Dupont M, Bertrand S, Masse A, et al. BET inhibitor OTX015 targets BRD2 and BRD4 and decreases c-MYC in acute leukemia cells. *Oncotarget*. 2015;**6**(19):17698–17712. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/25989842>
- [128] Sivin DD. Deepening our understanding of the linkage of working conditions to sexism and racism, restoring the regulatory state and learning lessons from the pandemic. *New Solutions: A Journal of Environmental and Occupational Health Policy*. 2022;**32**(3):168–170. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/36350313>
- [129] Gerlach D, Tontsch-Grunt U, Baum A, Popow J, Scharn D, Hofmann MH, et al. The novel BET bromodomain inhibitor BI 894999 represses super-enhancer-associated transcription and synergizes with CDK9 inhibition in AML. *Oncogene*. 2018;**37**(20):2687–2701. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/29491412>
- [130] Rathert P, Roth M, Neumann T, Muerdter F, Roe J-S, Muhar M, et al. Transcriptional plasticity promotes primary and acquired resistance to BET inhibition. *Nature*. 2015;**525**(7570):543–547. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/26367798>
- [131] Chandraprabha Vineetha R, Anitha Geetha Raj J, Devipriya P, Sreelatha Mahitha M, Hariharan S. MicroRNA-based therapies: Revolutionizing the treatment of acute myeloid leukemia. *International Journal of Laboratory Hematology*. 2024;**46**(1):33–41. DOI: 10.1111/ijlh.14211
- [132] Guo Y, Strickland SA, Mohan S, Li S, Bosompem A, Vickers KC, et al. MicroRNAs and tRNA-derived fragments predict the transformation of myelodysplastic syndromes to acute myeloid leukemia. *Leukemia & Lymphoma*. 2017;**58**(9):1–15. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28084850>
- [133] Jiang X, Hu C, Arnovitz S, Bugno J, Yu M, Zuo Z, et al. miR-22 has a potent anti-tumour role with therapeutic potential in acute myeloid leukaemia. *Nature Communications*. 2016;**7**:11452. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/27116251>
- [134] Hourigan CS, Gale RP, Gormley NJ, Ossenkoppele GJ, Walter RB. Measurable residual disease testing in acute myeloid leukaemia.

Leukemia. 2017;**31**(7):1482–1490.
Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28386105>

[135] Dorrance AM, Neviani P, Ferencak GJ, Huang X, Nicolet D, Maharry KS, et al. Targeting leukemia stem cells in vivo with antagonomiR-126 nanoparticles in acute myeloid leukemia. *Leukemia*. 2015;**29**(11): 2143–2153. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/26055302>

[136] Yin JAL, O'Brien MA, Hills RK, Daly SB, Wheatley K, Burnett AK. Minimal residual disease monitoring by quantitative RT-PCR in core binding factor AML allows risk stratification and predicts relapse: Results of the United Kingdom MRC AML-15 trial. *Blood*. 2012;**120**(14):2826–2835. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/22875911>

[137] Wallace JA, O'Connell RM. MicroRNAs and acute myeloid leukemia: Therapeutic implications and emerging concepts. *Blood*. 2017;**130**(11):1290–1301. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28751524>

[138] He P, Liang J, Zhang W, Lin S, Wu H, Li Q, et al. Hematopoietic stem cell transplantation for acute myeloid leukemia: An overview of systematic reviews. *International Journal of Clinical Practice*. 2022;**2022**:1828223. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/36277468>

[139] Walter RB. Where do we stand with radioimmunotherapy for acute myeloid leukemia? Expert Opinion on Biological Therapy. 2022;**22**(5):555–561. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/35350938>

[140] Walter RB. Where do we stand with radioimmunotherapy for acute myeloid leukemia? Expert Opinion on Biological Therapy. 2022;**22**(5):555–561

[141] O'Brien G, Cecotka A, Manola KN, Pagoni MN, Polanska J, Badie C. Epigenetic signature of ionizing radiation in therapy-related AML patients. *Heliyon*. 2024;**10**(1): e23244. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/38163095>

[142] Sun Q-Y, Ding L-W, Tan K-T, Chien W, Mayakonda A, Lin D-C, et al. Ordering of mutations in acute myeloid leukemia with partial tandem duplication of MLL (MLL-PTD). *Leukemia*. 2017;**31**(1):1–10. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/27389053>

[143] Cao F, Townsend EC, Karatas H, Xu J, Li L, Lee S, et al. Targeting MLL1 H3K4 methyltransferase activity in mixed-lineage leukemia. *Molecular Cell*. 2014;**53**(2):247–261. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/24389101>

[144] Shi Q, Xu M, Kang Z, Zhang M, Luo Y. Menin–MLL1 interaction small molecule inhibitors: A potential therapeutic strategy for leukemia and cancers. *Molecules*. 2023;**28**(7):3026. Available from: <https://www.mdpi.com/1420-3049/28/7/3026>

[145] Rücker FG, Lang KM, Fütterer M, Komarica V, Schmid M, Döhner H, et al. Molecular dissection of valproic acid effects in acute myeloid leukemia identifies predictive networks. *Epigenetics*. 2016;**11**(7):517–525. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/27309669>

[146] Hayatigolkhatmi K, Valzelli R, El Menna O, Minucci S. Epigenetic

alterations in AML: Deregulated functions leading to new therapeutic options. *International Review of Cell and Molecular Biology*. 2024;**387**:27–75. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/39179348>

[147] Zhao A, Zhou H, Yang J, Li M, Niu T. Epigenetic regulation in hematopoiesis and its implications in the targeted therapy of hematologic malignancies. *Signal Transduction and Targeted Therapy*. 2023;**8**(1):71. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/36797244>

[148] Allodji RS, Tucker MA, Hawkins MM, Le Deley M-C, Veres C, Weathers R, et al. Role of radiotherapy and chemotherapy in the risk of leukemia after childhood cancer: An international pooled analysis. *International Journal of Cancer*. 2021;**148**(9):2079–2089. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/33105035>

Exercise-Induced Micro-RNA Alteration Impact on Carcinogenesis within the Context of Rheumatoid Arthritis

Vimolmas Tansathitaya

Abstract

Rheumatoid arthritis (RA) patients face an increased risk of cancer diagnosis due to long-term inflammation. To lower this risk and help prevent cancer development, physical exercise stands out as an easily implementable prevention strategy. Chronic inflammation triggers a cascade of reactions, leading to the accumulation of immune cells, cytokines, and reactive oxygen and nitrogen species, all of which contribute to cellular instability and dysregulation. Micro-RNAs (miRNAs) are biomarkers, acting as both oncogenes and tumor suppressors, making them key indicators for cancer. Interestingly, physical activity and exercise can alter miRNA expression, subsequently influencing gene expression even at the epigenetic level. The miRNAs are secreted not only from muscle tissue during physical activity but also from other organs, including cancer cells, reflecting systemic changes. Studies have shown that physical activity can modify the tumor microenvironment and suppress cancer-promoting miRNAs. The interplay between RA, exercise, and cancer development involves complex interactions across multiple pathways and genes. In this chapter, we investigate how miRNAs, based on current human and animal model research, can help prevent cancer development in the persistent inflammatory state associated with RA.

Keywords: rheumatoid arthritis, physical activity, exercise, micro-RNA, cancer

1. Introduction

Rheumatoid arthritis is an autoimmune disorder with a recognized association with genetic inheritance [1, 2]. The disease can cause stiffness and reduced mobility in the bones and joints, significantly impacting the daily lives of many patients and sometimes hindering their ability to enjoy the world around them. Interestingly, a significant number of patients diagnosed with rheumatoid arthritis subsequently develop cancer. One of the explanations for this incidence lies in chronic inflammation as a risk factor and starting point for various cancer formations, but the underlying mechanism is still not clearly understood and may be more complex than

many people realize [3–5]. One of the mechanisms involved in these relationships is micro-RNA (miRNA).

The miRNAs are short, non-coding RNA molecules, about 20–22 nucleotides long, that participate in the post-transcriptional regulation of messenger RNAs (mRNAs), leading to either the up-regulation or downregulation of gene expression. The miRNAs can serve as prognostic markers for numerous diseases [6]. The levels of miRNAs can be influenced by a variety of environmental factors, including external factors such as air pollution, lifestyle, diet, physical activity, stress, and exercise, as well as internal factors like cell–cell communication, diseases, and viruses, including autoimmune conditions [7]. These miRNAs are sensitive to environmental variations, making them potentially good prognostic biomarkers for many diseases, including cancer [8]. As rheumatoid arthritis drives changes in miRNA expression, which may impact cancer development, another factor that also alters miRNA expression is physical activity [9, 10]. It is understandable that miRNA expression can be altered as diseases progress and physical activity varies. In addition, a notable characteristic of miRNAs is their capacity to function as both tumor suppressors and prognostic indicators in cancer [11, 12]. Given that all living beings are inherently dynamic, engaging in movement and diverse lifestyles can play a role in either promoting or inhibiting cancer development. Therefore, we can observe miRNA as one of the connectors between rheumatoid arthritis, cancer, and physical activity. This miRNA role may be useful not only as a marker but also as a preventive or even therapeutic tool, especially considering changes driven by physical activity that are readily accessible. The lucrative opportunities to use exercise as a medical tool for epigenetic change through miRNA manipulation to achieve better health outcomes in rheumatoid arthritis and cancer patients are worth exploring.

In this chapter, we will examine the relationship between rheumatoid arthritis, cancer, miRNA, and physical activity, as well as its potential as a preventive and therapeutic option.

2. Cancer incidence in rheumatoid arthritis

Despite the primary pathology of rheumatoid arthritis being focused on bones and joints, multiple studies have indicated an increased likelihood of developing cancer in rheumatoid arthritis patients. Studies have found that women with rheumatoid arthritis have an increased risk of developing breast cancer compared to their counterparts without autoimmune disorders. In addition, breast cancer in women with rheumatoid arthritis tends to present with more aggressive characteristics and is associated with a 40% higher mortality rate than in the general female population [13, 14]. However, the evidence of correlation between rheumatoid arthritis and cancer is not limited to breast cancer or factors like gender. The association varies among multiple factors and types of cancer, affecting outcomes differently. For instance, the incidence of colon cancer seems to be lower in individuals with rheumatoid arthritis compared to the general population [15, 16]. Another example is studies on lymphoma occurring after a rheumatoid arthritis diagnosis, which is generally associated with a lower mortality rate than in the general population across genders; however, this trend appears to differ in the Hispanic population [17]. These findings highlight an intricate mechanism that

differentiates based on multiple intertwined factors. One explanation for this phenomenon in rectal cancer cases is the elevated expression of genes involved in immune-mediated inflammation caused by rheumatoid arthritis [18]. Similarly, the relationship between rheumatoid arthritis and other cancers, as well as how race and nationality might factor into these associations, has been explored [17, 19]. These findings reveal that rheumatoid arthritis patients experience varying levels and types of risk depending on multiple factors, which still require much further research.

3. Understanding the connection between chronic inflammation and cancer

Human inflammation can arise from diverse causes, ranging from autoimmune diseases to infections caused by viruses and imbalances in gut microbes [20]. These factors frequently activate inflammatory signaling, a process increasingly recognized for its role in promoting chronic inflammation and, consequently, tumor development. The often silent and intricate nature of these mechanisms contributes to a lack of awareness among many patients regarding the long-term development of cancer.

Chronic inflammation can induce the accumulation of numerous macrophages and neutrophils, reactive oxygen species (ROS), and reactive nitrogen species (RNS). These molecules can cause DNA damage and genomic instability [21]. This accumulation of oxidative stress stimulates an inflammatory response that increases cytokines and chemokines [22]. During chronic inflammation, the increased release of cytokines such as TNF, IL-1, and IL-6 mediates inflammatory responses. However, sustained elevated cytokine levels in chronic inflammation contribute to the condition's progression. This release, coupled with the resulting increase in ROS and RNS, can lead to the formation of premalignant cells. Consequently, the escalation of ROS and RNS contributes to the development of several cancers by affecting mismatch repair genes and TP53, which can result in chromosomal instability and the dysregulation of epigenetic machinery, including DNA- and histone-modifying enzymes, miRNAs, and long non-coding RNAs (lncRNAs), ultimately driving tumor development [23, 24].

In animal models like rodents, chronic inflammation can be induced in two ways: by breeding offspring with a predisposition to inflammatory conditions or by injecting a substance, such as pristane, to induce arthritis and mimic human rheumatoid arthritis. The similarities observed in these animal models could significantly inform our understanding. Despite physiological differences between rodents and humans, their basic mechanisms are often comparable. Animal studies frequently utilize pristane-induced arthritis to establish a model for rheumatoid arthritis as a chronic inflammatory condition. In this model, the immune system becomes active, responding to infiltrating antigens. This activation leads to the production of pro-inflammatory T-cell cytokines, such as TNF- α , IL-1 β , and IL-6, which are central to the pathogenesis of rheumatoid arthritis [25].

Pristane-induced arthritis can also trigger similar pathways as those observed in humans, ultimately leading to widespread changes in miRNA regulation [26]. The expression of miRNA-466-4-3p can be altered due to the PIA-induced shift in the cellular environment or by other immune system activities, such as the up-regulation of cytokines when inflammation increases [9]. This increased inflammation has

broader consequences, impacting not only the ROS pathway but also the JAK/STAT signaling pathway. Dysregulation of the JAK/STAT pathway can result in mutations in key components like JAK1 and JAK3, potentially contributing to the development of leukemia and other malignancies.

In addition, STAT proteins, once phosphorylated by JAKs (a process often driven by chronic inflammation or potentially by loss-of-function mutations in SOCS6), can become dysregulated [27]. This altered STAT activity is significant because STAT proteins, upon activation, typically dimerize, translocate to the nucleus, and bind to DNA, thereby modulating the transcription of target genes. Errors or persistent activation in this pathway can lead to aberrant gene expression, potentially contribute to genomic instability, and cause cancer.

As an autoimmune disease, rheumatoid arthritis leads to chronic inflammation in various joints throughout the body [28]. Pristane-induced arthritis, for instance, may contribute to the development of rheumatoid arthritis by stimulating cytokine production and broader immune system activation. This persistent inflammatory state is associated with an elevated risk of cancer.

4. Physical activity interplay in rheumatoid arthritis and cancer development

As established earlier, miRNAs are one of the mechanisms the body uses to regulate gene expression, which are sensitive to environmental factors both external and internal [7]. Interestingly, exercise and physical activity are among such factors that induce changes in miRNA and subsequent gene expression, which are also known to play a role in cancer prevention [29]. This introduces complexity when examining miRNA involvement, as their expression profiles can be independently modulated by both physical exercise and the immune response itself, suggesting distinct mechanistic pathways.

Although our daily lives are filled with movement, what differentiates these movements, and how might they alter our epigenetics, specifically miRNAs? This question becomes particularly relevant when distinguishing between exercise and general physical activity. Exercise is a planned and systematic endeavor, defined by elements like intensity, duration, repetitions, and frequency. In contrast, physical activity broadly describes the energy expended through any movement involving major muscles in daily activity [30]. Movement, in its various forms, has the capacity to alter miRNA regulation, thereby impacting the expression of their target genes [31]. Notably, the intensity of both exercise and general physical activity can lead to distinct patterns of miRNA regulation in an individual [32]. For example, specific miRNAs might be downregulated or upregulated in response to low-intensity exercise or physical activity, while a different set of miRNAs may exhibit similar changes at higher intensities [33]. Moreover, miRNAs can modulate the expression of their target genes across different organs in the body, both during and following exercise [10]. This complex relationship between exercise and miRNAs provides a compelling explanation for how physical activity can contribute to cancer prevention as a key aspect of health promotion.

The immune system can be activated by a multitude of factors. It is well-established that exercise and physical activity enhance immune function, thereby contributing to disease prevention, including cancer [34]. However, within the

miRNA	Gene target and function	Exercise groups and regulation	Cancer risk	References
rno-miRNA 877	IITPR 3 (Tumor suppressor)	Upregulated in EX vs. EX + PIA groups	Decreased risk of proteoglycan cancer	genome.jp/kegg.ncbi.gov
rno-miRNA 466b-4-3p	SOCS 6 (Oncogene)	Downregulated in N-EX + PIA vs. EX + PIA groups	Increased risk of leukemia	genecards.org
rno-miRNA 128-2-3p	ITGA9 (Oncogene)	Downregulated in N-EX vs. EX groups	Increased risk of lung cancer	genecards.org
rno-miRNA 3064-3	NKX2-1 (Oncogene)	Downregulated in N-EX vs. N-EX + PIA groups	Increased risk of lung cancer	cancerindex.org.ncbi.gov

rno-miRNA: Rattus norvegicus micro-RNA; EX: exercise group; N-EX: non-exercise group; PIA: pristane-induced arthritis.

Table 1.
Exercise-induced rno-miRNA regulation and association with cancer.

specific context of rheumatoid arthritis, the interplay is more complex; exercise and physical activity might act as co-factors that influence immune activity and, consequently, cancer development. Exercise, categorized by its intensity, can exert considerable influence on both the immune system and cancer prevention [35]. Research in animal models, specifically a study by Tansathitaya et al., indicated that high-intensity exercise effectively inhibited proteoglycan cancer development in non-rheumatoid arthritis subjects, as opposed to control groups (see **Table 1**).

The expression of proteoglycan protein genes can be induced by miRNAs, a mechanism also observed in other genes. However, the specific gene responsible for this process is not widely known or well-documented in the scientific literature [36].

Many people may not be familiar with proteoglycans; they are a large family of proteins, and some members can be found in various cancer tumors. Exercise is known to alter miRNAs, which are small non-coding RNAs capable of regulating gene expression. This is relevant because such regulatory molecules can affect proteoglycans [37]. One specific proteoglycan, biglycan (*BGN*), normally plays roles in activating immune cells, promoting bone growth, and supporting muscle development [38]. However, alterations in *BGN*, whether through DNA methylation or regulation by small non-coding RNAs (which include miRNAs and others like small interfering RNAs (siRNAs)), can contribute to cancer development. For example, one study found that altered *BGN* expression in colon cancer tumors was regulated by siRNA. Understanding these connections is key to clarifying how exercise might influence cancer progression through the modulation of proteoglycans like *BGN* and small non-coding RNAs [38, 39]. This beneficial effect is believed to be mediated by alterations in key biomarkers, such as miRNAs, which are known to be modulated by external environmental factors like exercise, lifestyle, diet, stress, etc. [9].

4.1 Does exercise prevent cancer by influencing JAK/STAT signaling?

In Pristane-induced arthritis studies, numerous factors can contribute to alterations in JAK/STAT signaling throughout the long-term progression from chronic inflammation to cancer. A particularly relevant daily activity impacting

these pathways is exercise or intense physical activity [40]. It is important to recognize that JAK/STAT signaling can be modulated by the specific characteristics of physical activity, including its duration, type, frequency, and intensity [41]. This presents a complex picture. While exercise has these positive influences, alterations in DNA methylation of JAK/STAT genes are also implicated in cancer development. Therefore, a key question emerges: how do physical activity and exercise, by modulating these DNA methylation processes (especially within the context of erythropoiesis), ultimately affect the potential risk or progression of cancer – a condition already recognized as a possible outcome? When specifically considering the interplay of exercise, erythropoiesis, and cancer, unraveling these intricate relationships clearly requires further investigation and more future studies [42].

4.2 How physical activity exerts control and alteration through miRNA

The miRNAs can be secreted from muscle tissue during both exercise and general physical activity [43]. Concurrently, different sets of miRNAs can be secreted from various tissues [44]. While movement generally regulates miRNAs as discussed, certain highly expressed miRNAs within specific tissue organs can exhibit expression levels up to 20 times greater than other miRNAs not directly related to physical activity in other organs [32]. This differential expression of numerous miRNAs in tissues linked to energy expenditure holds significant promise [45]. It suggests that the up- or down-regulation of these miRNAs in targeted tissues could actively inhibit cancer formation. A pertinent example is observed in colon cancer, where miR-106a, miR-31, and miR-21 are often upregulated, promoting colorectal cancer, while miR-143 and miR-145 are known to reduce its incidence [46, 47]. During exercise, miRNAs are not only secreted from healthy tissues but can also be released by cancer cells. Nevertheless, a growing body of research suggests that engaging in physical activity and exercise can significantly alter the tumor microenvironment [48–50]. Crucially, these activities have been shown to inhibit the secretion of miRNAs from cancer cells that would otherwise induce pro-tumorigenic cytokines. Analysis of numerous breast cancer tumor cell samples has revealed differential miRNA expression patterns in response to varying exercise and physical activity conditions [51]. The significance of this understanding lies in the fact that regular exercise and daily movements are known to modify miRNA profiles in both healthy human biology and cancerous conditions. Research further demonstrates that exercise can regulate miRNAs across various compartments, including extracellularly in human organs, within the bloodstream, and even directly inside cancer tumor cells. In addition, the same miRNAs found within tumor cells can exhibit varied roles and utilize different pathways. For instance, miRNA-29a-3p, which is highly expressed in both stromal and tumor cells, demonstrates a context-dependent influence on M2 macrophage polarization (either promotion or inhibition), varying with the type of exercise and physical activity, and even across different cell types and their respective pathways [51, 52]. This understanding is crucial because regular exercise and daily movements have been shown to alter miRNAs in both healthy physiological states and internal cancerous conditions.

Several studies have linked the involvement of miRNAs to the pathology of both rheumatoid arthritis and cancer. The primary connection among these three is rheumatoid arthritis-induced inflammation, which alters miRNA expression. This alteration involves the upregulation of oncogenic miRNAs (oncomiRs) and the

downregulation of tumor-suppressive miRNAs (TSmiRs). In rheumatoid arthritis, activation of fibroblast-like synoviocytes (FLS) occurs with over-expression of miR-126, which promotes their proliferation, while miR-26b, which controls FLS proliferation, is downregulated due to inflammation; both are TSmiRs [53–56]. Interestingly, both miR-126 and miR-26b are upregulated during exercise but are suppressed in lung cancer [57–59]. However, not all miRNAs behave in this contrasting manner. For instance, miR-21, a pro-inflammatory oncomiR, is upregulated in all three scenarios: rheumatoid arthritis, cancer, and exercise. While miR-21 is a well-established oncomiR, physical activity also increases its circulating levels, creating an apparent paradox [60–62]. This paradox can be resolved by considering the context. The post-exercise increase in miR-21 is acute and transient, likely contributing to a beneficial physiological response such as tissue repair. This is fundamentally different from the chronic, sustained overexpression found within a tumor. More importantly, exercise does not modulate miR-21 in isolation. The simultaneous upregulation of tumor suppressors like miR-126 and miR-26b creates a systemic environment that balances its effects, likely ensuring the net systemic response is beneficial [63, 64]. Another key miRNA, miR-146a, acts as an inflammatory reset signal but is often downregulated during chronic inflammation. In rheumatoid arthritis, its negative feedback control of the NF- κ B pathway is impaired, and in cancer, it modulates the tumor immune microenvironment [65–67]. In contrast, physical activity consistently upregulates miR-146a, particularly in response to endurance exercise [68, 69]. Therefore, regular physical exercise, by inducing the repeated, systemic release of miR-146a, may help re-establish homeostatic control over the NF- κ B pathway, beneficially influencing both chronic inflammation and the tumor microenvironment.

4.3 The role of miRNAs as biomarkers for cancer therapy

The evidence detailing how physical activity systematically modulates key miRNAs provides a strong foundation for using these molecules as more than just mechanistic links. Their dynamic response to exercise positions them as powerful biomarkers and hints at a novel therapeutic framework for cancer prevention, particularly in high-risk populations, such as those with rheumatoid arthritis.

Despite promising preclinical research, the clinical development of miRNA therapeutics for cancer is still in its early stages, having yet to successfully pass a Phase III trial or receive FDA approval. The field faces significant hurdles in translating this concept to clinical practice [70]. For example, the Phase I trial for MRX34, an miR-34a mimic, was terminated due to severe and fatal immune-related adverse events, despite showing modest signs of efficacy [71]. In contrast, Cobomarsen, an miR-155 inhibitor, was well-tolerated but ultimately failed its Phase II trial because it lacked sufficient efficacy compared to an existing treatment [72, 73]. In addition, TargomiR, an miR-16 mimic, completed its Phase I trial but demonstrated a poor therapeutic window, with concerning cardiac toxicities that outweighed its very modest clinical benefit of a single partial response [74].

These clinical challenges highlight a fundamental issue. Current miRNA-based treatments often suffer from adverse events linked to the broad-spectrum nature of miRNAs, as each miRNA has multiple targets with different effects on multiple fronts. In contrast, exercise induces a systemic, coordinated activation of multiple miRNAs, where the integrated response of the entire system produces a net health

benefit. This suggests that miRNAs as a treatment still require a larger body of studies to fully understand each miRNA's scope of action to limit adverse effects while retaining efficacy, and that physical activity serves as a low-cost, effective preventive measure against cancer, especially for high-risk groups such as rheumatoid arthritis patients.

5. Future research directions

Despite the compelling mechanistic links between exercise, miRNA modulation, and cancer prevention in the context of rheumatoid arthritis, significant work is required to translate these findings into precise clinical recommendations. This body of research provides a strong hypothesis, but several gaps must be addressed to harness the full potential of exercise and miRNAs as targeted molecular medicine.

A primary gap in the literature is the scarcity of studies measuring this convergent miRNA signature within a single cohort of rheumatoid arthritis and cancer patients undergoing a structured exercise intervention. Most research investigates the link between only two of these components, rarely examining all three within the context of a dedicated physical activity program. Bridging this gap by conducting such longitudinal studies is a critical next step to validate these biomarkers and therapeutic pathways in a clinically relevant setting.

Moreover, the field is hampered by a lack of standardized protocols for sample collection, miRNA extraction, quantification, and, critically, exercise prescription. The intensity, duration, and type of exercise can all lead to distinct patterns of miRNA regulation. Therefore, future research must focus on defining the optimal dose and type of exercise required to produce robust, beneficial results in RA and cancer patients. Answering these questions will be essential for developing personalized, evidence-based exercise prescriptions designed to leverage epigenetic mechanisms for disease prevention and improved health outcomes.

6. Conclusion

Exercise acts as a systems-level modulator of epigenetics, particularly through its interaction with miRNAs. Its ability to orchestrate a complex, multi-pronged, and beneficial shift in circulating miRNAs represents an advantage that is currently difficult to replicate with conventional pharmaceuticals. However, realizing its full potential requires a concerted research effort to move from these foundational molecular insights to clinically validated, personalized exercise prescriptions. Future translational studies that directly link exercise-induced miRNA changes to clinical outcomes in patients are essential to harness the power of exercise as a preventive intervention.

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Conflict of interest

The authors declare no conflicts of interest.

Notes/Thanks/Other declarations

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References

- [1] Kurkó J, Besenyei T, Laki J, Glant TT, Mikecz K, Szekanecz Z, et al. Genetics of Rheumatoid Arthritis — A comprehensive review. *Clinical Reviews in Allergy & Immunology*. 2013;**45**:170–179
- [2] Jahid M, Khan KU, Rehan-Ul-Haq, et al. Overview of Rheumatoid Arthritis and Scientific Understanding of the Disease. *Mediterranean Journal of Rheumatology*. 2023;**34**:284
- [3] Abou Hjeily B, Nevaneeth BC, Samborski W, et al. Inflammatory pathways to carcinogenesis: Deciphering the Rheumatoid Arthritis-lung cancer connection. *Cancers (Basel)*. 2025;**17**:1330
- [4] Beydon M, Pinto S, De Rycke Y, et al. Risk of cancer for patients with rheumatoid arthritis versus general population: A national claims database cohort study. *The Lancet Regional Health. Europe*. 2023;**35**:100768
- [5] De Cock D, Hyrich K. Malignancy and rheumatoid arthritis: Epidemiology, risk factors and management. *Best Practice & Research: Clinical Rheumatology*. 2018;**32**:869–886
- [6] Ptb H, Clark IM, Ltt L. MicroRNA-based diagnosis and therapy. *International Journal of Molecular Sciences*. 2022;**23**:7167
- [7] Nalbant E, Akkaya-Ulum YZ. Exploring regulatory mechanisms on miRNAs and their implications in inflammation-related diseases. *Clinical and Experimental Medicine*. 2024;**24**:142
- [8] Budakoti M, Panwar AS, Molpa D, et al. Micro-RNA: The darkhorse of cancer. *Cellular Signalling*. 2021;**83**:109995
- [9] Tansathitaya V, Sarasin W, Phakham T, et al. Regulation of mi-RNAs target cancer genes between exercise and non-exercise in rat Rheumatoid Arthritis induction: Pilot study. *Epigenetics Insights*. 2022;**15**:25168657221110485
- [10] Balchin C, Tan AL, Wilson OJ, et al. The role of microRNAs in regulating inflammation and exercise-induced adaptations in rheumatoid arthritis. *Rheumatology Advances in Practice*. 2023;**7**:rkac110
- [11] Zhou K, Liu M, Cao Y. New insight into microRNA functions in cancer: Oncogene–microRNA–tumor suppressor gene network. *Frontiers in Molecular Biosciences*. 2017;**4**:46
- [12] Shenouda SK, SK A. MicroRNA function in cancer: Oncogene or a tumor suppressor? *Cancer & Metastasis Reviews*. 2009;**28**:369–378
- [13] Hellgren K, Smedby KE, Feltelius N, et al. Do rheumatoid arthritis and lymphoma share risk factors?: A comparison of lymphoma and cancer risks before and after diagnosis of rheumatoid arthritis. *Arthritis & Rheumatism*. 2010;**62**:1252–1258
- [14] Nayak P, Luo R, Elting L, et al. Impact of Rheumatoid Arthritis on the mortality of elderly patients who develop cancer: A population-based study. *Arthritis Care & Research*. 2017;**69**:75–83

- [15] Chang SH, Park JK, Lee YJ, et al. Comparison of cancer incidence among patients with rheumatic disease: A retrospective cohort study. *Arthritis Research and Therapy*. 2014;**16**:428
- [16] Long Y, Pan Y, Yang H, et al. The genetic causal effect of Autoimmune Diseases on pan-cancers: Evidence from mendelian randomization. *Journal of Cancer*. 2025;**16**:567–576
- [17] Parikh-Patel A, White RH, Allen M, et al. Risk of cancer among rheumatoid arthritis patients in California. *Cancer Causes & Control : CCC*. 2009;**20**:1001–1010
- [18] Li Q, Zhou L, Xia D, et al. Rheumatoid arthritis reduces the risk of colorectal cancer through immune inflammation mediation. *Journal of Cellular & Molecular Medicine*. 2024;**28**:e18515
- [19] Mikuls TR, Endo JO, Puumala SE, et al. Prospective Study of Survival Outcomes in Non-Hodgkin's Lymphoma Patients With Rheumatoid Arthritis. *Journal of Clinical Oncology*. 2006;**24**:1597–1602
- [20] Hibino S, Kawazoe T, Kasahara H, et al. Inflammation-induced tumorigenesis and metastasis. *International Journal of Molecular Sciences*. 2021;**22**:5421
- [21] Canli Ö, Nicolas AM, Gupta J, et al. Myeloid cell-derived reactive oxygen species induce epithelial mutagenesis. *Cancer Cell*. 2017;**32**:869–883.e5
- [22] Yu W, Tu Y, Long Z, et al. Reactive oxygen species bridge the gap between chronic inflammation and tumor development. *Oxidative Medicine and Cellular Longevity*. 2022;**2022** (1):2606928
- [23] Ahn J, Xia T, Konno H, et al. Inflammation-driven carcinogenesis is mediated through STING. *Nature Communications*. 2014;**5**:5166
- [24] Li T, Chen ZJ. The cGAS–cGAMP–STING pathway connects DNA damage to inflammation, senescence, and cancer. *Journal of Experimental Medicine*. 2018;**215**:1287–1299
- [25] Tuncel J, Haag S, Hoffmann MH, et al. Animal models of rheumatoid arthritis (I): Pristane-induced arthritis in the rat. *PLoS ONE*. 2016;**11**: e0155936
- [26] Vigar ND, Cabrera WH, Araujo LM, et al. Pristane-induced arthritis in mice selected for maximal or minimal acute inflammatory reaction. *European Journal of Immunology*. 2000;**30**:431–437
- [27] Croker BA, Kiu H, Nicholson SE. SOCS regulation of the JAK/STAT signalling pathway. *Seminars in Cell and Developmental Biology*. 2008;**19**: 414–422
- [28] Cooney JK, Law R-J, Matschke V, et al. Benefits of exercise in rheumatoid arthritis. *Journal of Aging Research*. 2011;**2011**:1–14
- [29] Mctiernan A, Friedenreich CM, Katzmarzyk PT, et al. Physical activity in cancer prevention and survival: A systematic review. *Medicine and Science in Sports and Exercise*. 2019;**51**:1252–1261
- [30] Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: Definitions and distinctions for health-related research. *Public Health Reports (Washington, D.C. : 1974)*. 1985;**100**:126–131

- [31] O'Brien J, Hayder H, Zayed Y, et al. Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Frontiers in Endocrinology*. 2018;**9**:402
- [32] Afzal M, Greco F, Quinzi F, et al. The effect of physical activity/exercise on miRNA expression and function in non-communicable diseases—A systematic review. *International Journal of Molecular Sciences*. 2024;**25**:6813
- [33] Dos Santos JAC, Veras ASC, Batista VRG, et al. Physical exercise and the functions of microRNAs. *Life Sciences*. 2022;**304**:120723
- [34] Fiuza-Luces C, Valenzuela PL, Gálvez BG, et al. The effect of physical exercise on anticancer immunity. *Nature Reviews Immunology*. 2024;**24**:282–293
- [35] Wang Q, Zhou W. Roles and molecular mechanisms of physical exercise in cancer prevention and treatment. *Journal of Sport and Health Science*. 2021;**10**:201–210
- [36] Ibrahim SA, Hassan H, Götte M. Micro RNA regulation of proteoglycan function in cancer. *The FEBS Journal*. 2014;**281**:5009–5022
- [37] Li F, Bai M, Xu J, et al. Long-term exercise alters the profiles of circulating micro-RNAs in the plasma of young women. *Frontiers in Physiology*. 2020;**11**:372
- [38] Lu B, Zhu Y, Li G, et al. Biglycan promotes tumour proliferation and invasion in human colon cancers. *Journal of International Medical Research*. 2024;**52**:03000605241300067
- [39] Chen D, Qin Y, Dai M, et al. BGN and COL11A1 regulatory network analysis in colorectal cancer (CRC) reveals that BGN influences CRC cell biological functions and interacts with miR-6828-5p. *Cancer Management and Research*. 2020;**12**:13051–13069
- [40] Moresi V, Adamo S, Berghella L. The JAK/STAT pathway in skeletal muscle pathophysiology. *Frontiers in Physiology*. 2019;**10**:500
- [41] Chen Z, Li B, Zhan R-Z, et al. Exercise mimetics and JAK inhibition attenuate IFN- γ -induced wasting in engineered human skeletal muscle. *Science Advances*. 2021;**7**:eabd9502
- [42] Cha HJ. Erythropoiesis: Insights from a genomic perspective. *Experimental & Molecular Medicine*. 2024;**56**:2099–2104
- [43] Gaál Z, Fodor J, Oláh T, et al. Implication of microRNAs as messengers of exercise adaptation in junior female triathlons. *Scientific Reports*. 2024;**14**:22858
- [44] Guo Z, Maki M, Ding R, et al. Genome-wide survey of tissue-specific microRNA and transcription factor regulatory networks in 12 tissues. *Scientific Reports*. 2014;**4**:5150
- [45] Shamsi F, Zhang H, Tseng Y-H. MicroRNA Regulation of Brown Adipogenesis and Thermogenic Energy Expenditure. *Frontiers in Endocrinology*. 2017;**8**:205
- [46] Luo X, Burwinkel B, Tao S, et al. MicroRNA signatures: Novel biomarker for colorectal cancer? *Cancer Epidemiology Biomarkers and Prevention*. 2011;**20**:1272–1286
- [47] Akao Y, Nakagawa Y, Hirata I, et al. Role of anti-oncomirs miR-143 and -145 in human colorectal tumors.

Cancer Gene Therapy. 2010;17:398–408

[48] Spiliopoulou P, Gavriatopoulou M, Kastritis E, et al. Exercise-induced changes in tumor growth via tumor immunity. *Sports*. 2021;9:46

[49] Zhang X, Ashcraft KA, Betof WA, et al. Can exercise-induced modulation of the tumor physiologic microenvironment improve antitumor immunity? *Cancer Research*. 2019;79:2447–2456

[50] Koelwyn GJ, Quail DF, Zhang X, et al. Exercise-dependent regulation of the tumour microenvironment. *Nature Reviews Cancer*. 2017;17:620–632

[51] Mei J, Luo Z, Cai Y, et al. Altered Atlas of exercise-responsive microRNAs revealing miR-29a-3p attacks armored and cold tumors and boosts anti-B7-H3 therapy. *Research*. 2025;8:0590

[52] Miyamoto T, Murakami R, Hamanishi J, et al. B7-H3 suppresses antitumor immunity via the CCL2–CCR2–M2 macrophage Axis and contributes to ovarian cancer progression. *Cancer Immunology Research*. 2022;10:56–69

[53] Huang Z, Xing S, Liu M, et al. MiR-26a-5p enhances cells proliferation, invasion, and apoptosis resistance of fibroblast-like synoviocytes in rheumatoid arthritis by regulating PTEN/PI3K/AKT pathway. *Bioscience Reports*. 2019;39:BSR20182192

[54] Gao J, Liu Q-G. The role of miR-26 in tumors and normal tissues (Review). *Oncology Letters*. 2011;2:1019–1023

[55] Qu Y, Wu J, Deng J-X, et al. MicroRNA-126 affects rheumatoid

arthritis synovial fibroblast proliferation and apoptosis by targeting PIK3R2 and regulating PI3K-AKT signal pathway. *Oncotarget*. 2016;7:74217–74226

[56] Casciaro M, Di Salvo E, Brizzi T, et al. Involvement of miR-126 in autoimmune disorders. *Clinical and Molecular Allergy*. 2018;16:11

[57] Liu Z, Ke S, Wan Y. miR-126: A bridge between cancer and exercise. *Cancer Cell International*. 2025;25:145

[58] Liu Y, He L, Wang W. Systematic assessment of microRNAs associated with lung cancer and physical exercise. *Frontiers in Oncology*. 2022;12:917667

[59] Chen X, Xu X, Li Y, et al. Role of miR-26b in regulating non-small cell lung cancer progression via targeting RCN1. *Journal of Thoracic Disease*. 2025;17:3873–3885

[60] Singh A, AK S, Giri R, et al. The Role of microRNA-21 in the onset and progression of cancer. *Future Medicinal Chemistry*. 2021;13:1885–1906

[61] Zhou Q, Shi C, Lv Y, et al. Circulating microRNAs in response to exercise training in healthy adults. *Frontiers in Genetics*. 2020;11:256

[62] Moness H, Ibrahim RA, Soliman SA, et al. Association of cell-free DNA, micro-RNA 21, and micro-RNA 146a levels with rheumatoid arthritis activity. *Molecular Biology Reports*. 2025;52:200

[63] Wahl P, Wehmeier UF, Jansen FJ, et al. Acute effects of different exercise protocols on the circulating vascular microRNAs -16, -21, and -126 in trained subjects. *Frontiers in Physiology*. 2016;7:643

- [64] Li Y, Yao M, Zhou Q, et al. Dynamic regulation of circulating microRNAs during acute exercise and long-term exercise training in basketball athletes. *Frontiers in Physiology*. 2018;**9**:282
- [65] Garo LP, Ajay AK, Fujiwara M, et al. MicroRNA-146a limits tumorigenic inflammation in colorectal cancer. *Nature Communications*. 2021;**12**:2419
- [66] Saba R, Sorensen DL, Booth SA. MicroRNA-146a: A dominant, negative regulator of the innate immune response. *Frontiers in Immunology*. 2014;**5**:578. DOI: 10.3389/fimmu.2014.00578
- [67] Liu W, Wu Y-H, Zhang L, et al. MicroRNA-146a suppresses rheumatoid arthritis fibroblast-like synoviocytes proliferation and inflammatory responses by inhibiting the TLR4/NF- κ B signaling. *Oncotarget*. 2018;**9**: 23944–23959
- [68] Soplinska A, Zareba L, Wicik Z, et al. MicroRNAs as biomarkers of systemic changes in response to endurance exercise—A comprehensive review. *Diagnostics*. 2020;**10**:813
- [69] Huang L-Y, Lim AY, Hsu -C-C, et al. Sustainability of exercise-induced benefits on circulating MicroRNAs and physical fitness in community-dwelling older adults: A randomized controlled trial with follow up. *BMC Geriatrics*. 2024;**24**:473
- [70] Seyhan AA. Trials and tribulations of microRNA therapeutics. *International Journal of Molecular Sciences*. 2024;**25**:1469
- [71] Hong DS, Kang Y-K, Borad M, et al. Phase 1 study of MRX34, a liposomal miR-34a mimic, in patients with advanced solid tumours. *British Journal of Cancer*. 2020;**122**:1630–1637
- [72] SOLAR. *SOLAR: Efficacy and Safety of Cobomarsen (MRG-106) Vs. Active Comparator in Subjects with Mycosis Fungoides (SOLAR)*. ClinicalTrials.Gov ID NCT03713320, miRagen Therapeutics, Inc. Available from: <https://clinicaltrials.gov/study/NCT03713320> [Accessed: 2022-April-8]
- [73] Querfeld C, Foss FM, Kim YH, et al. Phase 1 trial of cobomarsen, an inhibitor of Mir-155, in cutaneous T Cell Lymphoma. *Blood*. 2018;**132**:2903–2903
- [74] Van Zandwijk N, Pavlakis N, Kao SC, et al. Safety and activity of microRNA-loaded minicells in patients with recurrent malignant pleural mesothelioma: A first-in-man, phase 1, open-label, dose-escalation study. *The Lancet Oncology*. 2017;**18**:1386–1396

Impact of Ribosomal Protein Mutations on Inflammation, Metastasis, and Therapeutic Resistance in Cancer and Ribosomopathies

Gazmend Temaj and Zafer Gashi

Abstract

Ribosomal proteins (RPs), beyond their canonical roles in protein synthesis, serve as crucial modulators of inflammation, immune responses, and cellular homeostasis. The ribosome, composed of 40S and 60S subunits with distinct RPs and rRNAs, ensures translation fidelity and responds to cellular stress. Specific RPs, including RPS3, RPS6, RPS19, RPL13a, RPL22, and RACK1 exhibit diverse extra-ribosomal functions that regulate inflammatory signaling pathways such as NF- κ B, MAPK, and interferon responses. Mutations in RPs underlie ribosomopathies like Diamond-Blackfan Anemia (DBA), characterized by defective erythropoiesis and innate immune activation. Notably, RPS3 promotes inflammatory gene expression and cancer progression, RPS6 phosphorylation controls selective translation of inflammatory mediators, while RPL13a forms the GAIT complex to resolve inflammation by silencing inflammatory mRNAs. RPL22 influences ER stress and chemokine-mediated immune cell migration, and RACK1 integrates ribosomal translation control with immune signaling pathways. Additionally, RPs modulate the p53–MDM2 axis, linking ribosomal stress to tumor suppression and inflammation-driven carcinogenesis. Viruses exploit ribosomal heterogeneity and host translation machinery via internal ribosome entry sites (IRES) to maintain viral protein synthesis during inflammation. Collectively, these findings highlight RPs as pivotal nodes connecting translation, immune regulation, and disease pathogenesis, offering promising targets for therapeutic intervention in inflammatory diseases, cancers, and viral infections.

Keywords: ribosomal proteins (RPS), inflammation regulation, diamond-blackfan anemia (DBA), NF- κ B signaling, ribosome biogenesis, ribosomal protein mutations, viral IRES-mediated translation

1. Introduction

All living organisms fundamentally rely on the ribosome as the essential molecular machinery responsible for protein synthesis, a hallmark capability that defines life's autonomy at the cellular level. The ribosome is a complex ribonucleoprotein particle composed of two distinct subunits: the small 40S subunit and the large 60S subunit. The 40S subunit contains 33 unique ribosomal proteins (RPs) along with 18S rRNA, whereas the 60S subunit comprises 47 RPs together with three rRNAs (28S, 5.8S, and 5S). Their assembly demands a highly coordinated and intricate process involving transcription, translation, rRNA processing, and extensive chemical modifications [1, 2]. Evolutionary advances have coincided with increased cellular complexity and size, necessitating an upregulation in ribosome biogenesis to meet heightened demands for protein synthesis.

Ribosome biogenesis, however, is a vulnerable process that can be disrupted by various stressors such as ultraviolet (UV) radiation, oncogenic signals, cellular stress, nutritional imbalances, and altered growth factor signaling. These insults can impair ribosomal function and trigger compensatory mechanisms mediated by specific RPs to restore cellular homeostasis [3–5]. As the central cellular machinery for translating messenger RNA into polypeptide chains, the ribosome exhibits remarkable fidelity during translation, ensuring accuracy in codon recognition and peptide bond formation. This high precision is critical to maintain functional proteomes and cellular viability. The molecular basis of this fidelity involves stringent codon–anticodon base pairing within the A site, careful selection of aminoacyl-tRNAs, and quality control pathways that edit misincorporated amino acids during and following peptide bond formation [6–9]. Quantitative analyses using mass spectrometry reveal that translation error rates typically range from 10^{-7} to 10^{-5} per codon, with specific “hotspots” such as G–U mismatches in the second codon position, exhibiting a relatively higher tolerance [10–13]. Complementing these data, transcription error frequencies, which are estimated to be higher but less influenced by growth conditions, further highlight the precision required at multiple levels of gene expression [14, 15].

1.1 Ribosomal proteins as modulators of inflammatory responses

Given the ribosome's centrality in cellular physiology, dysfunction of RPs contributes to a broad spectrum of human diseases [16–20], collectively termed ribosomopathies. These include genetic disorders such as Diamond-Blackfan Anemia (DBA), Treacher Collins Syndrome (TCS), congenital aplasia (CA), various cancers, and inflammatory pathologies like atherosclerosis [21–27]. Importantly, specific RPs (e.g., RPS3, RPS6, RPS19, RPL13a, RPL22, RPL15, RPL39) have been shown to play pivotal roles not only in ribosome assembly and canonical translation but also as critical modulators of inflammation and tumor biology [28] (**Figure 1**). This emerging understanding redefines RPs as dynamic regulators at the intersection of protein synthesis, cellular stress responses, and immune signaling.

Beyond their canonical roles, RPs participate in noncanonical signaling pathways that distinctly influence the cellular inflammatory milieu. For example, the study of ribotoxic stress induced by the mycotoxin deoxynivalenol (DON) in

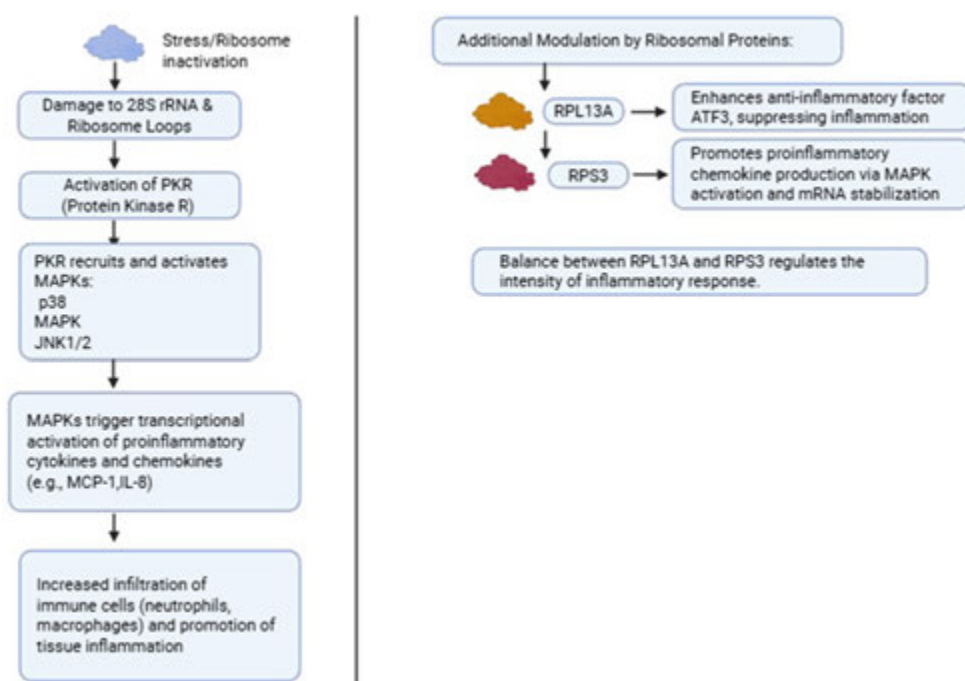


Figure 1.

This schematic summarizes key molecular players and steps linking ribosomal protein function and ribosomal stress to inflammation based on current research findings:

Ribosomal stress or inactivation, such as damage to the 28S rRNA, activates PKR, a key kinase that senses ribosomal damage.

PKR then activates MAPK signaling pathways (p38, JNK), which lead to the production of proinflammatory cytokines and chemokines.

These cytokines recruit immune cells like neutrophils and macrophages, amplifying inflammation.

Specific ribosomal proteins like RPL13A act to reduce inflammation by enhancing anti-inflammatory transcription factors (e.g., ATF3).

Conversely, RPS3 promotes inflammation by activating MAPKs and stabilizing mRNAs of proinflammatory genes.

The interplay between these ribosomal proteins finely tunes the inflammatory response.

gut epithelial cells reveals dual and opposing regulatory functions mediated by RPL13A and RPS3. RPL13A promotes anti-inflammatory programs via the ATF3 transcription factor, suppressing chemokine production, whereas RPS3 amplifies inflammatory responses through activation of MAPK signaling, induction of EGR-1, and mRNA stabilization via HuR. Similarly, ribosomal inactivation by toxins (ricin, anisomycin) triggers inflammasome activation and pro-inflammatory cytokine secretion independently of potassium efflux [29, 30]. Furthermore, cationic RPs like RPL9 and RPS5 play roles in modulating macrophage cytokine responses, which are antagonized by pathogenic ligands such as advanced glycation end products (AGEs) [31, 32]. The recruitment of protein kinase R (PKR) to stalled ribosomes subsequently activates key inflammatory pathways, including p38 MAPK and NF- κ B, while endoplasmic reticulum (ER) stress responses augment this inflammatory signaling by enhancing mRNA stability through HuR translocation [33, 34]. Collectively, these findings highlight RPs as versatile and critical modulators of both mucosal and systemic inflammation, offering promising avenues for developing therapeutic strategies targeting ribosomal function and its regulatory networks (**Table 1**).

Ribosomal protein	Mutation type	Associated disease	Inflammatory manifestation	Molecular mechanism
RPS19	Heterozygous loss-of-function	Diamond-Blackfan Anemia (DBA)	Failure of bone marrow function, with heightened vulnerability to infections	Impaired erythroid differentiation, chronic anemia; p53 activation, apoptosis [35]
RPL5/ RPL11	Haploinsufficiency	DBA and 5q – syndrome	Macrocytic anemia, autoimmune disorders	Defective ribosome assembly, nucleolar stress, and p53-mediated cell cycle arrest [35]
RPS7	Frameshift/nonsense	DBA and cancer predisposition	T-cell deficiency, recurrent infections	Reduced ribosome biogenesis impaired lymphocyte proliferation [35, 36]
RPS10	Somatic mutations	Chronic lymphocytic leukemia (CLL)	Tumor microenvironment inflammation	Altered ribosome function, NF- κ B activation, cytokine overproduction [35]
RPS24	Missense variants	DBA and immunodeficiency	Neutropenia, inflammatory bowel disease	Disrupted rRNA processing, ribosomopathy, and gut barrier dysfunction [35, 36].
RPL26	Overexpression	Myelodysplastic syndromes (MDS)	Inflammasome activation, cytokine storms	Enhanced translation of inflammatory mRNAs (e.g., IL-6, TNF- α) [35]

Table 1.

Ribosomal protein mutations: disease associations, inflammatory manifestations, and underlying molecular mechanisms.

In essence, this manuscript seeks to integrate current knowledge on ribosomal protein dysfunction with their expanding roles in inflammatory control and disease pathogenesis, emphasizing the potential to harness these insights for translational research in inflammation and cancer.

2. Ribosomal protein S3 (RPS3) as a multifaceted regulator of NF- κ B signaling, cancer metastasis, and therapeutic resistance

The RPS3 is a member of the small ribosome subunits. During the process, the RPS3 is assembled into the “beack structure” of the pre – 40S subunits. After the processing and maturation of RPS3, it is shown that RPS3 in its free state has the ability to interact with NF- κ B p65 [37]. The association between RPS3 and p65 facilitates the translocation and accumulation of NF- κ B within the nucleus. Many studies report how RPS3 is associated with and how it regulates p65. Under normal conditions, RPS3 can associate with MIF (macrophage inhibitory factor) and prevent it from becoming part of the NF- κ B complex. In lung cancer cells, dissociation of the RPS3–MIF complex and subsequent binding to p65 lead to the expression of pro-inflammatory genes such as CXCR4, IL-1 β , and IL-6 [28, 38].

Stanborough and colleagues showed that ribosomal protein S3 (RPS3) can bind to the NF- κ B inhibitor I κ B α in HEK293 cells. Significantly, strong co-immunoprecipitation between the NF- κ B subunit p65 and RPS3 was detected exclusively when I κ B α was overexpressed. Furthermore, *in vitro* assays demonstrated a strong interaction between RPS3 and I κ B α , whereas the direct binding affinity between RPS3 and p65 was relatively weak.

Crucially, I κ B α promoted the formation of a ternary complex containing p65 and RPS3 *in vitro*. These findings suggest that I κ B α functions to sequester both p65 and RPS3 within the cytoplasm, thereby maintaining a cytoplasmic reservoir of RPS3 for the NF- κ B signaling pathway and enabling their coordinated, equimolar release upon cellular stimulation [39].

Beyond its traditional functions, growing research reveals that RPS3 participates in various extra-ribosomal activities across different cellular contexts. Importantly, RPS3 promotes activation of the NF- κ B signaling pathway through its interaction with the p65 subunit of the NF- κ B complex. Given the central role of the NF- κ B pathway in cancer development and chemotherapy resistance, the involvement of RPS3 indicates it may drive tumor progression and hinder therapeutic efficacy. Consistent with this, increased levels of RPS3 have been detected in several cancers, even though RPS3 mRNA and protein are typically expressed ubiquitously in normal tissues without specific cell-type preference. However, despite these findings, the precise functions of RPS3 in cancer biology and its potential clinical relevance remain inadequately understood and warrant further investigation. In this review, we analyze the current literature to assess the role of RPS3 in cancer development and drug resistance [40].

Chen and co-workers were the first to show that RPS3 is critical for both the survival and drug resistance of multiple myeloma (MM) cells. The study revealed that RPS3 expression was markedly increased in MM and that its deletion suppressed cell proliferation and induced apoptosis in MM models both *in vitro* and *in vivo*. Moreover, elevated RPS3 levels were associated with resistance to proteasome inhibitors and shortened survival of animals bearing MM tumors. This study also discovered an association between RPS3 and TRIP13, a critical oncogene linked to MM tumorigenesis and chemotherapy resistance. The study shows that phosphorylation of RPS3, mediated by TRIP13 through PKC δ , is required for activation of the canonical NF- κ B pathway, thereby supporting cell survival and increasing drug resistance in MM. Significantly, the small-molecule TRIP13 inhibitor DCZ0415 was able to suppress NF- κ B signaling and induce synergistic cytotoxic effects when used alongside bortezomib in drug-resistant MM. This research underscores RPS3 as a potential biomarker and therapeutic target for MM treatment [41]. Metastasis accounts for nearly 90% of deaths caused by solid tumors. A key characteristic of cancer cells is their modified metabolism, which significantly contributes to the onset and advancement of tumors. The serine *de novo* synthesis pathway serves as an important metabolic detour from glycolysis, supplying essential materials and energy through one-carbon metabolism to support rapid tumor cell proliferation. This metabolic shift is especially prominent in hepatocellular carcinoma (HCC), where cancer cells often exhibit strong reliance on glycolysis. Nevertheless, the link between serine metabolism and HCC metastasis is not yet well understood. Using comprehensive bioinformatics analyses, we identified CCT6A, a gene involved in serine metabolism, as being strongly linked to metastatic events in HCC. Further experiments revealed that CCT6A expression is

elevated in HCC cells with high metastatic capacity. Functional analyses revealed that CCT6A facilitates the migration and invasion of HCC cells. Functionally, CCT6A interacts with RPS3 and may facilitate metastasis in HCC by modulating metabolic pathways. Overall, the findings suggesting that metabolic reprogramming driven by the CCT6A-RPS3 interaction facilitates HCC metastasis, positioning the CCT6A/RPS3 axis as a potential target for therapeutic strategies [42].

2.1 Ribosomal protein S6 (RPS6): Structure, phosphorylation dynamics, and its role in translational control and inflammation

Ribosomal protein S6 (RPS6) is located in the small head domain of the 40S ribosomal subunit and contains a structural motif consisting of a four-stranded antiparallel β -sheet flanked by two α -helices, as determined from the 2.0 Å-resolution crystal structure of *Thermus thermophilus* [43]. Positioned at the mRNA binding site, RPS6 directly interacts with initiation factors, tRNA, and the mRNA 5'-cap structure (m⁷GpppG), facilitating translation initiation [44, 45]. Functionally, RPS6 undergoes ordered C-terminal phosphorylation (Ser-235, Ser-236, Ser-240, Ser-244, Ser-247) in response to mitogenic signals, enhancing its affinity for the cap complex and promoting selective mRNA translation [45]. Phosphorylation – mediated sequentially by S6K, RSK, and CK1 kinases – progressively dephosphorylates during elongation, preferentially boosting translation of short coding sequence (CDS) mRNAs while excluding 5'TOP motif mRNAs from this regulation [46]. This dynamic modification integrates mTORC1 and RAS/ERK signaling to modulate translational efficiency and cell proliferation [47].

The RPS6 is shown to play a pivotal role in the inflammation process. Modification phosphorylation of the RPS6 at Ser235/236 in signaling pathways such as ERK has the ability to influence specific translation – macrophage CXCL-8 (IL8) and TNFAIP-6 [48]. This process is very important in acute inflammation because IL8 directs neutrophils to the inflammation site [49]. The RPS6 decreases signaling pathways of mTOR and Ras-Raf-ERK, and treatment with Lipopolysaccharide (LPS) could induce phosphorylation of the RPS6 via activation of the signaling pathway mTORC1-S6K [50]. During inflammation, the demand for protein synthesis increases, and numerous studies have shown that RPS6 plays a pivotal role in regulating this process [51].

2.2 Ribosomal protein S19: Structural features and functional roles in translation

The RPS19 gene, located on chromosome 19q13.2, consists of six exons and covers a region of 11 kilobases. The first exon, which is 372 base pairs long, is located within the 5' untranslated region (UTR). Similar to many other ribosomal protein-encoding mRNAs, it features a 13-nucleotide oligopyrimidine tract known as the terminal oligopyrimidine (TOP) sequence, which plays a key role in regulating the translation of the mRNA. The other five exons (435bp) encode a 145-amino acid (aa) protein (molecular weight [MW] 16kDa); the 30 UTR spans 40nt. Therefore, the entire RPS19 transcript is estimated to be 872 base pairs in length (GenBank NM_001022.3) [52].

The six new mutations (p.Phe21Ser, p.Trp52Cys, p.Thr76Pro, p.Ala135Thr, c.412delG, and c.417delA), four mutations are reported for the first time: p.

Mutation class	Mutations	Notes
New mutations (6 total)	p.Phe21Ser, p.Trp52Cys, p.Thr76Pro, p.Ala135Thr, c.412delG, c.417delA	Four reported for the first time: p.Phe21Ser, p.Trp52Cys, p.Thr76Pro, p.Ala135Thr
Class I	p.Val15Phe, p.Leu18Pro, p.Phe21Ser, p.Ala57Pro, p.Ala61Glu, p.Leu64Pro, p.Gly127Glu, p.Leu131Pro, p.Ala135Thr	Includes two new mutations: p.Phe21Ser, p.Ala135Thr
Class II	p.Pro47Leu, p.Trp52Arg, p.Trp52Cys, p.Arg56Gln, p.Ser59Phe, p.Arg62Trp, p.Arg62Gln, p.Arg101His, p.Gly120Ser	Located on the surface of RPS19; includes a new mutation, p.Trp52Cys

Table 2.
“Classification and characterization of RPS19 mutations including newly identified variants.”

Phe21Ser, p.Trp52Cys, p.Thr76Pro, and p.Ala135Thr. p.Trp52Cys and p.Thr76Pro. This class includes the variants p.Val15Phe, p.Leu18Pro, p.Phe21Ser, p.Ala57Pro, p.Ala61Glu, p.Leu64Pro, p.Gly127Glu, p.Leu131Pro, and p.Ala135Thr. Class II mutations – specifically p.Pro47Leu, p.Trp52Arg, p.Trp52Cys, p.Arg56Gln, p.Ser59Phe, p.Arg62Trp, p.Arg62Gln, p.Arg101His, and p.Gly120Ser – are positioned on the surface of RPS19.

[52] (**Table 2**).

Ribosomal protein S19 (RPS19) is a highly conserved 16-kDa protein that forms part of the 40S ribosomal subunit. Its structure, determined at high resolution in *Thermus thermophilus*, reveals a mixed α/β fold accompanied by flexible N and C-terminal tails. Positioned near the mRNA decoding site, RPS19 facilitates rRNA processing and 40S subunit assembly, with its C-terminal domain implicated in binding 18S rRNA. RPS19 controls translation initiation by binding to the 5' untranslated region (5'UTR) of its own mRNA, thereby regulating its own synthesis through a feedback loop. It further enhances translation of specific viral mRNAs by interacting with hantavirus nucleocapsid protein (N), which recruits the 40S subunit to viral transcripts through simultaneous engagement of the mRNA cap and conserved 5'UTR motifs. RPS19 enhances translation initiation efficiency by stabilizing the interaction between the 40S ribosomal subunit and the eukaryotic initiation factor eIF2 α . Mutations in RPS19 disrupt these functions, impairing ribosome biogenesis and contributing to DBA through defective erythropoiesis [53–57].

RPS19 is present in serum, where it interacts with various proteins and has the ability to modulate their function [58]. RPS19 interacts with chemokines such as MIF (macrophage migration inhibitory factor), which is involved in the regulation of migration of monocytes and T cells [59, 60]. This results in the disruption of the interaction between MIF and the receptors CD74 and CXCR2 [61]. Moreover, the RPS19 dimer acts as a ligand for the C5a receptor, also known as CD88 [61]. CD88 is reported to regulate the inflammatory process [62]. The review by Das et al. shows that recombinant RPS19 has the capacity to reduce ERK activation and inhibit the nuclear accumulation of NF- κ B [28].

Diamond-Blackfan anemia (DBA) is a genetic condition characterized by a failure to produce red blood cells, known as pure erythroid aplasia. Around 30%

of affected individuals present with congenital anomalies, most often affecting the hands, face, heart, and urogenital tract. Diamond–Blackfan anemia (DBA) exhibits an autosomal dominant inheritance pattern, with numerous cases resulting from de novo mutations, while familial cases display marked clinical variability. Approximately 25% of patients carry mutations in the ribosomal protein S19 (RPS19) gene, whereas alterations in other ribosomal protein genes – including RPS24, RPS17, RPL35A, RPL11, and RPL5 – are less frequent. These genes encode essential structural components of the ribosome. Although the precise cause of DBA remains unclear, there is a recognized connection between ribosomal dysfunction and erythroid aplasia. It is widely believed that impaired protein synthesis in rapidly dividing tissues like erythroid bone marrow underlies the defective red blood cell production. To date, 77 mutations of the RPS19 gene have been reported, most of which involve complete gene deletions, translocations, or truncating alterations such as nonsense and frameshift variants, supporting haploinsufficiency as the primary disease mechanism. Additionally, 22 missense mutations have been reported, with several studies providing functional analyses of these variants *in vitro*. This review summarizes the mutation data, offers a functional classification, and reports six novel mutations. Importantly, individuals harboring RPS19 mutations generally exhibit a reduced response to steroid therapy and experience worse long-term prognoses compared to other DBA cases [52].

An uncommon inherited disorder, DBA involves a failure to produce red blood cells and generally becomes evident during early infancy. Approximately 25% of affected individuals carry mutations in the ribosomal protein S19 (RPS19) gene, while variants in RPS24, RPS17, RPL35A, RPL11, and RPL5 have also been reported. The mechanism by which ribosomal protein deficiencies specifically impair red blood cell development in DBA remains unclear. To replicate the main ribosomal defect observed in DBA, a zebrafish *rps19* knockout was generated using TALEN-based gene editing. Molecular analysis revealed that loss of *rps19* recapitulates key DBA features, including impaired erythropoiesis and activation of p53. Importantly, *rps19* mutants exhibited significantly reduced globin protein synthesis despite unchanged or increased globin mRNA levels, a discrepancy also observed in zebrafish mutants lacking *rpl11*. Using transgenic zebrafish expressing mCherry specifically in erythroid cells, Zhang et al. found that mutations in either *rps19* or *rpl11* reduced protein production in these cells. Treatment with L-leucine enhanced protein synthesis and partially reversed anemia in both mutant models. These findings suggest that impaired protein synthesis in erythroid cells contributes to the blood-specific manifestations of DBA. The newly established *rps19* zebrafish model offers a valuable platform for investigating DBA pathogenesis and developing potential therapies [63].

Deficiencies in RPs lead to Diamond–Blackfan anemia (DBA), a disorder marked by anemia, congenital anomalies, and an elevated risk of cancer. Although p53 activation explains many of the clinical features of DBA, the contribution of the immune system remains poorly defined. In DBA, endogenous nucleic acids derived from unprocessed pre-rRNAs, along with DNA damage and apoptosis, can stimulate activation of the innate immune system. Recognition by toll-like receptors (TLRs) and Mda5-like sensors triggers interferon (IFN) production and inflammation, while dying cells may also stimulate the complement system. In studies of zebrafish lacking RPs, elevated levels of interferons, inflammatory cytokines, signaling molecules, and components of the

complement system were detected. Additionally, receptors involved in IFN signaling, including Mda5, Tlr3, and Tlr9, were upregulated. Members of the TGF- β family, including activin, were found to be elevated in both RP-deficient zebrafish and RPS19-deficient human cells, encompassing a lymphoid cell line from a DBA patient as well as fetal liver and K562 cells with RPS19 knockdown. In RP-deficient zebrafish, treatment with a TLR3 inhibitor reduced IFN activation, acute-phase responses, and apoptosis, thereby improving hematopoiesis and overall morphology. Inhibitors targeting complement and activin also produced beneficial effects. Collectively, these findings suggest that innate immune activation plays a contributory role in the pathology of RPS19-deficient zebrafish and human cellular models of DBA [64].

2.3 Extraribosomal functions of rpl13a in selective translation control and immune modulation

Ribosomal protein L13a (RPL13a) is a highly conserved component of the large 60S ribosomal subunit, characterized by a unique eukaryote-specific C-terminal α -helical extension of approximately 55 amino acids. This extension is essential for its integration into the ribosome during biogenesis, particularly at the 90S preribosome stage within the nucleolus. Structurally, the Arg68 residue within the N-terminal globular domain is essential for RPL13a's stable association with rRNA and ribosomal incorporation, while residues Arg59, Lys60, and Arg61 are crucial for its nuclear import. Although RPL13a is not essential for the canonical ribosomal role in global protein synthesis, it has a crucial extraribosomal function in the translational silencing of inflammatory mRNAs containing gamma interferon-activated inhibitor of translation (GAIT) elements. This occurs through its phosphorylation-dependent release from the 60S subunit, which prevents the recruitment of the 43S preinitiation complex to these target mRNAs. Additionally, RPL13a is essential for proper rRNA methylation, a process that facilitates internal ribosome entry site (IRES)-dependent translation of specific mRNAs. These dual functions position RPL13a as a critical regulator linking ribosome biogenesis, selective translation control, and the resolution of inflammation [65, 66].

RPL13a plays a pivotal role in the translation of pro-inflammatory genes in IFN- γ -stimulated monocyte cells [19]. Interferon- γ (IFN- γ) promotes the polarization of macrophages into the pro-inflammatory M1 phenotype [67], while it also mediates anti-inflammatory effects in M2 macrophages [68]. The active form of zipper-interacting protein kinase (ZIPK, also referred to as DAPK3) needs approximately 16 hours to phosphorylate RPL13a at a particular serine site [69]. This encourages the RPL13a releasing from the large subunits of the ribosome (60S subunits) [19, 70].

Gamma interferon-activated inhibitor of translation (GAIT) elements [71] are specific cis-acting RNA stem-loop structures located in the 3'-untranslated regions (3'-UTRs) of certain inflammatory mRNAs, such as ceruloplasmin, which mediate transcript-selective translational silencing in response to interferon-gamma (IFN- γ) stimulation [72, 73]. Upon IFN- γ activation in myeloid cells, ribosomal protein L13a (RPL13a) becomes phosphorylated and dissociates from the 60S ribosomal subunit. It then associates with glutamyl-prolyl-tRNA synthetase (EPRS), NS1-associated protein-1 (NSAP1), and GAPDH to assemble the heterotetrameric GAIT complex [72]. This complex binds to GAIT elements within target mRNAs and suppresses their translation by preventing the

recruitment of the 43S ribosomal pre-initiation complex, specifically through the disruption of eIF4G binding, thereby preventing excessive production of inflammatory proteins [72]. This post-transcriptional regulatory mechanism serves as a critical feedback system to resolve inflammation and protect tissues from injury by attenuating the synthesis of proinflammatory mediators during the late phase of the immune response [72–74].

Chemokines and certain kinases, such as VEGFA, CCL22, APOL2, CCR3, CCR4, CCR6, CXCL13, DAPK1, and ZIPK, are involved in mRNA encoding [73]. The expression of GAIT is reported to be modulated by IFN- γ , bacterial endotoxin (LPS), and proatherogenic high-fat diet (HFD) through modification processes such as phosphorylation — to moderately release the RPL13a in mouse macrophages [74, 75]. Knockout of the ribosomal protein RPL13a results in increased serum levels of CCL22, CXCL13, and CCL8 in LPS-treated mice [74]. The excision of RPL13a can increase the macrophage and neutrophil in the peritoneum and spleen [74]. A recent study demonstrated that deletion of RPL13a promotes M1 macrophage polarization in atherosclerotic ApoE knockout (ApoE $^{-/-}$) mice [75]. These data suggest for strong role of the RPL13a as a “extra-ribosomal” role as an endogenous molecule who can control the inflammation process.

2.4 Ribosomal protein L22 (RPL22) in ER stress, immune regulation, and inflammatory pathogenesis

The ribosomal protein L22 is assembled into the large 60S ribosomal subunit within the nucleolus [76]. Researchers have demonstrated that RPL22 is not essential for ribosome biogenesis. In T cells, loss of RPL22 leads to a developmental arrest of $\alpha\beta$ T cells, whereas $\gamma\delta$ T cells remain unaffected. This arrest is thought to be mediated by p53-induced ER stress, with p53 subsequently shown to activate PERK and ATF4 in RPL22-deficient $\alpha\beta$ T cells [77].

In RPL22-deficient cells, the absence of ribosomal protein L22 results in hyperactivation of the PERK (protein kinase RNA-like endoplasmic reticulum kinase) [78, 79] pathway, as evidenced by increased phosphorylation of PERK and its substrate eIF2 α , which, in turn, drives the translational induction of the transcription factor ATF4 (activating transcription factor 4) [77, 80]. Activation of the PERK–eIF2 α –ATF4 signaling cascade is a key component of the cellular response to ER stress. In RPL22-deficient cells, this pathway activation drives elevated expression of ATF4 target genes – including CHOP, ATF3, Trib3, and BiP – which contribute to cellular stress adaptation. However, in hematopoietic cells, this process can drive pathological effects such as heightened NF- κ B signaling and the development of leukemic transformation [77, 80]. Importantly, knockdown of PERK in RPL22-deficient cells abrogates ATF4 induction and its downstream effects, demonstrating that the PERK–ATF4 pathway is essential for mediating the cellular response to RPL22 loss [80].

Ribosomal protein L22 (RPL22) exerts an important regulatory function in inflammation by controlling the expression of key chemokines and cytokines that mediate immune cell recruitment and tissue responses. In particular, in LPS-activated myeloid cells, RPL22 binds to the 5' untranslated region (UTR) of CCL2 mRNA, facilitating its post-transcriptional degradation and thereby modulating monocyte infiltration during inflammatory reactions; loss of RPL22 leads to increased CCL2 expression and excessive monocyte migration, contributing to

chronic inflammation [20]. In the context of skin inflammation, such as psoriasis, RPL22 is upregulated in lesional keratinocytes, where its overexpression enhances keratinocyte proliferation, inhibits apoptosis, and increases the production of chemokines like CXCL10 and pro-inflammatory cytokines including IL-1 β , IL-23, and IL-17A, thus aggravating local inflammatory responses through recruitment of CD4⁺ T cells and amplification of cytokine signaling [81]. These findings highlight RPL22 as a key post-transcriptional regulator that both maintains immune homeostasis and, when dysregulated, can drive pathological inflammation. Up-frameshift-1 (UPF1) has been implicated in the suppression of multiple cancers, with reduced UPF1 levels associated with unfavorable outcomes. It is widely acknowledged as a central regulator of the nonsense-mediated mRNA decay (NMD) pathway [82]. Ribosomal protein L22 (RPL22) associates with the RNA helicase UPF1 in a LPS-dependent fashion to modulate inflammation by post-transcriptionally regulating the stability of CCL2 mRNA, an important chemokine responsible for recruiting monocytes. Upon inflammatory stimulation, both RPL22 and UPF1 translocate to the nucleus, where their interaction promotes the degradation of CCL2 mRNA in the cytoplasm, thereby fine-tuning monocyte infiltration and preventing excessive inflammation. This RPL22-UPF1 complex-mediated mRNA decay represents a critical mechanism to maintain immune homeostasis and resolve inflammation, as depletion of RPL22 results in increased CCL2 expression and exacerbated monocyte migration. Notably, the interaction between RPL22 and UPF1 is RNA-independent, highlighting a direct protein-protein regulatory axis in inflammatory gene expression control [20].

2.5 RACK1 as a multifunctional scaffold: Integrating ribosomal translation control with immune signaling and inflammation

RACK1 (Receptor for Activated C Kinase 1) is a highly conserved, multifunctional scaffolding protein belonging to the WD-repeat family, structurally characterized by a seven-bladed β -propeller that facilitates diverse protein-protein interactions. Initially identified as an intracellular receptor for protein kinase C [83]. RACK1 is currently recognized as an essential core component of the eukaryotic 40S ribosomal subunit [84]. Positioned on the solvent-exposed surface, it interacts with 18S rRNA and RPs, including uS3, uS9, and eS17. This strategic location enables RACK1 to coordinate translation regulation, including IRES-mediated translation, ribosome quality control, and stress responses. Beyond its ribosomal role, RACK1 acts as a dynamic hub linking signaling pathways – such as MAPK, Src, and protein kinase C – with translational machinery and cellular receptors, thereby integrating extracellular signals with protein synthesis [85]. It also participates in shuttling proteins within the cell, anchoring them at specific sites, and modulating transcriptional and epigenetic processes through nuclear translocation. Overall, RACK1 is crucial for cellular functions, including development, migration, immune responses, and circadian rhythm regulation, and its dysregulation is implicated in various diseases, including cancer [86].

Receptor for Activated C Kinase 1 (RACK1) serves a multifaceted role in the immune system as a scaffold protein that modulates key signaling pathways governing innate immunity and inflammation. It suppresses NF- κ B activation by disrupting the recruitment of the IKK complex to TRAF2, thereby regulating TNF-induced inflammatory cytokine production and preventing excessive inflammatory

responses. Furthermore, RACK1 facilitates activation of the NLRP3 inflammasome by promoting its active conformation and assembly, a process critical for IL-1 β maturation and secretion during innate immune responses. RACK1 also modulates antiviral immunity by interacting with the type I interferon receptor complex and STAT1, influencing interferon signaling; importantly, a deficiency of this protein in myeloid cells leads to increased production of type I interferons and provides *in vivo* resistance to viral infections. Furthermore, in plants, RACK1 functions within immune complexes and regulates reactive oxygen species production via NADPH oxidase, highlighting its conserved role across species in immune regulation. Collectively, these findings establish RACK1 as a critical regulator that balances immune activation and resolution to maintain homeostasis and protect against pathogens [87–90].

2.6 Diverse roles of ribosomal proteins RPL7, RPS3, RPL3, and RPS25 in immune regulation, host defense, and autoimmunity

Ribosomal proteins RPL7, RPS3, RPL3, and RPS25 play diverse and significant roles in the immune system beyond their canonical functions in protein synthesis. RPL7 acts as an autoantigen in systemic autoimmune diseases, including systemic lupus erythematosus (SLE), and its N-terminal basic leucine zipper-like domain enables binding to RNA and DNA, suggesting regulatory roles in translation and immune signaling [91–93]. RPS3 is a multifunctional protein that promotes innate immune responses through activation of NF- κ B signaling; however, certain pathogens, like *Escherichia coli* O157:H7, can suppress RPS3-mediated NF- κ B activation to evade host immunity, highlighting its critical role in host defense [94]. RPS25 specifically promotes the initiation of translation for viral mRNAs, affecting viral replication and immune escape, since reducing RPS25 levels inhibits viral translation without impacting overall protein synthesis [94, 95]. Although less directly studied in immunity, RPL3 has been implicated in ribosome function that may indirectly affect immune responses through regulation of protein synthesis during stress or infection [94]. Collectively, these RPs contribute to immune regulation by modulating inflammatory signaling, antiviral defense, and autoimmunity [94].

3. Regulatory roles of ribosomal proteins in the p53–MDM2 axis: Linking cellular stress, inflammation, and cancer development

The interaction between RPs, p53, and MDM2 plays a pivotal role in linking cellular stress responses to inflammation. Under normal conditions, p53 is restrained by MDM2 and MDM4, which promote its ubiquitin-dependent degradation and transcriptional repression. However, when ribosome biogenesis is disrupted – such as during cellular stress or inflammation – certain RPs (notably RPL5, RPL11, and S27a) bind to and inhibit MDM2, preventing it from degrading p53 and thereby stabilizing and activating p53 [96, 97]. This activation of p53 can lead to cell cycle arrest or apoptosis, acting as a protective mechanism against malignant transformation [98, 99]. In the context of chronic inflammation, cytokines like IL-6 can stimulate ribosome biogenesis, which paradoxically enhances MDM2-mediated degradation of p53 by reducing the availability of RPs

to bind MDM2, thus lowering p53 levels and promoting cellular changes associated with inflammation-driven tumorigenesis [100]. Therefore, the dynamic interplay between RPs, MDM2, and p53 not only serves as a cellular quality control system in response to ribosomal or metabolic stress but also influences inflammatory processes and contributes to the likelihood of cancer arising in tissues experiencing chronic inflammation [101, 102].

3.1 Ribosomal protein S27 (RPS27): A multifunctional regulator of antiviral immunity, inflammation, and p53-mediated stress responses

Ribosomal protein S27 (RPS27), also known as metallopanstimulin-1, is important in the immune system, where it influences antiviral defense mechanisms and inflammatory signaling pathways. In shrimp, RPS27 expression is upregulated upon viral infection and exerts antiviral effects by activating the NF- κ B pathway, which leads to increased expression of antimicrobial peptides and immune effectors; it also interacts directly with viral envelope proteins to inhibit virion assembly, thereby suppressing viral replication. In mammalian cells, RPS27 regulates immune-related gene expression and alternative splicing, with a particular impact on genes involved in inflammatory and immune responses, including those in the TLR signaling pathway. Additionally, RPS27 participates in the p53-MDM2 regulatory axis, stabilizing p53 and thus linking ribosomal stress to immune surveillance and inflammation. Collectively, these findings highlight RPS27 as a multifunctional ribosomal protein that integrates translational control with innate immune defense and inflammatory regulation [103–105].

3.2 Ribosomal protein L4 (RPL4): A key modulator of the immune microenvironment, viral infection, and cancer immunotherapy

Ribosomal protein L4 (RPL4) influences the immune system primarily through its role in remodeling the tumor immune microenvironment and modulating immune cell infiltration, which affects cancer progression and chemoresistance. Pan-cancer analyses reveal that RPL4 expression correlates with altered immune infiltration levels and impacts signaling pathways involved in immune regulation, such as those related to the tumor necrosis factor (TNF) receptor family member CD40. Additionally, RPL4 participates in ribosome biogenesis and translation regulation, processes that are crucial for immune cell function and response. Beyond its canonical ribosomal role, RPL4 exerts extra-ribosomal functions by interacting with key regulators like MDM2 and p53, influencing cell cycle arrest and apoptosis under stress, which can modulate immune surveillance and inflammation. Notably, RPL4 expression is upregulated during Epstein–Barr virus (EBV) infection in B lymphocytes, where it redistributes to the nucleus and may facilitate viral protein translation and immune evasion. The wide-ranging roles of RPL4 emphasize its promise as a biomarker as well as a therapeutic target in immunotherapy and cancer treatment [106–108].

3.3 Ribosomal protein L7 (RPL7): A multifaceted regulator in translation, autoimmunity, and host-pathogen interactions

Ribosomal protein L7 (RPL7) plays a pivotal role in the immune system, primarily functioning as an autoantigen associated with systemic autoimmune diseases such as

SLE. It possesses an N-terminal basic leucine zipper (BZIP)-like domain that facilitates RNA and DNA binding, enabling the regulation of translation through the inhibition of cell-free mRNA translation. The capacity of RPL7 to bind both 28S rRNA and mRNA indicates its role in modulating protein synthesis during immune responses. Moreover, RPL7 has been shown to interact with viral proteins such as HIV-1 Gag, indicating a role in host-pathogen interactions. Its recognition by autoreactive T cells highlights its contribution to autoimmune pathogenesis, where immune responses target RPL7, potentially leading to tissue damage. Thus, RPL7 functions both as a regulatory ribosomal protein and as a key player in immune tolerance and autoimmunity [91, 94].

4. Control of ribosomal RNA after infection of the cell by viruses

The viruses, as parasitic organisms with the ability to cause cell infections, are divided into two categories: DNA viruses and RNA viruses. This classification is based on genomic characterization. Over the last decade, RNA viruses have gained increased attention from researchers because they have been shown to possess the ability to adapt to host cells and exhibit strong pathogenicity[109–111]. RNA viruses are classified according to their genomic structure as double-stranded RNA viruses, positive-sense single-stranded RNA viruses, or negative-sense single-stranded RNA viruses.

By binding with cell membrane receptors, the viruses invade host cells and release the viral genome into the cell. After that, they start to use all mechanisms of translation initiation from the host cell to translate viral proteins, replicate their genome, and, in the end, complete the viral replication cycle. The ribosome, as a machine for protein synthesis, is formed by the assembly of RPs and rRNAs. It is known that not all RPs are involved in the maturation of the ribosome machine [112]. The size and composition of the ribosome can differ depending on the specific microenvironment [113]. Ribosome heterogeneity refers to all changes that occur based on the stoichiometry of rRNAs and RPs. This may occur during post-transcriptional and post-translational modifications [114–116]. Metabolic abnormalities in the body are linked to various ribosomopathies, including DBA, several types of cancer, and neurodevelopmental disorders [25, 117].

5. Strategies for viral RNA to bind with ribosomes in inflammation

Viruses have evolved sophisticated strategies to hijack host ribosomes and ensure translation of their own RNA during inflammation, when host cap-dependent translation is often inhibited. One key mechanism involves viral IRES, structured RNA elements within the 5' untranslated regions of viral genomes that directly recruit the 40S ribosomal subunit and initiate translation without dependence on the 5' cap or certain canonical initiation factors. Different classes of IRES elements, such as those found in picornaviruses, hepatitis C virus (HCV), and dicistroviruses, vary in structure and factor requirements but commonly remodel the ribosome to preferentially translate viral RNAs. Additionally, viral proteins can interact with and modulate host translation initiation factors (eIFs) and RNA-binding proteins (RBPs) to promote ribosome recruitment and stabilize IRES

structures, thereby enhancing viral protein synthesis during stress conditions. Some viruses also produce proteins that degrade or inhibit host mRNAs or translation factors to suppress host protein synthesis, further favoring viral RNA translation. These strategies allow viruses to bypass host translational control during immune responses and inflammation, ensuring efficient viral replication despite cellular antiviral defenses [113, 118–125].

6. Discussion

The data presented throughout this review collectively highlight the ribosome's fundamental and multifaceted role beyond its canonical function as the hub of cellular protein synthesis. Emerging evidence reveals that RPs operate as dynamic regulators at the intersection of translation, cell fate, and immune signaling. Several RPs, including RPS3, RPS6, RPS19, RPL13a, and RPL22, possess extra-ribosomal activities that profoundly influence inflammatory processes and disease pathogenesis.

Mutations or alterations in RP expression underpin a diversity of human disorders – ribosomopathies such as DBA, myelodysplastic syndromes, CA, and various cancers. Mechanistic studies demonstrate that RP dysfunction can elicit cellular stress responses, trigger innate immune activation, and dysregulate inflammatory signaling. For instance, RPS3 modulates NF- κ B signaling and interacts with inflammatory mediators in cancer; RPS6 undergoes phosphorylation-dependent control, impacting selective mRNA translation during inflammation; while RPS19 plays a crucial role in both ribosome biogenesis and immune regulation – substantiated by zebrafish DBA models linking impaired protein production to defective erythropoiesis and immune activation.

The review also delineates how RPs, such as RPL13a and RPL22 carry out selective translational silencing of inflammatory mRNAs, fine-tune chemokine responses, and maintain immune homeostasis, emphasizing the precision and specificity RPs bring to post-transcriptional regulation. Dysregulation of these processes can tip the balance toward chronic inflammation or autoimmunity, as shown in conditions like atherosclerosis and psoriasis.

Intriguingly, we observe that RPs serve as mediators of the crosstalk between ribosome biogenesis and key signaling axes, such as the p53-MDM2 network, linking ribosomal stress, cell cycle control, and tumorigenesis, particularly in inflammation-driven cancer contexts. The expanding repertoire of RP mutations and their clinical correlations – such as the novel RPS19 variants in DBA – underlines significant genotype-phenotype associations relevant for both prognosis and therapeutic intervention.

Finally, viral exploitation of the host's translational machinery via direct engagement with ribosomes or manipulation of ribosomal protein (RP) functions emerges as a critical determinant of infection, inflammation, and immune evasion. The ability of viruses to reprogram ribosome activity or bypass translational checkpoints highlights the ribosome itself as a central node in the host-pathogen interaction landscape.

Taken together, these findings position RPs as multifunctional molecules – integral not only to ribosome assembly and translational fidelity but also to the regulation of immune signaling and homeostatic responses. This duality underscores

the importance of further unraveling the molecular basis of RP-mediated pathways in health and disease.

7. Conclusion

Ribosomal proteins are increasingly recognized as pivotal modulators of inflammation, immunity, and cellular homeostasis, far beyond their classical roles in protein translation. Disruption of RP function, whether through genetic mutations, altered expression, or pathogenic hijacking, contributes to the onset and progression of ribosomopathies, malignancies, and chronic inflammatory diseases. The intricate roles of select RPs in regulating inflammatory signaling, immune cell differentiation, and the balance of cell proliferation and death highlight their therapeutic potential.

Understanding the dualistic and context-dependent functions of RPs – in both ribosome assembly and in non-canonical signaling pathways – opens new avenues for translational research and targeted therapy. Future investigations should focus on unraveling the precise molecular interactions and regulatory networks controlled by RPs, elucidating their utility as disease biomarkers, and harnessing their function for therapeutic intervention in inflammation and cancer. In sum, RPs stand at the crossroads of translation, immunity, and disease, representing a promising frontier for biomedical innovation.

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References

- [1] Pelletier J, Sonenberg N. The organizing principles of eukaryotic ribosome recruitment. *Annual Review of Biochemistry*. 2019;**88**:307–335.
- [2] Vadivel Gnanasundram S, Fåhræus R. Translation stress regulates ribosome synthesis and cell proliferation. *International Journal of Molecular Sciences*. 2018;**19**(12):3757.
- [3] Maehama T, Nishio M, Otani J, Mak TW, Suzuki A. Nucleolar stress: Molecular mechanisms and related human diseases. *Cancer Science*. 2023;**114**(5):2078–2086.
- [4] Russo A, Russo G. Ribosomal proteins control or bypass p53 during nucleolar stress. *International Journal of Molecular Sciences*. 2017;**18**(1):140.
- [5] Lu Y, Wang S, Jiao Y. The effects of deregulated ribosomal biogenesis in cancer. *Biomolecules*. 2023;**13**(11):1593.
- [6] Pavlov MY, Ehrenberg M. Substrate-induced formation of ribosomal decoding center for accurate and rapid genetic code translation. *Annual Review of Biophysics*. 2018;**47**(1):525–548. DOI: 10.1146/annurev-biophys-060414-034148.
- [7] Rodnina MV. Translation in prokaryotes. *Cold Spring Harbor Perspectives in Biology*. 2018;**10**(9):a032664.
- [8] Rodnina MV, Fischer N, Maracci C, and Stark H. Ribosome dynamics during decoding. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2011;**366**:160182.
- [9] Zaher HS, and Green R. Quality control by the ribosome following peptide bond formation. *Nature*. 2009;**457**(7226):161–6.
- [10] Garofalo R, Wohlgemuth I, Pearson M, Lenz C, Urlaub H, Rodnina MV. Broad range of missense error frequencies in cellular proteins. *Nucleic Acids Research*. 2019;**47**(6):2932–2945.
- [11] Loughran G, Chou MY, Ivanov IP, Jungreis I, Kellis M, Kiran AM, et al. Evidence of efficient stop codon readthrough in four mammalian genes. *Nucleic Acids Research*. 2014;**42**(14):8928–8938. DOI: 10.1093/nar/gku608.
- [12] Wohlgemuth I, Garofalo R, Samatova E, Günenç AN, Lenz C, Urlaub H, et al. Translation error clusters induced by aminoglycoside antibiotics. *Nature Communications*. 2021;**12**(1):1830.
- [13] Zhang Z, Shah B, Bondarenko PV. G/U and certain wobble position mismatches as possible main causes of amino acid misincorporations. *Biochemistry*. 2013;**52**(45):8165–8176. DOI: 10.1021/bi401002c.
- [14] Allan Drummond D, Wilke CO. The evolutionary consequences of erroneous protein synthesis. *Nature Reviews Genetics*. 2009;**10**(10):715–724.
- [15] Traverse CC, Ochman H. Conserved rates and patterns of transcription errors across bacterial growth states and lifestyles. *Proceedings of the National Academy of Sciences*. 2016;**113**(12):3311–3316. DOI: 10.1073/pnas.1525329113.

- [16] Zhang Y, O’Leary MN, Peri S, Wang M, Zha J, Melov S, et al. Ribosomal proteins Rpl22 and Rpl22l1 control morphogenesis by regulating pre-mRNA splicing. *Cell Reports*. 2017;**18**:545–556.
- [17] Zhang Y, Duc ACE, Rao S, Sun XL, Bilbee AN, Rhodes M, et al. control of hematopoietic stem cell emergence by antagonistic functions of ribosomal protein paralogs. *Developmental Cell* 2013;**24**:411–425.
- [18] Harris B, Perrigoue J, Kessel RM, Fahl S, Sykes SM, Verma A, et al. Rpl22 deficiency predisposes hematopoietic stem and progenitor cells to leukemogenesis. *Blood* 2015;**126**:899–899.
- [19] Mazumder B, Sampath P, Seshadri V, Maitra RK, DiCorleto PE, Fox PL. Regulated release of L13a from the 60S ribosomal subunit as a mechanism of transcript-specific translational control. *Cell*. 2003;**115**(2):187–198.
- [20] Das AS, Basu A, Kumar R, Borah PK, Bakshi S, Sharma M, et al. Post-transcriptional regulation of C-C motif chemokine ligand 2 expression by ribosomal protein L22 during LPS-mediated inflammation. *The FEBS Journal* 2020;**287**:3794–3813.
- [21] Ellis SR, Gleizes PE. Diamond blackfan anemia: Ribosomal proteins going rogue. *Seminars in Hematology*. 2011;**48**(2):89–96.
- [22] Fazen LE. Mandibulo-facial dysostosis: (Treacher-collins syndrome). *American Journal of Diseases of Children*. 1967;**113**(4):405.
- [23] Bolze A, Mahlaoui N, Byun M, Turner B, Trede N, Ellis SR, et al. Ribosomal protein SA haploinsufficiency in humans with isolated congenital asplenia. *Science* 2013;**340**:976–978.
- [24] Shenoy N, Kessel R, Bhagat TD, Bhattacharyya S, Yu Y, McMahon C, et al. Alterations in the ribosomal machinery in cancer and hematologic disorders. *Journal of Hematology & Oncology*. 2012;**5**(1):32. DOI: 10.1186/1756-8722-5-32.
- [25] Temaj G, Saha S, Dragusha S, Ejupi V, Buttari B, Profumo E, et al. Ribosomopathies and cancer: Pharmacological implications. *Expert Review of Clinical Pharmacology* 2022;**15**:729–746.
- [26] Temaj G, Chichiarelli S, Eufemi M, Altieri F, Hadziselimovic R, Farooqi AA, et al. Ribosome-Directed Therapies in Cancer. *Biomedicines* 2022;**10**:2088.
- [27] Basu A, Poddar D, Robinet P, Smith JD, Febbraio M, Baldwin WM, et al. Ribosomal protein L13a deficiency in macrophages promotes atherosclerosis by limiting translation control-dependent retardation of inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2014;**34**(3):533–542. DOI: 10.1161/ATVBAHA.113.302573.
- [28] Das AS, Basu A, Mukhopadhyay R. Ribosomal proteins: The missing piece in the inflammation puzzle? *Molecular & Cellular Biochemistry*. 2025;**480**(2):785–797. DOI: 10.1007/s11010-024-05050-9.
- [29] Kim J, Song B, Kim KH, and Moon Y. Ribosomal proteins mediate non-canonical regulation of gut inflammatory signature by crop contaminant deoxynivalenol.

Ecotoxicology & Environmental Safety. 2025;**289**:117645. DOI: 10.1016/j.ecoenv.2024.117645.

[30] Vyleta ML, Wong J, Magun BE. Suppression of ribosomal function triggers innate immune signaling through activation of the NLRP3 inflammasome. PLoS ONE. 2012;**7**(5): e36044. DOI: 10.1371/journal.pone.0036044.

[31] Watanabe M, Toyomura T, Wake H, Nishinaka T, Hatipoglu OF, Takahashi H, et al. Identification of ribosomal protein L9 as a novel regulator of proinflammatory damage-associated molecular pattern molecules. Molecular Biology Reports. 2022;**49**(4):2831–2838. DOI: 10.1007/s11033-021-07096-0.

[32] Watanabe M, Toyomura T, Wake H, Nishinaka T, Hatipoglu OF, Takahashi H, et al. Cationic ribosomal proteins can inhibit pro-inflammatory action stimulated by LPS+HMGB1 and are hindered by advanced glycation end products. Biotechnology and Applied Biochemistry. 2024;**71**(2):264–271. DOI: 10.1002/bab.2538.

[33] Lu B, Nakamura T, Inouye K, Li J, Tang Y, Lundbäck P, et al. Novel role of PKR in inflammasome activation and HMGB1 release. Nature. 2012;**488**(7413):670–4

[34] Moon Y. Ribosomal alteration-derived signals for cytokine induction in mucosal and systemic Inflammation: Noncanonical pathways by ribosomal inactivation. Mediators of Inflammation. 2014;**2014**:1–10.

[35] Kang J, Brajanovski N, Chan KT, Xuan J, Pearson RB, Sanij E. Ribosomal proteins and human diseases: Molecular mechanisms and targeted therapy.

Signal Transduction and Targeted Therapy. 2021;**6**(1):323.

[36] Narla A, Ebert BL. Ribosomopathies: Human disorders of ribosome dysfunction. Blood. 2010;**115**(16):3196–3205.

[37] Wan F, Anderson DE, Barnitz RA, Snow A, Bidere N, Zheng L, et al. Ribosomal protein S3: A KH domain subunit in NF- κ B complexes that mediates selective gene regulation. Cell. 2007;**131**:927–939.

[38] Youn H, Son B, Kim W, Jun SY, Lee JS, Lee J, et al. Dissociation of MIF-rpS3 complex and sequential NF- κ B activation is involved in IR-induced metastatic conversion of NSCLC. Journal of Cellular Biochemistry. 2015;**116**(11):2504–2516. DOI: 10.1002/jcb.25195.

[39] Stanborough T, Niederhauser J, Koch B, Bergler H, Pertschy B. Ribosomal protein S3 interacts with the NF- κ B inhibitor I κ B α . FEBS Letters. 2014;**588**(5):659–664. DOI: 10.1016/j.febslet.2013.12.034.

[40] Kim TS, Yi YW, Kim DJ, and Seong YS. A review on ribosomal protein S3 (RPS3): Roles in cancer and its resistance to drugs. International Journal of Biological Macromolecules. 2025;**318**:144955.

[41] Chen G, Gao X, Jia X, Wang Y, Xu L, Yu D, et al. Ribosomal protein S3 mediates drug resistance of proteasome inhibitor: Potential therapeutic application in multiple myeloma. haematol. 2024;**109**(4):1206–1219.

[42] Cui H, Jiang L, Zhou Y, Zhou Y, Li F, Zhang Z. Serine related gene CCT6A promotes metastasis of hepatocellular carcinoma via interacting with RPS3.

Functional & Integrative Genomics. 2024;**24**(6):225. DOI: 10.1007/s10142-024-01497-7.

[43] SL LM, SS LA, FN EIA, GM NSV, and Muranova TA. Crystal structure of the ribosomal protein S6 from *Thermus thermophilus*. *EMBO Journal* 1994;**13**(6):1249–54.

[44] Williams AJ, Werner-Fraczek J, Chang IF, Bailey-Serres J. Regulated phosphorylation of 40S ribosomal protein S6 in root tips of maize. *Plant Physiology*. 2003;**132**(4):2086–2097.

[45] Hutchinson JA, Shanware NP, Chang H, Tibbetts RS. Regulation of ribosomal protein S6 phosphorylation by casein kinase 1 and protein phosphatase 1. *Journal of Biological Chemistry*. 2011;**286**(10):8688–8696.

[46] Bohlen J, Roiuk M, Teleman AA. Phosphorylation of ribosomal protein S6 differentially affects mRNA translation based on ORF length. *Nucleic Acids Research*. 2021;**49**(22):13062–13074.

[47] Carriere A, Romeo Y, Acosta-Jaquez HA, Moreau J, Bonneil E, Thibault P, et al. ERK1/2 phosphorylate raptor to promote ras-dependent activation of mtor complex 1 (mTORC1). *Journal of Biological Chemistry* 2011;**286**:567–577.

[48] Ang Z, Koean RAG, Er JZ, Lee LT, Tam JKC, Guo H, et al. Novel AU-rich proximal UTR sequences (APS) enhance CXCL8 synthesis upon the induction of rpS6 phosphorylation. *PLOS Genetics*. 2019;**15**(4):e1008077. DOI: 10.1371/journal.pgen.1008077.

[49] De Oliveira S, Reyes-Aldasoro CC, Candel S, Renshaw SA, Mulero V, Â C. Cxcl8 (IL-8) mediates neutrophil

recruitment and behavior in the zebrafish inflammatory response. *The Journal of Immunology*. 2013;**190**(8):4349–4359.

[50] Lee PS, Wilhelmson ASK, Hubner AP, Reynolds SB, Gallacchi DA, Chiou TT, et al. mTORC1-S6K activation by endotoxin contributes to cytokine up-regulation and early lethality in animals. *PLoS ONE*. 2010;**5**(12):e14399. DOI: 10.1371/journal.pone.0014399.

[51] Ruvinsky I, Sharon N, Lerer T, Cohen H, Stolovich-Rain M, Nir T, et al. Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis. *Genes and Development*. 2005;**19**(18):2199–2211. DOI: 10.1101/gad.351605.

[52] Campagnoli MF, Ramenghi U, Armiraglio M, Quarello P, Garelli E, Carando A, et al. RPS19 mutations in patients with diamond-blackfan anemia. *Human Mutation* 2008;**29**:911–920.

[53] Suzuki K, Olvera J, Wool IG. The primary structure of rat ribosomal protein S19. *Biochimie*. 1990;**72**(4):299–302.

[54] Helgstrand M, Rak AV, Allard P, Davydova N, Garber MB, Härd T. Solution structure of the ribosomal protein S19 from *Thermus thermophilus* 1. Edited by Wright PE. *Journal of Molecular Biology*. 1999;**292**(5):1071–1081.

[55] Schuster J, Fröjmark AS, Nilsson P, Badhai J, Virtanen A, Dahl N. Ribosomal protein S19 binds to its own mRNA with reduced affinity in diamond-blackfan anemia. *Blood Cells, Molecules, and Diseases*. 2010;**45**(1):23–28.

[56] Cheng E, Haque A, Rimmer MA, Hussein ITM, Sheema S, Little A, et al. Characterization of the Interaction between Hantavirus Nucleocapsid

- Protein (N) and Ribosomal Protein S19 (RPS19). *Journal of Biological Chemistry* 2011;**286**:11814–11824.
- [57] Juli G, Gismondi A, Monteleone V, Caldarola S, Iadevaia V, Aspesi A, et al. Depletion of ribosomal protein S19 causes a reduction of rRNA synthesis. *Scientific Reports*. 2016;**6**(1)35026
- [58] Nishiura H, Tanase S, Tsujita K, Sugiyama S, Ogawa H, Nakagaki T, et al. Maintenance of ribosomal protein S19 in plasma by complex formation with prothrombin. *European Journal of Haematology*. 2011;**86**(5):436–441. DOI: 10.1111/j.1600-0609.2011.01585.x.
- [59] Bernhagen J, Krohn R, Lue H, Gregory JL, Zerneck A, Koenen RR, et al. MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Nature Medicine* 2007;**13**:587–596.
- [60] Filip AM, Klug J, Cayli S, Fröhlich S, Henke T, Lacher P, et al. Ribosomal protein S19 Interacts with macrophage migration inhibitory factor and attenuates its pro-inflammatory function. *Journal of Biological Chemistry* 2009;**284**:7977–7985.
- [61] Revollo I, Nishiura H, Shibuya Y, Oda Y, Nishino N, Yamamoto T. Agonist and antagonist dual effect of the cross-linked S19 ribosomal protein dimer in the C5a receptor-mediated respiratory burst reaction of phagocytic leukocytes. *Inflammation Research*. 2005;**54**(2):82–90. DOI: 10.1007/s00011-004-1327-4.
- [62] Brennan FH, Gordon R, Lao HW, Biggins PJ, Taylor SM, Franklin RJM, et al. The complement receptor C5aR controls acute inflammation and astrogliosis following spinal cord injury. *The Journal of Neuroscience*. 2015;**35**(16):6517–6531. DOI: 10.1523/JNEUROSCI.5218-14.2015.
- [63] Zhang Y, Ear J, Yang Z, Morimoto K, Zhang B, Lin S. Defects of protein production in erythroid cells revealed in a zebrafish Diamond–Blackfan anemia model for mutation in RPS19. *Cell Death and Disease*. 2014;**5**(7):e1352–e1352.
- [64] Danilova N, Wilkes M, Bibikova E, Youn MY, Sakamoto KM, Lin S. Innate immune system activation in zebrafish and cellular models of Diamond Blackfan Anemia. *Scientific Reports*. 2018;**8**(1):5165.
- [65] Das P, Basu A, Biswas A, Poddar D, Andrews J, Barik S, et al. Insights into the mechanism of ribosomal incorporation of mammalian L13a protein during ribosome biogenesis. *Molecular & Cellular Biology*. 2013;**33**(15):2829–2842. DOI: 10.1128/MC.B.00250-13.
- [66] Kour R, Komar AA, Mazumder B. Mutually exclusive amino acid residues of L13a are responsible for its ribosomal incorporation and translational silencing leading to resolution of inflammation. *RNA*. 2019;**25**(10):1377–1392. DOI: 10.1261/rna.071118.119.
- [67] Baxter VK, Griffin DE. Interferon-gamma modulation of the local T cell response to alphavirus encephalomyelitis. *Viruses*. 2020;**12**(1):113.
- [68] Lee AJ, Ashkar AA. The dual nature of type I and type II interferons. *Frontiers in Immunology*. 2018;**9**:2061. DOI: 10.3389/fimmu.2018.02061/full.

- [69] Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. *Antioxidants & Redox Signaling*. 2014;**20**(7):1126–1167. DOI: 10.1089/ars.2012.5149.
- [70] Mukhopadhyay R, Ray PS, Arif A, Brady AK, Kinter M, Fox PL. DAPK-ZIPK-L13a axis constitutes a negative-feedback module regulating inflammatory gene expression. *Molecular Cell*. 2008;**32**(3):371–382.
- [71] Mazumder B. GAITing the GUT. *Cellular and Molecular Immunology*. 2018;**15**(12):1082–1084.
- [72] Mukhopadhyay R, Jia J, Arif A, Ray PS, Fox PL. The GAIT system: A gatekeeper of inflammatory gene expression. *Trends in Biochemical Sciences*. 2009;**34**(7):324–331.
- [73] Arif A, Yao P, Terenzi F, Jia J, Ray PS, Fox PL. The GAIT translational control system. *WIREs RNA*. 2018;**9**(2): e1441. DOI: 10.1002/wrna.1441.
- [74] Poddar D, Basu A, Baldwin WM, Kondratov RV, Barik S, Mazumder B. An extraribosomal function of ribosomal protein L13a in macrophages resolves inflammation. *The Journal of Immunology*. 2013;**190**(7): 3600–3612.
- [75] Basu A, Dvorina N, Baldwin WM, Mazumder B. High-fat diet-induced GAIT element-mediated translational silencing of mRNAs encoding inflammatory proteins in macrophage protects against atherosclerosis. *The FASEB Journal*. 2020;**34**(5):6888–6906. DOI: 10.1096/fj.201903119R.
- [76] Ohmayer U, Gamalinda M, Sauert M, Ossowski J, Pöll G, Linnemann J, et al. Studies on the assembly characteristics of large subunit ribosomal proteins in *S. cerevisiae*. *PLoS ONE*. 2013;**8**(7):e68412. DOI: 10.1371/journal.pone.0068412.
- [77] Solanki NR, Stadanlick JE, Zhang Y, Duc AC, Lee SY, Lauritsen JPH, et al. Rpl22 loss selectively impairs $\alpha\beta$ T cell development by dysregulating endoplasmic reticulum stress signaling. *The Journal of Immunology* 2016;**197**:2280–2289.
- [78] Kemp K, Poe CS. The unfolded protein response in T cell development, activation, and function. *International Journal of Molecular Sciences*. 2019;**20**(7):1792.
- [79] Fahl SP, Sertori R, Zhang Y, Contreras AV, Harris B, Wang M, et al. Loss of ribosomal protein paralog Rpl22-like1 blocks lymphoid development without affecting protein synthesis. *The Journal of Immunology* 2022;**208**:870–880.
- [80] Patel NS, Kushnir NG, Rao S, Peri S, Rhodes M, Nagahawatte P, et al. Abstract 1269: Role of ribosomal protein, Rpl22 in regulating leukemic transformation. *Cancer Research*. 2015;**75**(15_Supplement):1269–1269
- [81] Zeng J, Zhang Y, Zhang H, Zhang Y, Gao L, Tong X, et al. RPL22 overexpression promotes psoriasis-like lesion by inducing keratinocytes abnormal biological behavior. *Frontiers in Immunology*. 2021;**12**:1269–1269.
- [82] Temaj G, Chichiarelli S, Telkoparan-Akillilar P, Saha S, Nuhii N, Hadziselimovic R, et al. Advances in molecular function of UPF1 in Cancer. *Archives of Biochemistry and Biophysics*. 2024;**756**: 1269–1269.

- [83] Adams DR, Ron D, Kiely PA. RACK1, A multifaceted scaffolding protein: Structure and function. *Cell Communication and Signaling*. 2011;**9**(1):22. DOI: 10.1186/1478-811X-9-22.
- [84] Coyle SM, Gilbert WV, Doudna JA. Direct link between RACK1 function and localization at the ribosome in vivo. *Molecular & Cellular Biology*. 2009;**29**(6):1626–1634. DOI: 10.1128/MC.B.01718-08.
- [85] Gandin V, Senft D, Topisirovic I, Ronai ZA. RACK1 function in cell motility and protein synthesis. *Genes and Cancer*. 2013;**4**(9–10):369–377. DOI: 10.1177/1947601913486348.
- [86] Ron D, Adams DR, Baillie GS, Long A, O'Connor R, Kiely PA. RACK(1) to the future – A historical perspective. *Cell Communication and Signaling*. 2013;**11**(1):53. DOI: 10.1186/1478-811X-11-53.
- [87] Nakashima A, Chen L, Thao NP, Fujiwara M, Wong HL, Kuwano M, et al. RACK1 functions in rice innate immunity by interacting with the Rac1 immune complex. *The Plant Cell* 2008;**20**: 2265–2279.
- [88] Yao F, Long LY, Deng YZ, Feng YY, Ying GY, Bao WD, et al. RACK1 modulates NF- κ B activation by interfering with the interaction between TRAF2 and the IKK complex. *Cell Research* 2014;**24**:359–371.
- [89] Duan Y, Zhang L, Angosto-Bazarra D, Pelegrín P, Núñez G, and He Y. RACK1 mediates NLRP3 inflammasome activation by promoting NLRP3 active conformation and inflammasome assembly. *Cell Reports*. 2020;**33**(7):1269–1269
- [90] Qin C, Niu C, Shen Z, Zhang Y, Liu G, Hou C, et al. RACK1 T50 phosphorylation by AMPK potentiates its binding with IRF3/7 and inhibition of type 1 IFN production. *The Journal of Immunology* 2021;**207**:1411–1418.
- [91] Donauer J, Wochner M, Witte E, Peter HH, Schlesier M, Krawinkel U. Autoreactive human T cell lines recognizing ribosomal protein L7. *International Immunology*. 1999;**11**(2):125–132. DOI: 10.1093/intimm/11.2.125.
- [92] Von Mikecz A, Hemmerich P, Peter HH, and Krawinkel U. Characterization of eukaryotic protein L7 as a novel autoantigen which frequently elicits an immune response in patients suffering from systemic autoimmune disease. *Immunobiology*. 1994;**192**(1–2):1269–1269
- [93] Wu WC, Liu HW, Lin A. Human ribosomal protein L7 displays an ER binding property and is involved in ribosome-ER association. *FEBS Letters*. 2007;**581**(4):651–657. DOI: 10.1016/j.febslet.2007.01.023.
- [94] Zhou X, Liao WJ, Liao JM, Liao P, Lu H. Ribosomal proteins: Functions beyond the ribosome. *Journal of Molecular Cell Biology*. 2015;**7**(2): 92–104. DOI: 10.1093/jmcb/mjv014.
- [95] Landry DM, Hertz MI, Thompson SR. RPS25 is essential for translation initiation by the *Dicistroviridae* and hepatitis C viral IRESs. *Genes and Development*. 2009;**23**(23):2753–2764. DOI: 10.1101/gad.1832209.
- [96] Deisenroth C, Franklin DA, Zhang Y. The evolution of the ribosomal protein-MDM2-p53 pathway. *Cold Spring Harbor Perspectives in Medicine*.

2016;**6**(12):a026138. DOI: 10.1101/cshperspect.a026138.

[97] Temaj G, Chichiarelli S, Telkoparan-Akillilar P, Saha S, Nuhii N, Hadziselimovic R, et al. P53: A key player in diverse cellular processes including nuclear stress and ribosome biogenesis, highlighting potential therapeutic compounds. *Biochemical Pharmacology*. 2024;**226**:1269–1269.

[98] Deisenroth C, Zhang Y. The ribosomal protein-Mdm2-p53 pathway and energy metabolism: Bridging the gap between feast and famine. *Genes and Cancer*. 2011;**2**(4):392–403. DOI: 10.1177/1947601911409737.

[99] Sun XX, DeVine T, Challagundla KB, Dai MS. Interplay between ribosomal protein S27a and MDM2 protein in p53 activation in response to ribosomal stress. *Journal of Biological Chemistry*. 2011;**286**(26):22730–22741.

[100] Brighenti E, Calabrese C, Liguori G, Giannone FA, Trerè D, Montanaro L, et al. Interleukin 6 downregulates p53 expression and activity by stimulating ribosome biogenesis: A new pathway connecting inflammation to cancer. *Oncogene* 2014;**33**:4396–4406

[101] Liu Y, Deisenroth C, Zhang Y. RP-MDM2-p53 pathway: Linking ribosomal biogenesis and tumor surveillance. *Trends in Cancer*. 2016;**2**(4):191–204.

[102] Lindström MS. the central role of ribosomal proteins in p53 regulation. *Cancers*. 2025;**17**(10):1597.

[103] Diao MQ, Li C, Xu JD, Zhao XF, Wang JX. RPS27, a sORF-encoded

polypeptide, functions antivirally by activating the NF-κB pathway and interacting with viral envelope proteins in shrimp. *Frontiers in Immunology*. 2019;**10**:2763. DOI: 10.3389/fimmu.2019.02763/full.

[104] Wan J, Lv J, Wang C, Zhang L. RPS27 selectively regulates the expression and alternative splicing of inflammatory and immune response genes in thyroid cancer cells. *Advances in Clinical and Experimental Medicine*. 2022;**31**(8):889–901.

[105] Xiong X, Zhao Y, He H, Sun Y. Ribosomal protein S27-like and S27 interplay with p53-MDM2 axis as a target, a substrate and a regulator. *Oncogene*. 2011;**30**(15):1798–1811.

[106] Liu Y, Li W, Zhou S, Cui M, and Zhang L. Pan-cancer analysis of the prognostic and immunological role of RPL4. *Heliyon*. 2024;**10**(14):1269–1269

[107] Shen CL, Liu CD, You RI, Ching YH, Liang J, Ke L, et al. Ribosome protein L4 is essential for Epstein-Barr virus nuclear antigen 1 function. *Proceedings of the National Academy of Sciences*. 2016;**113**(8):2229–2234. DOI: 10.1073/pnas.1525444113.

[108] Green L, Houck-Loomis B, Yueh A, Goff SP. Large ribosomal protein 4 increases efficiency of viral recoding sequences. *Journal of Virology*. 2012;**86**(17):8949–8958. DOI: 10.1128/JVI.01053-12.

[109] Colson P, Gautret P, Delerce J, Chaudet H, Pontarotti P, Forterre P, et al. The emergence, spread and vanishing of a French SARS-CoV-2 variant exemplifies the fate of RNA virus epidemics and obeys the Mistigri

- rule. *Journal of Medical Virology*. 2023;**95**(1):e28102. DOI: 10.1002/jmv.28102.
- [110] Liu T, Wang Y, Tan TJC, Wu NC, Brooke CB. The evolutionary potential of influenza A virus hemagglutinin is highly constrained by epistatic interactions with neuraminidase. *Cell Host & Microbe*. 2022;**30**(10):1363–1369.e4.
- [111] Tomar PPS, Krugliak M, Singh A, Arkin IT. Zika M—A potential viroporin: Mutational study and Drug Repurposing. *Biomedicines*. 2022;**10**(3):641
- [112] Hamidi T, Singh AK, Veland N, Vemulapalli V, Chen J, Hardikar S, et al. Identification of Rpl29 as a major substrate of the lysine methyltransferase Set7/9. *Journal of Biological Chemistry* 2018;**293**:12770–12780
- [113] Wang X, Zhu J, Zhang D, Liu G. Ribosomal control in RNA virus-infected cells. *Frontiers in Microbiology*. 2022;**13**. 1026887. DOI: 10.3389/fmicb.2022.1026887/full.
- [114] Genuth NR, Barna M. The Discovery of Ribosome Heterogeneity and Its Implications for Gene Regulation and Organismal Life. *Molecular Cell*. 2018;**71**(3): 364–374.
- [115] Cheng CA, Luo JM, Chiang MH, Fang KY, Li CH, Chen CW, et al. Nervous necrosis virus coat protein mediates host translation shutoff through nuclear translocalization and degradation of polyadenylate binding protein. *James Ou JH*. 2021;**95**(17):e02364–20. DOI: 10.1128/JVI.02364-20.
- [116] Emmott E, Jovanovic M, Slavov N. Ribosome stoichiometry: From form to function. *Trends in Biochemical Sciences*. 2019;**44**(2):95–109.
- [117] Segev N, Gerst JE. Specialized ribosomes and specific ribosomal protein paralogs control translation of mitochondrial proteins. *Journal of Cell Biology*. 2018;**217**(1):117–126.
- [118] Kieft JS. Viral IRES RNA structures and ribosome interactions. *Trends in Biochemical Sciences*. 2008;**33**(6):274–283.
- [119] Jaafar ZA, Kieft JS. Viral RNA structure-based strategies to manipulate translation. *Nature Reviews, Microbiology*. 2019;**17**(2):110–123.
- [120] Sarnow P. Viral internal ribosome entry site elements: Novel ribosome-RNA complexes and roles in viral pathogenesis. *Journal of Virology*. 2003;**77**(5):2801–2806. DOI: 10.1128/JVI.77.5.2801-2806.2003.
- [121] Chaudhary R, Meher A, Krishnamoorthy P, and Kumar H. Interplay of host and viral factors in inflammatory pathway mediated cytokine storm during RNA virus infection. *Current Research in Immunology*. 2023;**4**:1269–1269.
- [122] Stern-Ginossar N, Thompson SR, Mathews MB, Mohr I. Translational control in virus-infected cells. *Cold Spring Harbor Perspectives in Biology*. 2019;**11**(3):a033001. DOI: 10.1101/cshperspect.a033001.
- [123] Mazumder B, Li X, Barik S. Translation control: A multifaceted regulator of inflammatory response. *The Journal of Immunology*. 2010;**184**(7):3311–3319.

[124] Gokhale NS, Smith JR, Van Gelder RD, Savan R. RNA regulatory mechanisms that control antiviral innate immunity. *Immunological Reviews*. 2021;**304**(1):77–96. DOI: 10.1111/imr.13019.

[125] Bushell M, Sarnow P. Hijacking the translation apparatus by RNA viruses. *The Journal of Cell Biology*. 2002;**158**(3):395–399.

Section 3

Epigenetic Regulation in
Cardiovascular and Metabolic
Diseases

Perspective Chapter: Epigenetic Modifications in Stroke – Molecular Mechanisms and Therapeutic Targets

Afzal Misrani, Shaohua Qi, Conelius Ngwa and Fudong Liu

Abstract

In recent years, research on epigenetic modifications has expanded rapidly, highlighting their crucial role in regulating gene expression. This area of study emphasizes how epigenetic mechanisms guide natural evolutionary processes and preserve distinct gene expression profiles in mammals. Unlike genetics, which focuses on DNA sequences, epigenetics examines changes to genetic information beyond DNA sequence alterations. Numerous studies have investigated the link between epigenetic changes and central nervous system (CNS) disorders, providing insights into the complex nature of these modifications in such conditions. This review specifically explores epigenetic changes in stroke, emphasizing their impact on disease onset and progression. Additionally, it discusses sex differences in different types of epigenetic modifications. Furthermore, the review surveys the development of epigenetics-based therapeutic strategies for stroke. By leveraging our growing understanding of epigenetic mechanisms, researchers aim to pioneer new approaches for stroke treatment and prevention, offering promising avenues for improving patient outcomes and reducing the burden of this devastating disease.

Keywords: epigenetics, stroke, DNA methylation, histone modification, chromatin remodeler, non-coding RNA

1. Introduction

Stroke is the world's second most common cause of death after ischemic heart disease and remains the leading cause of long-term disability worldwide. Even with the growing accessibility of acute thrombolytic therapies like tissue plasminogen activator (tPA) and advanced endovascular procedures, stroke continues to pose a major challenge to healthcare systems [1]. Although mortality from stroke has gradually decreased with advancements in hyperacute interventions, many patients still experience long-term disability and require institutional care. In the United States, stroke imposes an annual economic burden of about \$34 billion, covering healthcare costs, medications, and lost productivity [2]. Thus, discovering novel

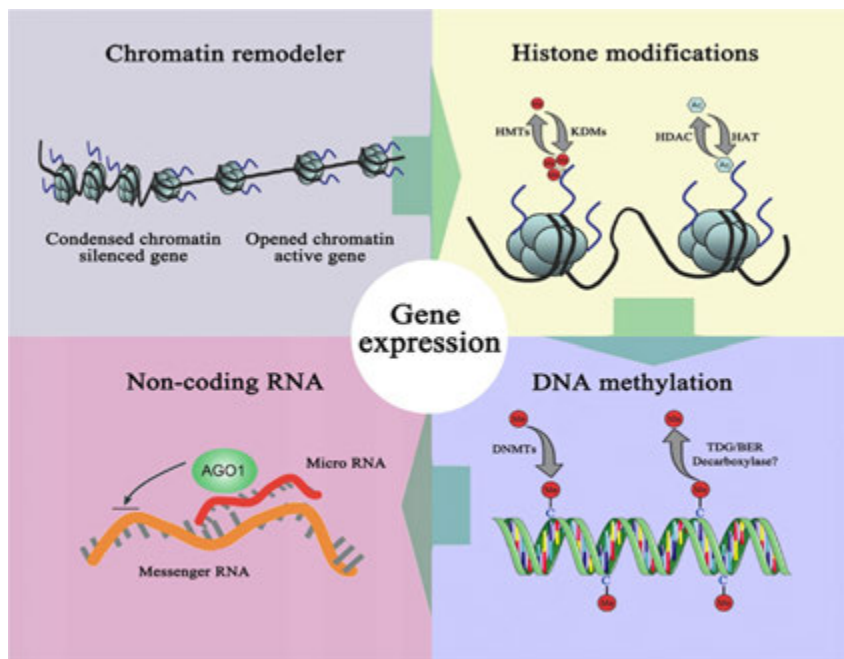


Figure 1.

Mechanistic diagram of four epigenetic modifications. Gene transcription is regulated by the combined effects of DNA methylation, histone modification, chromatin remodeling, and non-coding RNAs (ncRNAs). To initiate gene expression, chromatin must first loosen to allow DNA accessibility. Histone-modifying complexes add or remove groups (mainly on histone tails) to alter chromatin state and recruit regulatory proteins. DNA methylation, primarily at CpG islands, can either suppress or promote transcription. ncRNAs, via RNAi pathways, can silence target genes as needed (original figure created by the authors).

molecular pathways that can be targeted to promote neurological recovery in stroke survivors is of critical importance.

Recent research using cell lines and animal models strongly indicates that epigenetic factors contribute significantly to stroke development. These mechanisms exert substantial biological influence in the pathogenesis of many polygenic and multifactorial disorders, including stroke [3], and offer promise for research due to their potential reversibility through pharmaceutical intervention. Potential targets for epigenetic regulation include DNA methylation and demethylation, post-translational modifications of histones, and the activity of non-coding RNAs, particularly microRNAs (miRNAs) (**Figure 1**). Developing pharmaceuticals or cell-based therapies targeting these epigenetic alterations holds potential for promoting plasticity and enhancing long-term recovery. This review article aims to elucidate various epigenetic mechanisms and their roles in stroke pathogenesis. In addition, we will discuss the potential of epigenetic factors to serve as both therapeutic targets and biomarkers in stroke.

2. Sex differences in the epigenetic modifications in stroke

Biological sex, determined by genetic factors encoded in DNA, and gender, encompassing social, cultural, and psychological traits associated with being male or

female, both significantly influence health and disease outcomes. Stroke is a disease that exhibits clear differences between sexes in its clinical presentation and outcomes [4]. Younger women have a lower risk of stroke than their male counterparts, a disparity that is partially linked to the protective influence of estrogen [5]. However, this protective phenotype diminishes after menopause, with older women showing higher stroke incidence, increased mortality rates, and worse outcomes relative to age-matched men [6]. Epigenetics encompasses a variety of processes that modify gene expression without altering the DNA sequence itself. It is a key regulator of gene activity and is fundamental in creating sex-specific differences in mammals. Brain sexual differentiation starts before birth and persists throughout life, driven by a complex interaction among gonadal hormones, sex chromosomes, and environmental factors. These factors are thought to influence brain development, at least partially, by epigenetic mechanisms that alter chromatin structure and regulate transcriptional activity in neural cells. Consequently, epigenetic regulation is a key to orchestrating sexual differentiation in the brain from early development into adulthood. Experimental research targeting specific epigenetic pathways during the neonatal period has demonstrated that histone acetylation and DNA methylation play key roles in shaping sex-specific brain and behavioral development, especially in rodent models. Collectively, these studies underscore the critical importance of epigenetic mechanisms, particularly histone acetylation and DNA methylation, in guiding the developmental processes that drive sexual differentiation of the brain and behavior.

Adult females have been reported to exhibit about 30% higher methylation at a specific CpG site compared to males, with no significant sex difference observed during the neonatal period [7]. These differences are largely driven by hormonal regulation. Because hormones also affect epigenetic mechanisms, including DNA methylation, histone modifications, genomic imprinting, and microRNAs, it seems that epigenetic processes act in concert with gonadal hormones to maintain cellular homeostasis [8]. For example, estrogens contribute to the primary prevention of atherosclerosis in premenopausal women [9, 10]. Beyond hormonal effects, genetic and epigenetic influences also stem from the inheritance and differential dosage of genes located on the X and Y chromosomes [11]. Sex chromosomes lead to sex-specific differences in the expression of X–Y homologous genes in the brain, partly influenced by variations in the levels of epigenetic enzymes [12]. These findings point to mechanisms by which sex chromosomes shape differences in brain development and function [13]. Research on epigenetics and brain sexual differentiation has examined the genome-wide distribution of the histone mark H3K4me3 in sexually dimorphic brain regions of male and female mice [13]. Findings reveal potential sex differences in epigenetic marker usage, which may underlie sex-related variations in susceptibility to drugs or diseases affecting specific epigenetic pathways. Moreover, neonatal exposure to testosterone was found to masculinize DNA methylation patterns at numerous sexually dimorphic CpG sites in adulthood [14]. These epigenetic changes evolve over time and may be linked to aging and the modulation of cardiovascular disease risk [14].

Sex-specific differences in epigenetic markers may primarily serve to balance gene expression between males and females. Neural development diverges between sexes through processes like migration, apoptosis, and cell survival. Sex hormones influence the entire brain in both sexes via genomic and nongenomic receptors. Estrogen affects various neural and behavioral functions, including mood, cognition,

blood pressure regulation, motor coordination, pain perception, and opioid sensitivity [15]. A critical element is the neonatal surge of testosterone, which stimulates gene transcription via androgen receptors (AR) and, once converted to estradiol, through estrogen receptors (ER) [16]. Research indicates that AR and ER engage with histone-modifying enzymes, resulting in sex-specific methylation patterns of the ER α gene in the brains of rats following stroke [17]. Sex-specific differences have been reported in models of passive systemic anaphylaxis (PSA) and renal ischemia (RS). In PSA, female mice exhibited higher clinical scores, more pronounced hypothermia, and elevated serum histamine levels, while in RS, they showed increased intestinal permeability and heightened histamine responses compared to male mice [18]. These results underscore the interplay between genetic, hormonal, and epigenetic factors in shaping sex differences in stroke susceptibility, brain response, and recovery. Gaining insight into these mechanisms is essential for designing sex-specific approaches to stroke prevention and therapy.

3. Epigenetic modification and stroke

3.1 DNA methylation and its association with stroke

DNA methylation, an important epigenetic modification, controls gene expression by attaching methyl groups to the C-5 position of cytosine residues in DNA. This process was first identified around the same time that DNA was recognized as the genetic material in 1944 [19]. This modification is prevalent across various cell types and plays a role in processes like genomic imprinting [20], X-chromosome gene silencing [21], and age-related phenotypic changes [22, 23]. Methylating DNA within promoter regions diminishes its accessibility to binding proteins. Furthermore, other methylated sites may draw histone modification complexes, such as H3K9me3 methyltransferase, triggering additional histone modifications and ultimately silencing gene transcription [24]. However, DNA methylation can also be associated with increased gene expression depending on cell type and context [25]. Maintaining a balanced level of methylcytosine (5mC) across the genome is essential for healthy cell function. The DNA methyltransferase family, especially Dnmt1, preserves methylation patterns during cell division [26]. In 2011, Shinsuke et al. identified the Ten-Eleven Translocation 1 (TET) protein as responsible for converting 5mC to 5-hydroxymethylcytosine (5hmC), initiating DNA demethylation [27]. This pathway proceeds through the sequential oxidation of 5-hydroxymethylcytosine (5hmC) to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC), ultimately restoring cytosine through base excision repair facilitated by thymine DNA glycosylase (TDG) [28]. Early studies on DNA methylation focused on its role in gene regulation without altering the DNA sequence, such as random X-chromosome inactivation (XCI) in females [29]. Typically, methylation at CpG islands in promoter regions leads to gene silencing by recruiting repressors or inhibiting transcription factor binding [30].

The importance of DNA methylation in cellular processes has received growing focus, especially regarding embryonic development, XCI, and vascular conditions like stroke. Abnormal methylation patterns have been linked to worse stroke outcomes. In response to stroke, microglia and macrophages show altered methylation profiles, while DNMT1 and DNMT3a are critical for neuronal

depolarization, maturation, and survival [31]. After MCAO in mice, DNA methylation rises markedly in the ischemic striatum (4.1-fold) and cortex (3.2-fold), which is associated with more severe brain damage [32]. Treatment with DNA methylation inhibitors reduces ischemic damage [33], and research in *DnmtS*^{-/-} mice indicates that decreased methyltransferase activity offers protection in cases of mild cerebral ischemia [34]. Sex differences in DNMT expression have been observed, with females showing higher DNMT3b levels in the adult liver, and both DNMT3a and DNMT3b varying with age [35]. DNA methylation also serves as a “biological clock” for assessing age and predicting age-related disease risk. In comparison to young mice, aged mice exhibited increased hypermethylation in polycomb target genes as well as in multiple tumor suppressor genes [36], and the neuroprotective effects of estrogen are reduced with age, possibly due to methylation changes [37].

Several epigenetic drugs with DNMT-inhibitory effects have been explored for treating ischemic brain injury by targeting DNA hypermethylation. These include first-generation agents like 5-azacytidine, decitabine (5-aza-2'-deoxycytidine) [34], and zebularine [38]. These first-generation agents lack specificity and can cause significant toxicity. Second-generation drugs, including hydralazine, procainamide, and valproic acid, also influence DNA methylation, which may underlie both their therapeutic effects and associated side effects [39]. These drugs have shown potential not only in stroke but also in other CNS disorders [40], highlighting the significance of DNA methylation in tissue damage and recovery following ischemic stroke (Table 1).

3.2 Chromatin remodeling and its association with stroke

Genetic material is protected within chromatin, a structured complex of tightly packed DNA and histone proteins. During key cellular activities like DNA replication, transcription, and repair, specific regions of this compacted chromatin must unfold to provide enzyme access. The chromatin structure is like a dynamic spring, capable of adjusting its level of condensation based on the ongoing molecular activities within the nucleus. Far from being a passive entity, chromatin serves as an active mediator in nuclear processes, orchestrating both local and global structural and functional changes. Chromatin remodeling is essential for modifying nucleosome density, which relaxes the chromatin structure. This relaxation allows transcription factors to access DNA, promoting processes such as replication, transcription, and repair [73, 74]. Moreover, chromatin can adopt different hierarchical arrangements, ranging from open and functionally active euchromatin to densely packed, inactive heterochromatin, each maintaining genomic integrity in distinct ways [75]. By repositioning nucleosomes at specific sites, chromatin remodeling selectively controls the accessibility of genomic regulatory regions to transcription factors and other proteins. These local adjustments can also initiate wider chromatin remodeling events, emphasizing the complex and dynamic behavior of chromatin [76].

The regulation of a gene's expression (whether it is activated or silenced) depends on multiple factors, including histone modifications, nucleosome positioning, and the condition of the promoter and enhancer regions associated with the gene. Chromatin remodelers, acting as DNA translocases fueled by ATP, exert force to reposition nucleosomes, which are constantly in flux [77]. Chromatin remodelers equipped with Plant Homeodomain and bromodomains can detect histone

DNA methylation target	Methylation change after stroke	Reference
TIMP-2 promoter	Increased (hypermethylation)	[41]
TRAF3	Decreased (hypomethylation)	[42]
PPM1A	Decreased (hypomethylation)	[43, 44]
MTHFR promoter	Increased (hypermethylation)	[45]
Lef1 promoter	Increased (hypermethylation)	[46]
BDNF IV promoter	Decreased (hypomethylation)	[47, 48]
THBS1 promoter	Increased (hypermethylation)	[49]
ER α promoter	Decreased (hypomethylation)	[50]
Rhoa promoter	Increased (hypermethylation)	[51, 52]
Cdc42ep1 promoter	Decreased (hypomethylation)	[41, 53]
Wnt7b promoter	Decreased (hypomethylation)	[54, 55]
Fzd9 promoter	Decreased (hypomethylation)	[41, 56]
Foxo1 promoter	Decreased (hypomethylation)	[57, 58]
Klf8 promoter	Increased (hypermethylation)	[59]
Epha6 promoter	Increased (hypermethylation)	[41, 60]
Slit1 non-promoter	Increased (hypermethylation)	[41, 61]
Cldn5 promoter	Decreased (hypomethylation)	[62]
Ocln promoter	Decreased (hypomethylation)	[63, 64]
Ezr promoter	Decreased (hypomethylation)	[41, 65]
Myo3b promoter	Increased (hypermethylation)	[41, 66]
Sox17 promoter	Decreased (hypomethylation)	[67, 68]
Erg promoter	Decreased (hypomethylation)	[69]
Notch4 promoter	Decreased (hypomethylation)	[70]
THBS2	Decreased (hypomethylation)	[71]
LINE-1	Decreased (hypomethylation)	[72]

Table 1.

DNA methylation changes after stroke, showing the direction (increase or decrease) and supporting references for each gene.

modifications and adjust nucleosome positions accordingly, thereby influencing chromatin architecture. Moreover, certain histone modification marks attract specific chromatin restructuring complexes, enhancing the restructuring process and influencing gene expression through the “position effect” [78]. Nucleosome positioning is intricately regulated, with chromatin remodelers falling into four families in human cells: SWI/SNF, ISW1, CHD, and INO80 [79]. These complexes are vital for the normal growth and development of multicellular organisms as they modulate gene expression by altering chromatin structure [80]. Nucleosome remodeling targets specific gene loci, while higher-order chromatin formation affects broader genomic regions [76]. Abnormal chromatin regulators that disrupt normal chromatin structure are frequently associated with neurodevelopmental disorders [81] and cognitive impairments [82], and, in some cases, may also lead to weakened immune function [83].

Abnormal chromatin is a hallmark of both necrotic and apoptotic cell death in stroke-related neural injury. Although the impact of chromatin control in cerebral ischemia is not yet fully clarified, increasing research emphasizes its significance and the neuroprotective potential of chromatin-modifying agents. Histone modification enzymes and chromatin remodeling factors that alter chromatin structure may play a critical role in ischemic pathology. Notably, histone deacetylase inhibitors have helped identify new treatment strategies for stroke [84].

3.3 Histone modification and stroke

It is well known that nuclear DNA primarily associates with histones, forming chromosomes and chromatin [85]. Histones are composed of a central set of canonical histones (such as H2A, H2B, H3, and H4), linker histones (like H1), and histone variants (such as H2A.Z). Histone units can undergo depolymerization and reorganization both before and after the formation of DNA replication and transcription complexes [86]. In the nucleosome, histones come together to form octamers. The DNA then wraps around each octamer in a rope-like structure, completing approximately 1.67 turns, while some histone tails protrude outward [87]. The histone code refers to the specific patterns of chemical modifications added to histone proteins after translation, such as acetylation, methylation, phosphorylation, ubiquitination, SUMOylation, and ADP-ribosylation, each of which is mediated by dedicated enzymes [84]. During transcription, as DNA is ensconced within histone grooves, machinery containing RNA Pol II must locate and target the gene promoter region. Histones thus act as “gatekeepers,” influencing either the facilitation or suppression of DNA transcription. Histone modification occurs across nearly the entire amino acid sequence, with a focus on the histone tails, particularly at the N-terminals extending beyond the histone octamer core, though modifications can also occur within the octamer [88]. These chemical modifications, such as acetylation, methylation, ubiquitination, phosphorylation, and SUMOylation, regulate chromatin structure, influencing its compactness, flexibility, and stability, thereby affecting critical processes like transcription, DNA replication, and repair. Additionally, these histone tail modifications can recruit other functional complexes, collectively regulating gene expression.

To regulate gene expression effectively, both inhibitory and stimulatory factors need to be maintained at optimal levels. Acetylation is usually linked to turning genes on, whereas histone methylation can either enhance or inhibit gene expression, highlighting the need for a balanced regulation of these histone modifications. Histone alterations like H3K4me3 and H3K36me3 can boost gene expression by increasing transcription machinery access, while marks such as H3K9me3, H3K27me3, and H4K20me3 may result in decreased gene activity or transcriptional repression [89]. Histone modifications often interact with DNA methyltransferases and chromatin remodeling complexes, and some modification markers can recruit antagonistic complexes [90], collectively ensuring accurate and flexible gene expression. Even minor disruptions in these processes can lead to conditions like cancer [91, 92] or intellectual disability [93]. Cancer cells frequently exhibit a loss of active H3K4me3 and repressive H4K20me3, alongside an increase in repressive marks like H3K9me3 or H3K27me3 [94]. Methylation and acetylation represent the most widely investigated types of histone modifications, frequently occurring on lysine residues either together or in a mutually interchangeable manner.

Epigenetic regulatory processes are increasingly recognized as key modulators of ischemic stroke. In response to global ischemia, core histones H3 and H4 undergo deacetylation, while histone H3 at lysine-9 (H3K9) becomes dimethylated at the MOR-1 promoter. Reducing MOR-1 gene expression has been demonstrated to protect CA1 pyramidal neurons from ischemia-induced cell death [95]. Schweizer and colleagues demonstrated that blocking the activity of the histone methyltransferases SUV39H1 and G9a can protect neurons from oxidative damage caused by hypoxia and support their survival in an in vitro cerebral ischemia model [96]. Notably, multiple preclinical investigations have shown that treating animal models of cerebral ischemia with different HDAC inhibitors diminishes neuronal damage and improves functional recovery, indicating their potential as neuroprotective agents [97]. Research from the last 10 years indicates that HDAC inhibitors may be effective therapeutic agents for central nervous system disorders, including stroke [98]. Acetylation of histones at the Hsp70 promoter is associated with activating gene transcription and triggering signaling pathways that protect neurons from cell death [99]. In fact, brain tissue exposed to MCAO exhibits pronounced alterations in histone acetylation patterns [100]. Endres and colleagues reported that administering the deacetylase inhibitor trichostatin A (TSA) offered neuroprotection in mice, with TSA-treated animals showing a 48% reduction in infarct size after 45 minutes of MCAO and 48 hours of reperfusion [101]. Similarly, administering the MTase inhibitor 5-aza-2'-deoxycytidine along with the deacetylase inhibitor TSA provided neuroprotection against stroke in wild-type mice [34]. Additionally, histone acetylation helps shield neurons from oxidative damage by indirectly boosting the activity of neuroprotective antioxidant enzymes like peroxiredoxins [102]. Reports indicate that TSA by itself did not improve stroke recovery in *Hdac2* conditional knockout (*Hdac2* CKO) mice. However, administering suberoylanilide hydroxamic acid, a selective inhibitor of HDAC1 and HDAC2, during the delayed phase of stroke resulted in prolonged functional recovery in mice [103]. Clinical studies have associated treatment with valproic acid (VPA), a class I histone deacetylase inhibitor targeting HDACs 1, 2, 3, and 8, with a lower incidence of stroke [104]. Preclinical research also shows that post-stroke VPA treatment provides neuroprotection by reducing microglial activation, preserving hippocampal CA1 neurons, promoting neurogenesis, and improving cognitive function [105]. In subarachnoid hemorrhage (SAH) models, administering VPA after the injury enhanced H3 acetylation, lowered levels of apoptotic proteins, and alleviated vasospasm, indicating its potential neuroprotective role in hemorrhagic stroke [104]. Additionally, in rat MCAO models, overexpressing HDAC4 or HDAC5 led to reduced levels of the neuroprotective Na⁺/Ca²⁺ exchanger 3 (NCX3), whereas their knockdown caused its expression to rise. Histone acetylation promotes chromatin relaxation, facilitating transcription and protein synthesis, whereas deacetylation leads to chromatin condensation and gene suppression [106]. A recent proteomic study found decreased H3K9 acetylation in penumbra neurons within 24 hours of photothrombotic stroke in the rat cortex. Interestingly, even with this decline, levels of the histone acetyltransferases HAT1 and PCAF were elevated in the same model [107]. A further factor behind decreased histone acetylation in the ischemic penumbra could be the heightened activity of HDAC enzymes, which strip acetyl groups from histone lysines. Modifications like H4 acetylation enhance the activation of genes that support tissue regeneration and axonal repair. Higher AcH4 levels are linked to greater neuronal resilience following ischemia and better

functional recovery after stroke. Altogether, these observations underscore the promise of inhibiting histone deacetylation as a potential therapeutic approach for stroke.

3.4 Non-coding RNAs and their association with stroke

Non-coding RNAs (ncRNAs) are nucleic acid molecules transcribed from DNA within the nucleus that do not undergo translation into proteins. Among these, long non-coding RNAs (lncRNAs) constitute a subset of non-protein-coding transcriptomes widely distributed throughout the genome, pivotal in regulating cellular functions. Disruptions in their structure or turnover can lead to various diseases [108]. The well-explored repertoire of non-coding RNAs encompasses microRNAs (miRNAs), short interfering RNAs (siRNAs), piwi-interacting RNAs (piRNAs), and long non-coding RNAs (lncRNAs) [109]. While the family of ncRNAs is extensive, previously regarded as “junk RNAs,” recent studies have unveiled their pivotal roles in epigenetic gene regulation, influencing diverse biological processes [110]. A prominent mechanism through which ncRNAs modulate gene expression is interference RNA (RNAi) [111]. This pathway involves small double-stranded RNAs (dsRNA) and RNAi effectors like Dicer and Argonaute proteins. Dicer processes double-stranded RNA (dsRNA) into short interfering RNAs (siRNAs), which are subsequently loaded into RNA-induced silencing complexes (RISCs). Guided by siRNAs, Argonaute proteins within RISCs target and degrade mRNA, leading to translational inhibition [112]. This process not only controls gene expression but also acts as an effective method for post-transcriptional gene silencing. Recent research has underscored the crucial role of ncRNAs as master regulators of the epigenetic landscape, opening up new avenues for epigenetic investigations and promising therapeutic interventions [113]. The loss or modification of non-coding RNAs (ncRNAs) can lead to severe consequences such as tumorigenesis, neurological disorders, cardiovascular issues, developmental abnormalities, and other diseases [114]. For example, specific circulating miRNAs such as miR-208a, miR-1, miR-499, and miR-133 have been recognized as potential biomarkers for the early diagnosis and monitoring of ST-segment elevation in myocardial infarction [115]. The emergence of long non-coding RNAs (lncRNAs) across a spectrum of diseases indicates their potential as therapeutic targets for various cardiovascular and central nervous system disorders in the future [116].

Recent studies have revealed that microRNAs play a role in stroke pathophysiology, showing increased expression within brain tissue [117]. Genome-wide RNA-seq analyses conducted on mouse models of transient focal ischemia have revealed extensive changes in lncRNA expression within the cortex region, with transcript levels fluctuating during different reperfusion times [118]. Moreover, the involvement of lncRNAs in regulating endothelial responses to ischemic stress has been confirmed through in vitro experiments [119]. Following transient focal ischemia in rat brains, significant alterations in lncRNA expression were observed. Microarray analysis of lncRNAs revealed a subset that showed enhanced interaction with the corepressors of the key neuronal repressor RE-1 Silencing Transcription Factor (REST), namely Sin3A and coREST [120]. Moreover, lncRNAs have been implicated in anti-inflammatory functions within the brain microvasculature, mitigating ischemic cerebral vascular and parenchymal damages. Suppression of Malat1 using Malat1 GapmeR significantly elevated OGD-induced cell death and

Caspase 3 activity in brain microvascular endothelial cells [121]. lncRNAs play a key role in defining and preserving neural cell identity [122]. In human embryonic stem cells (hESCs), certain lncRNAs interact with SRY-related HMG-box 2 (SOX2) and the Polycomb Repressive Complex 2 (PRC2) subunit Suppressor of zeste 12 homolog (SUZ12), promoting effective and stable neuronal differentiation [123]. Additionally, modulating pro-inflammatory microRNAs like miR-125b and anti-inflammatory microRNAs such as miR-26a, miR-34a, miR-145, and let-7b has demonstrated beneficial effects on stroke recovery in preclinical studies [124, 125].

Non-coding RNAs (ncRNAs) have been recognized as crucial modulators of post-transcriptional gene regulation in stroke. Clinical studies have explored changes in long non-coding RNA (lncRNA) expression and genetic variations in stroke patients. In 2012, the Vemuganti lab used microarrays to analyze 8,314 lncRNAs in the rat cortex post-ischemia, identifying significant expression changes, 359 were upregulated and 84 downregulated after MCAO [126]. One such lncRNA, H19, known for its role in hypoxia-induced gene dysregulation and cancer, was elevated in circulation following stroke. Wang et al. found increased H19 in stroke patients and in mouse plasma, WBCs, and brain tissue post-MCAO. Inhibiting H19 protected neurons from oxygen-glucose deprivation, suggesting it may increase stroke risk and serve as a potential therapeutic target [127]. These findings highlight lncRNA dysregulation in stroke patients and suggest their potential as therapeutic targets for stroke therapy. However, unlike other medications, RNA lacks a suitable *in vivo* carrier for precise delivery to specific cells, necessitating further refinement of this treatment approach.

4. Crosstalk among different epigenetic modifications and therapeutic targets

Every type of epigenetic modification is dynamically regulated within cells, adapting to cues from changes in the cellular environment, including variations in nutrient availability, stress, hormone levels, and cellular injury [128]. Moreover, most genes, particularly those involved in cell differentiation and proliferation, are subject to multiple layers of regulatory mechanisms [129]. These mechanisms collaboratively ensure precise control of gene expression. For example, DNA methylation can stabilize histone modifications and influence chromatin architecture, ultimately resulting in the activation or repression of particular genes. At times, these mechanisms may even counterbalance each other to maintain homeostasis. Cooperative interactions between promotive histone modification markers and repressive chromatin structures can moderate gene expression levels or toggle genes “on and off” rapidly and efficiently [130]. Recent findings highlight the role of multiple epigenetic mechanisms, such as DNA methylation, histone modifications, and non-coding RNAs, in the development and progression of diverse cardiovascular diseases (**Table 2**). Consequently, the combined effects of these epigenetic modifications determine the ultimate outcome of gene transcription.

While epigenetic changes are vital for normal development and cellular stability, they can also contribute to disease. Dysregulated methylation of immune processes, for instance, has been linked to autoimmune disorders [141]. Given the reversible nature of epigenetic modifications, it is both necessary and feasible to intervene in these processes with epigenetic treatments. The primary focus of epigenetic

Epigenetic modification	Therapeutic targets/Strategies	References
DNA methylation	- DNMT inhibitors: 5-azacytidine, RG108, zebularine - Methyl donors: SAMe, folate - TET enzyme activation: Vitamin C	[131, 132]
Histone modification	- HDAC inhibitors: Valproic acid, SAHA, TSA, sodium butyrate - HAT activators: Curcumin - HDAC6 inhibitors: Tubastatin A (shown to reduce neuroinflammation in stroke)	[133–135]
Chromatin remodeling	- EZH2 inhibitors (e.g., GSK126): reduce neuroinflammation post-stroke - SWI/SNF complex (BRG1): modulates microglial response in stroke	[136, 137]
Non-coding RNAs	- miRNA mimics/inhibitors: miR-124 (promotes neurogenesis), miR-155 (pro-inflammatory, inhibited in stroke), miR-21 (involved in apoptosis) - lncRNA modulation: MEG3 (pro-apoptotic, inhibition beneficial), MALAT1 (dual role, context-dependent) - circRNAs: circDLGAP4 (protective in stroke model)	[138–140]

Table 2.
This table highlights epigenetic therapies shown to be effective in experimental stroke models, including inhibitors of DNA methylation, HDACs, and modulators of non-coding RNAs and chromatin remodeling.

therapies has been on DNA methylation and histone acetylation. Since these modifications are reversible, they present attractive opportunities for therapeutic intervention, with DNA methylation and histone acetylation being the most extensively investigated. These modifications not only offer therapeutic potential but may also serve as early diagnostic markers. In ischemic stroke, epigenetic changes may aid in tissue repair and influence the risk of recurrence [18]. Around two decades ago, Endres and colleagues observed increased DNA methylation levels after *in vivo* ischemia/reperfusion. Their study demonstrated that blocking DNA methyltransferase activity in these models could shield the brain from ischemic damage [34]. Furthermore, DNA methylation patterns are markedly altered during stroke. Earlier research has indicated that some drugs can lower DNA methylation levels, although they may also produce adverse effects [39]. Unlike histone acetylation and DNA methylation, studies on histone methylation in ischemic stroke remain limited. Nevertheless, these epigenetic modifications hold potential as therapeutic targets and diagnostic biomarkers. Utilizing epigenetic marks as biomarkers can enhance the identification of genetically high-risk populations, enabling earlier preventive measures and potentially reducing healthcare costs for patients.

Over the past decade, there has been a notable surge in the development of experimental tools aimed at detecting and modifying epigenetic changes, with the goal of preventing and treating associated diseases. For example, research has identified a link between DNA methyltransferase 1 (DNMT1) and histone deacetylases in the context of stroke [142]. Moreover, the HDAC inhibitor LBH589 has been found to induce DNMT1 degradation via the ubiquitin-proteasome pathway mediated by Hsp90, presenting a novel mechanism for HDAC inhibitors to downregulate DNMT1 [143]. Numerous potential epigenetic therapeutic agents, including DNMT inhibitors, histone acetyltransferase (HAT) and histone deacetylase (HDAC) inhibitors, histone methyltransferase (HMT) and histone

demethyltransferase (HDMT) inhibitors, as well as miRNA biomarkers, have been investigated and demonstrated translational value [144]. Notably, HDAC inhibitors have garnered attention for their potential role in leukemia treatment [145], with compounds like Trichostatin A (TSA) showing promise as anti-inflammatory agents [146].

5. Conclusion

The regulation of gene expression through epigenetic mechanisms is a complex and rapidly advancing area of research, with numerous processes yet to be fully elucidated. These modifications act synergistically to maintain transcriptional stability; however, their dysregulation can lead to abnormal gene expression and play a role in the development of diseases. Recognizing the central role of epigenetics in both normal development and pathogenesis opens new avenues for therapeutic interventions. Epigenetic therapies have proven effective in several cancers. However, treatment options for stroke are still limited, with recombinant tissue plasminogen activator (rtPA) being the only approved drug therapy [147]. Considering the essential function of epigenetic modifications in regulating gene expression, there is growing interest in developing epigenetic-based therapies for ischemic stroke. These modifications offer novel molecular targets for stroke diagnosis, neuroprotection, and tissue repair. Nonetheless, challenges remain regarding therapeutic specificity. For instance, histone deacetylase inhibitors (HDACi) affect a wide range of targets, which can lead to off-target effects and unintended outcomes. Nonetheless, advances in CRISPR/Cas9 genome editing and high-throughput sequencing technologies are enhancing our knowledge of how epigenetic modifications interact with gene regulation and contribute to disease. These tools hold great promise for advancing molecular diagnostics and personalized treatment strategies.

Furthermore, an expanding body of evidence suggests that epigenetic pathways are vital in vascular biology and may influence the heritability of cardiovascular diseases. The impact of epigenetic modifications, particularly intragenic chromatin changes, on transcription remains underappreciated in current genome-wide studies. Numerous epigenetic compounds are being explored for their ability to improve neuronal survival, facilitate brain tissue regeneration, and aid functional recovery after stroke. Epigenetic mechanisms are central to neuron development, homeostasis, and plasticity. When combined with conventional treatments, epigenetic therapies may allow for reduced drug dosages, minimized side effects, and enhanced overall quality of life for individuals recovering from stroke.

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Conflict of interest

None

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References

- [1] Cheng Y, et al. Projections of the stroke burden at the global, regional, and national levels up to 2050 based on the global burden of disease study 2021. *Journal of the American Heart Association*. 2024;**13**(23):e036142. DOI: 10.1161/JAHA.124.036142.
- [2] Gerstl JVE, et al. Global, regional, and national economic consequences of stroke. *Stroke*. 2023;**54**(9):2380–2389. DOI: 10.1161/STROKEAHA.123.043131.
- [3] Peng J, et al. Advancement of epigenetics in stroke. *Frontiers in Neuroscience*. 2022;**16**:981726. DOI: 10.3389/fnins.2022.981726.
- [4] Hanna M, Wabnitz A, Grewal P. Sex and stroke risk factors: A review of differences and impact. *Journal of Stroke & Cerebrovascular Diseases*. 2024; **33**(4):107624. DOI: 10.1016/j.jstrokecerebrovasdis.2024.107624.
- [5] Bushnell C, et al. Guideline for the primary prevention of stroke: A guideline from the american heart association/american stroke association. *Stroke*. 2024;**55**(12):e344–e424. DOI: 10.1161/STR.0000000000000475.
- [6] Bushnell CD, Kapral MK. 2023. Stroke in women and unique risk factors. *Stroke*. **54**(2):587–590. DOI: 10.1161/STROKEAHA.122.041734.
- [7] Shealy EP, et al. DNA methylation-based age prediction and sex-specific epigenetic aging in a lizard with female-biased longevity. *Science Advances*. 2025;**11**(5):eadq3589. DOI: 10.1126/sciadv.adq3589.
- [8] Vazquez MJ, Daza-Duenas S, Tena-Sempere M. 2021. Emerging roles of epigenetics in the control of reproductive function: Focus on Central Neuroendocrine Mechanisms. *Journal of the Endocrine Society*. 5(11):bvab152. DOI: 10.1210/jendso/bvab152.
- [9] Machuca JN, Rosales-Alvarez CP. 2024. Cardiovascular Disease In Women And The Role Of Hormone Replacement Therapy. *Cureus*. **16**(9):e69752. DOI: 10.7759/cureus.69752.
- [10] Hakamaa E, et al. Sex differences in coronary atherosclerosis during the pre- and postmenopausal period: The Tampere Sudden Death Study. *Atherosclerosis*. 2024;**390**:117459. DOI: 10.1016/j.atherosclerosis.2024.117459.
- [11] Cabrera Zapata LE, et al. Genetics and epigenetics of the x and y chromosomes in the sexual differentiation of the brain. *International Journal of Molecular Sciences*. 2022;**23**(20):12288. DOI: 10.3390/ijms232012288.
- [12] Lemos B, Branco AT, Hartl DL. 2010. Epigenetic effects of polymorphic Y chromosomes modulate chromatin components, immune response, and sexual conflict. *Proceedings of the National Academy of Sciences of the United States of America*. **107**(36):15826–15831. DOI: 10.1073/pnas.1010383107.
- [13] Kundakovic M, Tickerhoof M. 2024. Epigenetic mechanisms underlying sex differences in the brain and behavior. *Trends in Neurosciences*. **47**(1):18–35. DOI: 10.1016/j.tins.2023.09.007.
- [14] Ghahramani NM, et al. The effects of perinatal testosterone exposure on

the DNA methylome of the mouse brain are late-emerging. *Biology of Sex Differences*. 2014;5:8.

[15] Mosconi L, et al. In vivo brain estrogen receptor density by neuroendocrine aging and relationships with cognition and symptomatology. *Scientific Reports*. 2024;14(1):12680. DOI: 10.1038/s41598-024-62820-7.

[16] Fuentes N, and Silveyra P. Chapter Three - Estrogen receptor signaling mechanisms. In Donev R, editor. *Advances in Protein Chemistry and Structural Biology*. Academic Press; 2019. p. 135–170 DOI: 10.1016/bs.apcsb.2019.01.001.

[17] Westberry JM, Prewitt AK, Wilson ME. 2008. Epigenetic regulation of the estrogen receptor alpha promoter in the cerebral cortex following ischemia in male and female rats. *Neuroscience*. 152(4):982–989. DOI: 10.1016/j.neuroscience.2008.01.048.

[18] Mackey E, et al. Sexual dimorphism in the mast cell transcriptome and the pathophysiological responses to immunological and psychological stress. *Biology of Sex Differences*. 2016;7:60

[19] Avery OT, Macleod CM, McCarty M. Studies on the chemical nature of the substance inducing transformation of pneumococcal types: induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type iii. *The Journal of Experimental Medicine*. 1944;79(2):137–158.

[20] Rosenski J, et al. Atlas of imprinted and allele-specific DNA methylation in the human body. *Nature Communications*. 2025;16(1):2141. DOI: 10.1038/s41467-025-57433-1.

[21] Graves JAM. 2025. Imprinting of the paternal marsupial X chromosome by DNA methylation. *Trends in Genetics*. 41(1):9–11. DOI: 10.1016/j.tig.2024.10.011.

[22] Chan J, Rubbi L, Pellegrini M. 2025. DNA methylation entropy is a biomarker for aging. *Aging (Albany, NY)*. 17(3):685–698. DOI: 10.18632/aging.206220.

[23] Olecka M, et al. Nonlinear DNA methylation trajectories in aging male mice. *Nature Communications*. 2024;15(1):3074. DOI: 10.1038/s41467-024-47316-2.

[24] Buonocore F, et al. Transcriptomic sex differences in early human fetal brain development. *Communications Biology*. 2025;8(1):664. DOI: 10.1038/s42003-025-08070-3.

[25] Yang X, et al. Gene body methylation can alter gene expression and is a therapeutic target in cancer. *Cancer Cell*. 2014;26(4):577–590. DOI: 10.1016/j.ccr.2014.07.028.

[26] Cheng X, Blumenthal RM. 2008. Mammalian DNA methyltransferases: A structural perspective. *Structure*. 16(3):341–350. DOI: 10.1016/j.str.2008.01.004.

[27] Dawlaty MM, et al. Tet1 is dispensable for maintaining pluripotency and its loss is compatible with embryonic and postnatal development. *Cell Stem Cell*. 2011;9(2):166–175. DOI: 10.1016/j.stem.2011.07.010.

[28] Cortellino S, et al. Thymine DNA glycosylase is essential for active DNA demethylation by linked deamination-base excision repair. *Cell*. 2011;146

- (1):67–79. DOI: 10.1016/j.cell.2011.06.020.
- [29] Zore T, Palafox M, Reue K. Sex differences in obesity, lipid metabolism, and inflammation—A role for the sex chromosomes?. *Molecular Metabolism*. 2018;**15**:35–44. 10.1016/j.molmet.2018.04.003.
- [30] Cappelluti MA, et al. Durable and efficient gene silencing in vivo by hit-and-run epigenome editing. *Nature*. 2024;**627**(8003):416–423. DOI: 10.1038/s41586-024-07087-8.
- [31] Guida N, et al. Stroke causes DNA methylation at ncx1 heart promoter in the brain via dnmt1/mecp2/rest epigenetic complex. *Journal of the American Heart Association*. 2024;**13**(6):e030460. DOI: 10.1161/JAHA.123.030460.
- [32] Endres M, et al. Effects of cerebral ischemia in mice lacking DNA methyltransferase 1 in post-mitotic neurons. *Neuroreport* 2001;**12**:3763–3766.
- [33] Dock H, Theodorsson A, Theodorsson E. 2015. DNA methylation inhibitor zebularine confers stroke protection in ischemic rats. *Translational Stroke Research*. **6**(4):296–300. DOI: 10.1007/s12975-015-0397-7.
- [34] Endres M, et al. DNA methyltransferase contributes to delayed ischemic brain injury. *The Journal of Neuroscience*. 2000;**20**(9):3175–3181. DOI: 10.1523/JNEUROSCI.20-09-03175.2000.
- [35] Xiao Y, et al. Age and gender affect DNMT3a and DNMT3b expression in human liver. *Cell Biology & Toxicology*. 2008;**24**(3):265–272. DOI: 10.1007/s10565-007-9035-9.
- [36] Maegawa S, et al. Widespread and tissue specific age-related DNA methylation changes in mice. *Genome Research*. 2010;**20**(3):332–340. DOI: 10.1101/gr.096826.109.
- [37] Sunday L, et al. Age alters cerebrovascular inflammation and effects of estrogen. *American Journal of Physiology-Heart and Circulatory Physiology*. 2007;**292**(5):H2333–40. DOI: 10.1152/ajpheart.01057.2006.
- [38] Miller CA, Sweatt JD. 2007. Covalent modification of DNA regulates memory formation. *Neuron*. **53**(6):857–869. DOI: 10.1016/j.neuron.2007.02.022.
- [39] Csoka AB, Szyf M. 2009. Epigenetic side-effects of common pharmaceuticals: A potential new field in medicine and pharmacology. *Medical Hypotheses*. **73**(5):770–780. DOI: 10.1016/j.mehy.2008.10.039.
- [40] Gros C, et al. DNA methylation inhibitors in cancer: Recent and future approaches. *Biochimie*. 2012;**94**(11):2280–2296. DOI: 10.1016/j.biochi.2012.07.025.
- [41] Phillips CM, et al. Epigenetics and stroke: Role of DNA methylation and effect of aging on blood-brain barrier recovery. *Fluids and Barriers of the CNS*. 2023;**20**(1):14. DOI: 10.1186/s12987-023-00414-7.
- [42] Zhou Y, et al. TRAF3 mediates neuronal apoptosis in early brain injury following subarachnoid hemorrhage via targeting TAK1-dependent MAPKs and NF-kappaB pathways. *Cell Death and Disease*. 2021;**12**(1):10. DOI: 10.1038/s41419-020-03278-z.
- [43] Krupinski J, et al. DNA methylation in stroke. Update of latest

advances. Computational and Structural Biotechnology Journal. 2018;**16**:1–5.

[44] Feng D, et al. Astrocytic NDRG2-PPM1A interaction exacerbates blood-brain barrier disruption after subarachnoid hemorrhage. Science Advances. 2022;**8**(39):eabq2423. DOI: 10.1126/sciadv.abq2423.

[45] Xu S, et al. High MTHFR promoter methylation levels in men confer protection against ischemic stroke. Bosnian Journal of Basic Medical Sciences. 2020;**20**(4):477–486. DOI: 10.17305/bjbms.2020.4636.

[46] Yadava S, et al. Unravelling neuroregenerative and neuroprotective roles of WNT/beta-catenin pathway in ischemic stroke: Insights into molecular mechanisms. Neuroscience. 2025;**565**:527–547.

[47] Overberg LT, et al. Plasma levels of BDNF and EGF are reduced in acute stroke patients. Heliyon. 2022;**8**(6):e09661. DOI: 10.1016/j.heliyon.2022.e09661.

[48] Qiao HJ, et al. Association of lower serum brain-derived neurotrophic factor levels with larger infarct volumes in acute ischemic stroke. Journal of Neuroimmunology. 2017;**307**:69–73.

[49] Liu C, et al. THBS1 in macrophage-derived exosomes exacerbates cerebral ischemia-reperfusion injury by inducing ferroptosis in endothelial cells. Journal of Neuroinflammation. 2025;**22**(1):48. DOI: 10.1186/s12974-025-03382-x.

[50] Pradhyumn H, et al. Post-stroke periodic estrogen receptor-beta agonist improves cognition in aged female rats. Neurochemistry International.

2023;**165**:105521. DOI: 10.1016/j.neuint.2023.105521.

[51] Lu W, Chen Z, Wen J. The role of RhoA/ROCK pathway in the ischemic stroke-induced neuroinflammation. Biomedicine & Pharmacotherapy. 2023;**165**. 115141. DOI: 10.1016/j.biopha.2023.115141.

[52] Pinosanu EA, et al. Rhoa/rock, mtor and secretome-based treatments for ischemic stroke: New perspectives. Current Issues in Molecular Biology 2024;**46**(4):3484–3501. DOI: 10.3390/cimb46040219.

[53] Yu H, Ji X, Ouyang Y. 2023. Unfolded protein response pathways in stroke patients: A comprehensive landscape assessed through machine learning algorithms and experimental verification. Journal of Translational Medicine. **21**(1):759. DOI: 10.1186/s12967-023-04567-9.

[54] Mo Z, et al. Activation of Wnt/beta-catenin signaling pathway as a promising therapeutic candidate for cerebral ischemia/reperfusion injury. Frontiers in Pharmacology. 2022;**13**:914537. DOI: 10.3389/fphar.2022.914537.

[55] Callegari K, et al. Molecular profiling of the stroke-induced alterations in the cerebral microvasculature reveals promising therapeutic candidates. Proceedings of the National Academy of Sciences of the United States of America. 2023;**120**(16):e2205786120. DOI: 10.1073/pnas.2205786120.

[56] Song S, et al. Activation of endothelial Wnt/beta-catenin signaling by protective astrocytes repairs BBB damage in ischemic stroke. Progress in

- Neurobiology. 2021;**199**:101963. DOI: 10.1016/j.pneurobio.2020.101963.
- [57] Guo S, et al. Role of forkhead box protein o1 (foxo1) in stroke: A literature review. *Aging and Disease*. 2022;**13**(2):521–533. DOI: 10.14336/AD.2021.0826.
- [58] Wang B, et al. Foxo1 silencing facilitates neurological function recovery in intracerebral hemorrhage mice via the lncrna gas5/mir-378a-5p/hspa5 axis. *Journal of Stroke & Cerebrovascular Diseases*. 2022;**31**(7):106443. DOI: 10.1016/j.jstrokecerebrovasdis.2022.106443.
- [59] Pollak NM, et al. Kruppel-like factors: Crippling and un-crippling metabolic pathways. *JACC: Basic to Translational Science*. 2018;**3**(1):132–156. DOI: 10.1016/j.jacbs.2017.09.001.
- [60] Coulthard MG, et al. Eph/Ephrin signaling in injury and inflammation. *The American Journal of Pathology*. 2012;**181**(5):1493–1503. DOI: 10.1016/j.ajpath.2012.06.043.
- [61] Sherchan P, et al. The potential of Slit2 as a therapeutic target for central nervous system disorders. *Expert Opinion on Therapeutic Targets*. 2020;**24**(8):805–818. DOI: 10.1080/14728222.2020.1766445.
- [62] Hashimoto Y, et al. The CLDN5 gene at the blood-brain barrier in health and disease. *Fluids and Barriers of the CNS*. 2023;**20**(1):22. DOI: 10.1186/s12987-023-00424-5.
- [63] Torices S, et al. Mitochondrial antiviral pathways control anti-hiv responses and ischemic stroke outcomes via the rig-1 signaling and innate immunity mechanisms. *BioRxiv: The Preprint Server for Biology*. 2024. 10.1101/2024.06.07.598027.
- [64] Bencivenni S, et al. Early and late gut microbiota signatures of stroke in high salt-fed stroke-prone spontaneously hypertensive rats. *Scientific Reports*. 2024;**14**(1):19575. DOI: 10.1038/s41598-024-69961-9.
- [65] Morris-Blanco KC, et al. Epigenetic mechanisms and potential therapeutic targets in stroke. *Journal of Cerebral Blood Flow and Metabolism*. 2022;**42**(11):2000–2016. DOI: 10.1177/0271678X221116192.
- [66] Cirilo Jr JA, et al. The dynamics of actin protrusions can be controlled by tip-localized myosin motors. *Journal of Biological Chemistry*. 2024;**300**(1):105516. DOI: 10.1016/j.jbc.2023.105516.
- [67] Corada M, et al. Fine-tuning of Sox17 and canonical Wnt coordinates the permeability properties of the blood-brain barrier. *Circulation Research*. 2019;**124**(4):511–525. DOI: 10.1161/CIRCRESAHA.118.313316.
- [68] Corada M, et al. Sox17 is indispensable for acquisition and maintenance of arterial identity. *Nature Communications*. 2013;**4**:2609
- [69] Carnwath TP, Demel SL, Prestigiacomo CJ. 2024. Genetics of ischemic stroke functional outcome. *Journal of Neurology*. **271**(5): 2345–2369. DOI: 10.1007/s00415-024-12263-x.
- [70] Balaganapathy P, et al. Interplay between Notch and p53 promotes neuronal cell death in ischemic stroke. *Journal of Cerebral Blood Flow and Metabolism*. 2018;**38**

- (10):1781–1795. DOI: 10.1177/0271678X17715956.
- [71] Jimenez-Balado J, et al. DNA methylation and stroke prognosis: An epigenome-wide association study. *Clinical Epigenetics*. 2024;**16**(1):75. DOI: 10.1186/s13148-024-01690-2.
- [72] Lin RT, et al. LINE-1 methylation is associated with an increased risk of ischemic stroke in men. *Current Neurovascular Research*. 2014;**11**(1):4–9. DOI: 10.2174/1567202610666131202145530.
- [73] Bhat A, et al. A predictive chromatin architecture nexus regulates transcription and DNA damage repair. *Journal of Biological Chemistry*. 2025;**301**(3):108300. DOI: 10.1016/j.jbc.2025.108300.
- [74] He L, et al. The interplay between chromatin remodeling and DNA double-strand break repair: Implications for cancer biology and therapeutics. *DNA Repair (Amst.)*. 2025;**146**:103811. DOI: 10.1016/j.dnarep.2025.103811.
- [75] Kouzarides T. 2007. Chromatin modifications and their function. *Cell*. **128**(4):693–705. DOI: 10.1016/j.cell.2007.02.005.
- [76] Cairns BR. 2009. The logic of chromatin architecture and remodelling at promoters. *Nature*. **461**(7261):193–198. DOI: 10.1038/nature08450.
- [77] Langst G, Manelyte L. 2015. Chromatin remodelers: from function to dysfunction. *Genes (Basel)*. **6**(2):299–324. DOI: 10.3390/genes6020299.
- [78] Akhtar W, et al. Chromatin position effects assayed by thousands of reporters integrated in parallel. *Cell*. 2013;**154**(4):914–927. DOI: 10.1016/j.cell.2013.07.018.
- [79] Zhang P, et al. An overview of chromatin-regulating proteins in cells. *Current Protein & Peptide Science*. 2016;**17**:401–410.
- [80] Nair SS, Kumar R. 2012. Chromatin remodeling in cancer: A gateway to regulate gene transcription. *Molecular Oncology*. **6**(6):611–619. DOI: 10.1016/j.molonc.2012.09.005.
- [81] Lewis EM, Kroll KL. 2018. Development and disease in a dish: The epigenetics of neurodevelopmental disorders. *Epigenomics*. **10**(2):219–231. DOI: 10.2217/epi-2017-0113.
- [82] Kleefstra T, et al. Disruption of an ehmt1-associated chromatin-modification module causes intellectual disability. *The American Journal of Human Genetics*. 2012;**91**(1):73–82. DOI: 10.1016/j.ajhg.2012.05.003.
- [83] Verdin E, P. Jr. P, Van Lint C. Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcriptional activation. *The EMBO Journal*. 1993;**12**(8):3249–3259.
- [84] Yao W, Hu X, Wang X. 2024. Crossing epigenetic frontiers: The intersection of novel histone modifications and diseases. *Signal Transduct Target Ther*. **9**(1):232. DOI: 10.1038/s41392-024-01918-w.
- [85] Kloc M, Wosik J. Mechanical forces, nucleus, chromosomes, and chromatin. *Biomolecules*. 2025;**15**(3). DOI: 10.3390/biom15030354.
- [86] Zhang Y, et al. The role of histone modification in DNA replication-coupled nucleosome assembly and cancer. *International Journal*

- of Molecular Sciences. 2023;**24**(5). DOI: 10.3390/ijms24054939.
- [87] Luger K, et al. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature*. 1997;**389**:251.
- [88] Tropberger P, Schneider R. Scratching the (lateral) surface of chromatin regulation by histone modifications. *Nature Structural and Molecular Biology*. 2013;**20**:657. DOI: 10.1038/nsmb.2581.
- [89] Dong X, Weng Z. 2013. The correlation between histone modifications and gene expression. *Epigenomics*. 5(2):113–116. DOI: 10.2217/epi.13.13.
- [90] Strahl BD, Allis CD. 2000. The language of covalent histone modifications. *Nature*. **403** (6765):41–45. DOI: 10.1038/47412.
- [91] Declerck K, Berghe WV. Back to the future: Epigenetic clock plasticity towards healthy aging. *Mechanisms of Ageing and Development*. 2018;**174**:18–29. DOI: 10.1016/j.mad.2018.01.002.
- [92] Attar N, Kurdistani SK. Exploitation of EP300 and CREBBP lysine acetyltransferases by cancer. *Cold Spring Harbor Perspectives in Medicine*. 2017;**7**(3). DOI: 10.1101/cshperspect.a026534.
- [93] Pedersen MT, Helin K. 2010. Histone demethylases in development and disease. *Trends in Cell Biology*. **20** (11):662–671. DOI: 10.1016/j.tcb.2010.08.011.
- [94] Messier TL, et al. Histone H3 lysine 4 acetylation and methylation dynamics define breast cancer subtypes. *Oncotarget*. 2016;**7**(5):5094–5109. DOI: 10.18632/oncotarget.6922.
- [95] Formisano L, et al. Ischemic insults promote epigenetic reprogramming of μ opioid receptor expression in hippocampal neurons. *Proceedings of the National Academy of Sciences*. 2007;**104**(10):4170–4175. DOI: 10.1073/pnas.0611704104.
- [96] Schweizer S, et al. Inhibition of Histone Methyltransferases SUV39H1 and G9a Leads to Neuroprotection in an in vitro Model of Cerebral Ischemia. *Journal of Cerebral Blood Flow and Metabolism*. 2015;**35**(10):1640–1647. DOI: 10.1038/jcbfm.2015.99.
- [97] Langley B, Brochier C, Rivieccio MA. 2009. Targeting histone deacetylases as a multifaceted approach to treat the diverse outcomes of stroke. *Stroke*. **40**(8):2899–2905. DOI: 10.1161/strokeaha.108.540229.
- [98] Ling L, et al. A novel histone deacetylase inhibitor protects the blood-brain barrier by regulating NF-kappaB and Nrf2 signaling pathways in OGD/R injury. *Archives of Gerontology and Geriatrics*. 2025;**131**:105739. DOI: 10.1016/j.archger.2024.105739.
- [99] Giffard RG, et al. Regulation of apoptotic and inflammatory cell signaling in cerebral ischemia: The complex roles of heat shock protein 70. *Anesthesiology*. 2008;**109** (2):339–348. DOI: 10.1097/ALN.0b013e31817f4ce0.
- [100] Faraco G, et al. Pharmacological inhibition of histone deacetylases by suberoylanilide hydroxamic acid specifically alters gene expression and reduces ischemic injury in the mouse brain. *Molecular Pharmacology*.

2006;**70**(6):1876–1884. DOI: 10.1124/mol.106.027912.

[101] Endres M, et al. DNA methyltransferase contributes to delayed ischemic brain injury. *The Journal of Neuroscience*. 2000;**20**(9):3175–3181. DOI: 10.1523/jneurosci.20-09-03175.2000.

[102] Soriano FX, et al. Role of histone acetylation in the activity-dependent regulation of sulfiredoxin and sestrin 2. *Epigenetics* 2009;**4**:152–158.

[103] Tang Y, et al. Inhibiting histone deacetylase 2 (HDAC2) promotes functional recovery from stroke. *Journal of the American Heart Association*. 2017;**6**(10). DOI: 10.1161/jaha.117.007236.

[104] Brookes RL, et al. Sodium valproate, a histone deacetylase inhibitor, is associated with reduced stroke risk after previous ischemic stroke or transient ischemic attack. *Stroke*. 2018;**49**(1):54–61. DOI: 10.1161/STROKEAHA.117.016674.

[105] Xuan A, et al. Neuroprotective effects of valproic acid following transient global ischemia in rats. *Life Sciences*. 2012;**90**(11-12):463–468. DOI: 10.1016/j.lfs.2012.01.001.

[106] Uzdensky AB, Demyanenko S. 2021. Histone acetylation and deacetylation in ischemic stroke. *Neural Regeneration Research*. **16**(8):1529–1530. DOI: 10.4103/1673-5374.303024.

[107] Demyanenko SV, Dzreyan VA, Uzdensky AB. 2020. The expression and localization of histone acetyltransferases HAT1 and PCAF in neurons and astrocytes of the photothrombotic stroke-induced

penumbra in the rat brain cortex. *Molecular Neurobiology*. **57**(7):3219–3227. DOI: 10.1007/s12035-020-01959-6.

[108] Kopp F. 2019. Molecular functions and biological roles of long non-coding RNAs in human physiology and disease. *The Journal of Gene Medicine*. **21**(8):e3104. DOI: 10.1002/jgm.3104.

[109] Collins LJ, Schönfeld B, Chen XS. Chapter 4 - The epigenetics of non-coding RNA. In Tollefsbol T, editor. *Handbook of Epigenetics*. San Diego: Academic Press; 2011. p. 49–61

[110] Dakal TC, et al. Genetic and epigenetic regulation of non-coding RNAs: Implications in cancer metastasis, stemness and drug resistance. *Pathology - Research and Practice*. 2025;**266**:155728. DOI: 10.1016/j.prp.2024.155728.

[111] Rutenberg-Schoenberg M, Sexton AN, Simon MD. 2016. The properties of long noncoding RNAs that regulate chromatin. *Annual Review of Genomics & Human Genetics*. **17**(1):69–94. DOI: 10.1146/annurev-genom-090314-024939.

[112] Roberts TC. *The microRNA Machinery*, in *microRNA: Basic Science: From Molecular Biology to Clinical Practice*, Santulli G Editor. Springer International Publishing Cham 2015;15–30.

[113] Grillone K, et al. A systematic review of non-coding RNA therapeutics in early clinical trials: A new perspective against cancer. *Journal of Translational Medicine*. 2024;**22**(1):731. DOI: 10.1186/s12967-024-05554-4.

- [114] Esteller M. Non-coding RNAs in human disease. *Nature Reviews Genetics*. 2011;**12**:861. DOI: 10.1038/nrg3074.
- [115] Wang C, Jing Q. 2018. Non-coding RNAs as biomarkers for acute myocardial infarction. *Acta Pharmacologica Sinica*. **39**(7):1110–1119. DOI: 10.1038/aps.2017.205.
- [116] Adams BD, et al. Targeting noncoding RNAs in disease. *Journal of Clinical Investigation*. 2017;**127**(3):761–771. DOI: 10.1172/jci84424.
- [117] Abdel-Reheim MA, et al. Unveiling the regulatory role of miRNAs in stroke pathophysiology and diagnosis. *Pathology Research and Practice*. 2024;**253**:155085. DOI: 10.1016/j.prp.2023.155085.
- [118] Bhattarai S, et al. Deep sequencing reveals uncharted isoform heterogeneity of the protein-coding transcriptome in cerebral ischemia. *Molecular Neurobiology*. 2019;**56**(2):1035–1043. DOI: 10.1007/s12035-018-1147-0.
- [119] Zhao YH, et al. Endothelial lincRNA-p21 alleviates cerebral ischemia/reperfusion injury by maintaining blood-brain barrier integrity. *Journal of Cerebral Blood Flow Metabolism*. 2024;271678X241248907. DOI: 10.1177/0271678X241248907.
- [120] Dharap A, Pokrzywa C, Vemuganti R. 2013. Increased binding of stroke-induced long non-coding RNAs to the transcriptional corepressors Sin3A and coREST. *ASN Neuro*. **5**(4):283–289. DOI: 10.1042/an20130029.
- [121] Zhang X, et al. Long noncoding RNA Malat1 regulates cerebrovascular pathologies in ischemic stroke. *The Journal of Neuroscience*. 2017;**37**(7):1797–1806. DOI: 10.1523/jneurosci.3389-16.2017.
- [122] Alammari F, et al. Hidden regulators: The emerging roles of lncRNAs in brain development and disease. *Frontiers in Neuroscience*. 2024;**18**:1392688. DOI: 10.3389/fnins.2024.1392688.
- [123] Ng S-Y, Johnson R, Stanton LW. 2012. Human long non-coding RNAs promote pluripotency and neuronal differentiation by association with chromatin modifiers and transcription factors. *The EMBO Journal*. **31**(3):522–533. DOI: 10.1038/emboj.2011.459.
- [124] Tiedt S, et al. RNA-seq identifies circulating miR-125a-5p, miR-125b-5p, and miR-143-3p as potential biomarkers for acute ischemic stroke. *Circulation Research*. 2017;**121**(8):970–980. DOI: 10.1161/CIRCRESAHA.117.311572.
- [125] Loggini A, Hornik J, Hornik A. The role of microRNAs as super-early biomarkers in acute ischemic stroke: A systematic review. *Clinical Neurology and Neurosurgery*. 2024; **244**. 108416. DOI: 10.1016/j.clineuro.2024.108416.
- [126] Dharap A, Nakka VP, Vemuganti R. 2012. Effect of focal ischemia on long noncoding RNAs. *Stroke*. **43**(10):2800–2802. DOI: 10.1161/strokeaha.112.669465.
- [127] Wang J, et al. Long non-coding RNA H19 induces cerebral ischemia reperfusion injury via activation of autophagy. *Aging and Disease*. 2017;**8**(1):71–84. DOI: 10.14336/ad.2016.0530.
- [128] Alegría-Torres JA, Baccarelli A, Bollati V. 2011. Epigenetics and lifestyle. *Epigenomics*. **3**(3):267–277. DOI: 10.2217/epi.11.22.

- [129] Wu H, Sun YE. Epigenetic regulation of stem cell differentiation. *Pediatric Research*. 2006;**59**:21R. 10.1203/01.pdr.0000203565.76028.2a.
- [130] Tang H, et al. Characterization of combinatorial histone modifications on lineage-affiliated genes during hematopoietic stem cell myeloid commitment. *Acta Biochimica Et Biophysica Sinica*. 2014;**46**(10):894–901. DOI: 10.1093/abbs/gmu078.
- [131] Lyu TJ, et al. DNMT3A dysfunction promotes neuroinflammation and exacerbates acute ischemic stroke. *MedComm*. 2020;**5**(7):e652. DOI: 10.1002/mco2.652.
- [132] Zhang N, et al. Folic acid supplementation for stroke prevention: A systematic review and meta-analysis of 21 randomized clinical trials worldwide. *Clinical Nutrition*. 2024;**43**(7):1706–1716. DOI: 10.1016/j.clnu.2024.05.034.
- [133] Kuo TT, et al. Post-stroke delivery of valproic acid promotes functional recovery and differentially modifies responses of peri-infarct microglia. *Frontiers in Molecular Neuroscience*. 2021;**14**:639145. DOI: 10.3389/fnmol.2021.639145.
- [134] Wang H, et al. Fecal transplantation from db/db mice treated with sodium butyrate attenuates ischemic stroke injury. *Microbiology Spectrum*. 2021;**9**(2):e0004221. DOI: 10.1128/Spectrum.m.00042-21.
- [135] Du X, et al. Pharmacological intervention of curcumin via the NLRP3 inflammasome in ischemic stroke. *Frontiers in Pharmacology*. 2021;**12**:187249644. DOI: 10.3389/fphar.2023.1249644.
- [136] Zhou T, et al. GSK-126 attenuates cell apoptosis in ischemic brain injury by modulating the EZH2-H3K27me3-Bcl2l1 axis. *Molecular Neurobiology*. 2024;**61**(6):3369–3383. DOI: 10.1007/s12035-023-03808-8.
- [137] Guo K, et al. BRG1 alleviates microglial activation by promoting the KEAP1-NRF2/HO-1 signaling pathway and minimizing oxidative damage in cerebral ischemia-reperfusion. *International Immunopharmacology*. 2023;**119**:110201. DOI: 10.1016/j.intimp.2023.110201.
- [138] Shi Y, et al. Corrigendum: miR-124 alleviates ischemic stroke-induced neuronal death by targeting DAPK1 in mice. *Frontiers in Neuroscience*. 2021;**15**:745087. DOI: 10.3389/fnins.2021.745087.
- [139] Chelluboina B, et al. Therapeutic potential of intravenous mir-21 mimic after stroke following stair criteria. *Translational Stroke Research*. 2025;**16**(2):403–409. DOI: 10.1007/s12975-023-01223-8.
- [140] Khoshnam SE, et al. LncRNA MALAT1 and ischemic stroke: pathogenesis and opportunities. *Molecular Neurobiology*. 2024;**61**(7):4369–4380. DOI: 10.1007/s12035-023-03853-3.
- [141] Li J, et al. Insights into the role of DNA methylation in immune cell development and autoimmune disease. *Frontiers in Cell and Developmental Biology*. 2021;**9**:757318. DOI: 10.3389/fcell.2021.757318.
- [142] Su Y, et al. The progress of research on histone methylation in ischemic stroke pathogenesis. *Journal of Physiology and Biochemistry*. 2022;**78**(1):1–8. DOI: 10.1007/s13105-021-00841-w.

[143] Zhou Q, et al. Inhibition of histone deacetylases promotes ubiquitin-dependent proteasomal degradation of DNA methyltransferase 1 in human breast cancer cells. *Molecular Cancer Research*. 2008;**6**(5):873–883. DOI: 10.1158/1541-7786.mcr-07-0330.

[144] Ahuja N, Sharma AR, Baylin SB. Epigenetic therapeutics: a new weapon in the war against cancer. *Annual Review of Medicine*. 2016;**67**:73–89. 10.1146/annurev-med-111314-035900.

[145] Minucci S, Pelicci PG. 2006. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nature Reviews Cancer*. **6**(1):38–51. DOI: 10.1038/nrc1779.

[146] Toki S, et al. The histone deacetylase inhibitor trichostatin A suppresses murine innate allergic inflammation by blocking group 2 innate lymphoid cell (ILC2) activation. *Thorax*. 2016;**71**(7):633. DOI: 10.1136/thoraxjnl-2015-207728.

[147] Moretti A, Ferrari F, Villa RF. Neuroprotection for ischaemic stroke: Current status and challenges. *Pharmacology & Therapeutics*. 2015;**146**:23–34. 10.1016/j.pharmthera.2014.09.003.

Epigenetics of Heart Development and Disease

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Abstract

Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality worldwide, accounting for approximately 31% of global mortality. Despite advances in diagnosis, prevention, and treatment, the prevalence of CVD continues to rise, highlighting that traditional risk factors (hypertension, diabetes, and dyslipidemia) do not fully explain disease susceptibility. Epigenetics, defined as heritable modifications of gene expression independent of DNA sequence changes, has emerged as a critical factor influencing cardiovascular health and development. This regulation involves dynamic changes in chromatin structure through chromatin remodeling and DNA and histone chemical modifications, as well as the regulatory functions of noncoding RNAs. Epigenetic mechanisms modulate gene expression programs essential for cardiovascular development and response to injury, linking environmental exposures to disease onset and progression. Epigenetic dysregulation has been implicated in heart failure (HF) pathogenesis, often exacerbated by comorbid conditions like diabetes. Understanding epigenetic contributions offers promising avenues for novel therapeutic strategies targeting these molecular pathways. This chapter focuses on the evolving role of epigenetics as a key nongenetic determinant of cardiac development and heart failure.

Keywords: epigenetics, heart, development, cardiovascular disease, heart failure, aging

1. Introduction

Cardiovascular disease (CVD) remains the foremost cause of global morbidity and mortality, frequently resulting in heart failure (HF) and sudden cardiac death. Various underlying conditions contribute to HF, including cardiomyopathies, hypertension, myocardial infarction (MI), and aortic stenosis [1]. Following disease onset, the heart undergoes both pathological and physiological remodeling, characterized by cardiomyocyte hypertrophy, fibrosis, and inflammation. Over the past 20 years, significant advances have been made in unraveling the molecular and cellular mechanisms driving HF. These insights have informed the development of multidrug therapies, such as angiotensin II receptor blockers, diuretics, and

beta-adrenergic antagonists, which improve symptoms but often fail to fully restore myocardial function [2].

A key regulator of cardiac gene expression during maturation and differentiation is epigenetics, which is defined as a complex system of mechanisms that control gene expression without altering the DNA sequence itself [3–5]. Recent studies have demonstrated that epigenetic mechanisms, which establish cardiomyocyte identity during development, also contribute to the reactivation of fetal gene expression programs observed in HF [4, 5]. Therefore, elucidating the relationship between epigenetic regulation and cardiac remodeling throughout development and disease may reveal novel epigenetic drug targets aimed at modulating the cardiac epigenome (**Figure 1**).

This chapter explores the roles of epigenetic mechanisms in cardiac development and disease and discusses how these pathways may provide insight into novel therapeutic strategies for CVD and HF. More specifically, we focus our attention on studies involving DNA methylation, histone modifications, chromatin remodeling mechanisms, and noncoding RNAs (ncRNAs) in the context of both cardiac development and CVD. The evidence discussed in this chapter supports the role of chromatin structural modulation in cardiovascular development and also in the

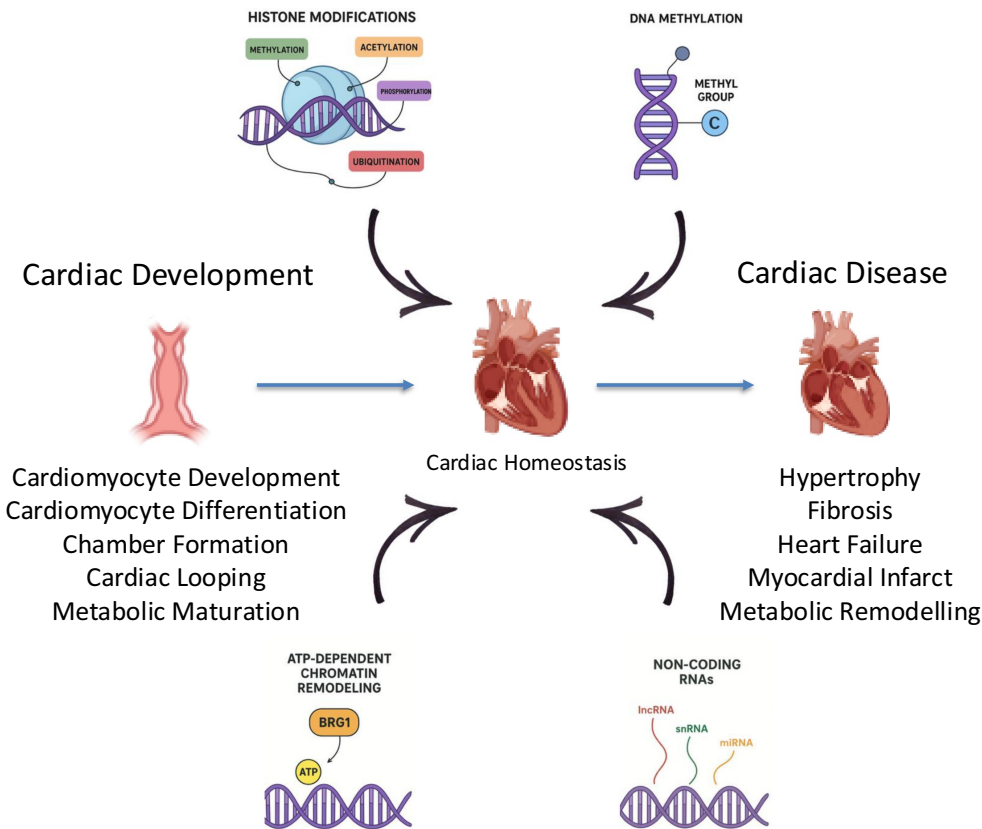


Figure 1. Summary of epigenetic mechanisms (histone modifications, DNA methylation, ATP-dependent chromatin remodeling and noncoding RNAs) involved in cardiac development and disease.

development of CVDs. The rising incidence of CVDs over recent decades underscores the urgent need to deepen our understanding of their underlying mechanisms. Research has increasingly focused on epigenetic processes as critical contributors to these conditions. Improved knowledge of individual epigenetic landscapes will facilitate the identification of disease causes and enable the design of personalized therapeutic strategies. Despite advances in the understanding of epigenetic mechanisms in the context of pathophysiology, a comprehensive understanding of cardiovascular epigenetic networks remains incomplete. Clarifying the intricate relationship between epigenetic regulation and tissue-specific transcriptional programs is essential for developing therapies capable of selectively reversing disease-associated gene expression patterns without causing off-target effects.

2. Molecular basis of epigenetics

Epigenetics refers to the study of heritable modifications in gene expression that occur without altering the underlying DNA sequence. These changes result from mechanisms such as transcriptional and translational regulation, as well as modifications in DNA structure and conformation [6]. Epigenetic processes play a crucial role in proper development [7], normal cellular function, and the maintenance of cellular homeostasis [8]. Dysregulation of these regulatory pathways is strongly associated with a wide range of pathological conditions [9]. The main mechanisms that encompass epigenetic regulation include DNA methylation, histone modification, chromatin remodeling, and ncRNAs [10]. Coordinated gene regulation through these mechanisms depends on complex interactions, among which are transcription factors and specific DNA regulatory regions [6]. DNA methylation typically involves the covalent addition of a methyl group to the 5-carbon of cytosine residues (5mC) within CpG sites, leading to transcriptional repression either by directly hindering transcription factor binding or by recruiting methyl-binding proteins (MBPs), which suppress gene expression via corepressor complexes [11]. Histone proteins undergo a variety of posttranslational modifications (PTMs) – including acetylation, methylation, ubiquitination, sumoylation, and phosphorylation – which influence chromatin dynamics and gene activity. For instance, acetylation can indirectly facilitate the recruitment of transcriptional machinery or structural proteins that modify chromatin architecture [10]. ncRNAs, which constitute a large portion of the genome, perform diverse and largely still-unknown functions. Recent research has highlighted their involvement in gene regulation, either by modulating gene expression at the posttranscriptional level (e.g., through microRNAs or miRNAs) or by serving as molecular scaffolds that mediate interactions between DNA and regulatory proteins, thereby influencing transcription factor binding and chromatin organization [12].

Two key aspects of epigenetic regulation are particularly important to consider in the context of disease. The first is its susceptibility to environmental influences, such as diet, circadian rhythms, and pollutants. This makes epigenetic mechanisms prime candidates for mediating the impact of environmental factors on disease onset and progression. The second aspect is the dynamic nature of epigenetic mechanisms, which makes them targetable for pharmacological modulation. This concept has given rise to “epigenetic therapy,” namely the possibility of interfering with

epigenetic changes responsible for disease onset. However, current epigenetic therapies have important limitations: available drugs often exert broad, pleiotropic, and toxic effects due to the ubiquitous expression of many epigenetic enzymes. To overcome these challenges, research is increasingly moving toward the development of more selective strategies, such as compounds capable of modulating specific epigenetic pathways in a cell-type-specific manner.

In the past decade, growing evidence reveals that multiple layers of epigenetic regulation contribute to the transcriptional alterations seen in HF. These include alterations in the genomic distribution of DNA methylation and histone modifications, variations in the function of enzymes that catalyze epigenetic marks, and changes in ncRNA expression critical for both cardiac development and adult heart function [13,14].

2.1 DNA methylation

DNA methylation refers to the enzymatic addition of a methyl group to the 5th carbon position of cytosine bases (5mC) within CpG dinucleotides, which are commonly found in CpG-rich regions of the genome known as “CpG islands.” This modification can also take place in repetitive genomic elements, including satellite DNA, centromeric repeats, and retrotransposons, where it is generally linked to transcriptional repression. In mature mammalian cells, approximately 70% of CpG sites are methylated. In contrast, embryonic stem cells show a distinctive methylation pattern, with about 25% of methylated cytosines occurring outside CpG sites, suggesting a unique regulatory role in pluripotent or undifferentiated states [15]. DNA methylation leads to transcriptional repression either by directly blocking transcription factor binding or by recruiting MBPs, which suppress gene expression via corepressor complexes [11].

The process of DNA methylation is driven by a conserved group of enzymes called DNA methyltransferases (DNMTs), which include DNMT1, DNMT3A, and DNMT3B. These enzymes utilize S-adenosyl-methionine as a methyl group donor. DNMT3A and DNMT3B are primarily involved in establishing *de novo* methylation patterns, while DNMT1 is mainly responsible for preserving existing methylation patterns after DNA replication [16]. Additionally, during the early stages of embryonic development, DNMT3A and DNMT3B play essential roles in initiating *de novo* DNA methylation [17]. Active DNA demethylation is mediated by the 10–11 translocation (TET) family of enzymes, which includes TET1, TET2, and TET3. These enzymes catalyze the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine (5hmc), which can be further oxidized to 5-formylcytosine (5fc) and 5-carboxylcytosine (5caC). These oxidized derivatives are recognized and excised by the base excision repair (BER) pathway, restoring unmodified cytosine. Interestingly, 5-hmC has been shown to act as an epigenetic mark capable of promoting transcription. Indeed, 5-hmC is enriched in regulatory regions (promoter and enhancer regions) of actively transcribed genes [18, 19].

2.2 Histone modifications

Chromatin is primarily composed of nucleosomes, which consist of DNA wrapped around a histone octamer made up of two copies each of histones H2A, H2B, H3, and H4. The N-terminal tails of these histone proteins extend outward

from the nucleosome core and serve as sites for various PTMs, including acetylation, methylation, ubiquitylation, sumoylation, and phosphorylation. These chemical modifications are critical players in epigenetic regulation and play crucial roles in numerous cellular processes, such as gene expression, differentiation, development, DNA replication, and repair [10].

Histone modifications can occur at several different amino acid residues: acetylation, ubiquitylation, and sumoylation typically target lysines; methylation can occur on lysine or arginine residues; and phosphorylation is generally found on serines and threonines [20]. Importantly, the specific pattern and combination of these modifications at any given time define the transcriptional state of the chromatin. Active gene expression is usually correlated with histone acetylation and methylation at lysines 4 and 36 of histone H3, while regions of transcriptional repression are commonly associated with methylation or deacetylation at lysines 9 and 27 of histone H3 [21].

Acetylation of histones H2B, H3, and H4 occurs at lysine residues and is controlled by the interplay between histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs transfer acetyl groups to lysine residues, neutralizing their positive charge and thereby loosening the interaction between DNA and histones. This leads to a more relaxed chromatin structure, which facilitates transcriptional activation. Conversely, HDACs remove these acetyl groups, resulting in a more compact chromatin state that suppresses transcription [22].

Histone methylation is a more nuanced regulatory mechanism, as it does not alter histone charge and can be correlated with either gene activation or repression, depending on the specific residue and the extent of methylation (mono-, di-, or trimethylation). This dynamic process is regulated by histone methyltransferase (HMT) enzymes, which add methyl groups, and histone demethylases (HDMTs), which remove these modifications [23]. Transcriptional activation is commonly marked by modifications such as H3K4me3, H3K36me3, and H3K79me3, whereas transcriptional repression is often indicated by methylation marks like H3K9me2, H3K27me3, and H4K20me3 [24].

2.3 Chromatin remodeling

ATP-dependent chromatin-remodeling complexes are large, multi-subunit protein assemblies that utilize the energy from ATP hydrolysis to modify chromatin structure. By repositioning, ejecting, or restructuring nucleosomes, these complexes enable access for transcription factors, thereby facilitating gene activation [10]. Among the four main families of ATP-dependent remodelers, the most functionally significant in cardiac tissue is the BAF complex, the mammalian equivalent of the yeast SWI/SNF complex. This complex plays a key role in regulating genes involved in cardiac muscle cell growth, proliferation, and the formation of heart chambers through the coordinated activity of its various subunits [25].

2.4 Noncoding RNA

A significant portion of the genome is classified as noncoding, meaning that while these regions are transcribed into RNA, they are not translated into functional proteins. ncRNAs are broadly categorized into two main groups: (1) short ncRNAs, including microRNAs (miRNAs), small interfering RNAs (siRNAs), and

PIWI-interacting RNAs (piRNAs), and (2) long noncoding RNAs (lncRNAs). Short ncRNAs typically function to suppress gene expression, whereas lncRNAs have more diverse and complex roles [26].

miRNAs are short RNA molecules, typically 19–25 nucleotides in length, that have been extensively investigated due to their regulatory roles in numerous biological processes, such as development, cell proliferation, and differentiation. They exert their function by repressing gene expression at the posttranscriptional level. miRNA biogenesis begins with transcription by RNA polymerase II, followed by processing of the primary miRNA transcript (pri-miRNA) into precursor miRNA (pre-miRNA) by the RNase III enzyme Drosha. The pre-miRNA is then transported to the cytoplasm via exportin-5, where it undergoes further processing by the enzyme dicer to produce the mature miRNA. This mature miRNA associates with argonaute proteins to form the RNA-induced silencing complex (RISC), which binds to the 3' untranslated region (3' UTR) of target mRNAs, leading to either translational inhibition or mRNA degradation [27].

In contrast, lncRNAs – defined as RNA transcripts longer than 200 nucleotides that do not code for proteins – remain less thoroughly characterized, and their functions are still being uncovered. The activity of lncRNAs is highly complex and appears to depend on their genomic origin, subcellular localization, and temporal expression patterns. These features allow lncRNAs to participate in a wide array of cellular functions, including the regulation of organ development [28]. Overall, ncRNAs function as central mediators linking genetic information to protein activity, exerting regulatory control through transcriptional and posttranscriptional mechanisms, while also coordinating intra and intercellular transport of regulatory factors crucial for cellular homeostasis [29].

3. Epigenetic mechanisms of heart development

During embryogenesis, the heart is the first fully functional organ, beginning around embryonic day 7.5 (E7.5) in mice and day 15 in humans, when cardiac progenitors migrate from the lateral plate mesoderm to form the cardiac crescent. This migration is driven by FGF8, while BMP signals from the endoderm establish cardiac progenitor identity by inducing early markers like NKX2.5 and TBX5. Conversely, WNT3A and WNT8 inhibit heart formation and must be blocked by antagonists such as Crescent and Dickkopf-1 [30]. By E8.5 in mice (day 21 in humans), the bilateral crescents fuse into a primitive, beating heart tube, which later develops into atria and arterial outflows [30].

As the heart loops, additional progenitors from the pharyngeal mesoderm – the secondary heart field – migrate into the heart, contributing to heart tube elongation and right ventricle formation [28]. The early heart tube, composed of myocardial and endocardial layers, is regulated by NKX2.5 and GATA4, which drive heart development. Between E9.5 and E10.5 in mice (day 28 in humans), rightward looping aligns the chambers and outflow tracts, controlled by pathways including SHH and nodal, and transcription factors MEF2C and HAND family members [29–32].

During this period, the proepicardium forms and generates the epicardium, coronary vessels, and parts of the conduction system. Epicardial cells migrate over

the myocardium and, through EMT, form coronary endothelial cells and vascular smooth muscle cells. Coronary arteries connect to systemic circulation and release endothelin-1, promoting Purkinje fiber differentiation. HAND1 and HAND2 transcription factors regulate ventricle development downstream of NKX2.5 [33, 34].

Cardiac growth results from cardiomyocyte proliferation and differentiation. Embryonic cardiomyocytes proliferate mainly on the heart's outer surface, stimulated by epicardial mitogens like retinoic acid and endocardial growth factors such as VEGF and angiopoietins. GSK3, β -catenin, Myc, and p38 MAPK also regulate proliferation, while transcription factors SRF, MEF2A/C, myocardin, and GATA4 promote differentiation and contractile gene expression [35–37].

By E10.5, endocardial cushions form and differentiate into fibrous valves under TGF- β influence, contributing to the septation of the atrioventricular canal and conotruncus. By E12.5, chamber formation is complete, including the atria, ventricles, and major arteries. Neural crest cells play essential roles in septation and in forming the vascular smooth muscle of the aortic and branchial arch arteries, remodeling into key vessels like the ductus arteriosus [28]. Epigenetic mechanisms involved in cardiac development are summarized in **Table 1**.

3.1 DNA methylation and cardiac development

Over the past two decades, numerous studies have highlighted the pivotal role of epigenetic mechanisms in controlling the activity of key transcription factors during cardiomyocyte specification, proliferation, and maturation. DNA methylation regulates the timing of gene expression essential for cardiac lineage commitment and functional maturation of cardiomyocytes. In embryonic stem cells, the genomic regions that bind pluripotency factors like OCT4 and NANOG are unmethylated, allowing the expression of genes involved in maintaining cellular stemness. Conversely, binding sites for cardiac-specific transcription factors such as GATA1–4 and MEF2C are methylated, preventing premature expression of cardiac genes. During differentiation, these methylation patterns undergo extensive remodeling: demethylation at regulatory regions of genes belonging to cardiomyocyte differentiation facilitates the binding of lineage-specific transcription factors and the activation of cardiac gene networks, while methylation of noncardiac genes ensures lineage fidelity [4].

In addition, several downstream targets of major developmental signaling pathways (Hedgehog, BMP, TGF β , FGF, Notch, and Wnt/ β -catenin) have been found to carry hypermethylated regions during cardiomyocyte maturation, correlating with their transcriptional repression [38]. During the neonatal period, completing DNA methylation-mediated silencing, the repressive histone mark K3K27me₃ accumulates at the methylated promoters of Notch-responsive genes, thereby ensuring stable repression of proliferative programs and preventing Notch-driven cell cycle reactivation in mature adult cardiomyocytes [39]. Over the past decade, 5-hydroxymethylcytosine, an intermediate product of DNA methylation, has also been implicated in defining the transcription program of cardiomyocytes during heart development, resulting in impaired homeostasis and altered responses to pathological stress [18, 19].

Category	Key mechanisms/factors	Developmental timing	Functional outcome/notes
Heart morphogenesis	FGF8 (progenitor migration), BMP (cardiac identity via NKX2.5, TBX5), WNT3A/WNT8 inhibition via Crescent, Dickkopf-1	E7.5 (mice), day 15 (humans)	Formation of the cardiac crescent and the primitive heart tube
Heart looping and chamber formation	SHH, Nodal, MEF2C, HAND1/2, NKX2.5, GATA4	E8.5–E10.5 (mice), day 21–28 (humans)	Rightward looping, alignment of chambers/outflow tracts
Epicardium and coronary development	Proepicardium → epicardium, EMT, endothelin-1, HAND1/2	E9.5–E12.5 (mice)	Coronary vessels, Purkinje fiber differentiation, ventricle formation
Cardiomyocyte growth and differentiation	Mitogens: retinoic acid, VEGF, angiopoietins; Signaling: GSK3, β -catenin, Myc, p38 MAPK; TFs: SRF, MEF2A/C, myocardin, GATA4	Embryonic stages	Proliferation on the outer heart surface, differentiation, and contractile gene expression
Valve and septa formation	TGF- β , neural crest cells	E10.5–E12.5 (mice)	Endocardial cushion differentiation, septation, and formation of major arteries and aortic structures
DNA methylation	Regulates transcription factor activity (GATA1–4, MEF2C); hypermethylation of noncardiac genes; 5-hydroxymethylcytosine	Embryonic → neonatal stages	Controls timing of cardiomyocyte differentiation, ensures lineage fidelity, and silences proliferative programs in mature cardiomyocytes
Histone modifications	WHSC1 (H3K36me3; NKX2.5 target genes), SMYD1 (H3K4me; cardiomyocyte maturation), DOT1L (H3K79; differentiation; cardioprotection)	Embryonic → postnatal	Promotes transcription of cardiac genes, essential for chamber formation and cardiomyocyte maturation
Chromatin remodeling	BAF complex, BRG1 (ATPase subunit), interacts with GATA TFs	Embryonic; reactivated in stressed adult hearts	Facilitates fetal cardiac gene expression (β -MHC) and represses adult isoforms (α -MHC); critical for development and maladaptive stress response
Noncoding RNAs (ncRNAs)	lncRNAs: Mhrt, Upperhand (<i>Upht</i>), Fendrr, Braveheart (<i>Bvht</i>).	Embryonic → adult stress response	Epigenetic regulation via chromatin remodeling, super-enhancer maintenance, inhibition of PRC2, and activation of TrxG/MLL complexes control cardiomyocyte proliferation, differentiation, and stress responses

Table 1.
Summary of epigenetic mechanisms and factors involved in cardiovascular development.

3.2 Histone modifications and cardiac development

The HMT WHSC1 plays a regulatory role in cardiac development by interacting directly with the transcription factor NKX2.5. Through this interaction, WHSC1 promotes the transcription of NKX2.5 target genes by catalyzing histone H3 lysine 36 trimethylation (H3K36me3). Disruption of WHSC1 function results in atrial and

ventricular septal defects, mirroring those observed in NKX2.5 mutations [40]. In addition, the lysine methyltransferase SMYD1 (SET and MYND domain-containing 1) functions as a transcriptional activator through its SET domain, which catalyzes the methylation of histone 3 lysine 4 (H3K4). Loss of SMYD1 impairs cardiomyocyte maturation and leads to the absence of right ventricular structures [41].

Another histone-modifying enzyme involved in transcriptional activation, DOT1L, which specifically methylates H3K79, has been identified as essential for heart development. Mice lacking DOT1L experience postnatal lethality accompanied by cardiac defects. Beyond its developmental role, DOT1L supports cardiomyocyte differentiation and cardiomyogenesis, and emerging evidence suggests that it may help protect the heart from pathological remodeling processes [42].

3.3 Chromatin remodeling and cardiac development

The Brg1/Brm-associated factor (BAF) chromatin remodeling complex is a critical regulator of cardiac development. Its catalytic ATPase subunit, primarily BRG1 (encoded by *Smarca4*), utilizes energy from ATP hydrolysis to remove nucleosomes from DNA, thereby increasing DNA accessibility at gene promoters and enhancers. This activity facilitates the binding of key cardiac transcription factors, including the GATA family, with which BRG1 directly interacts to synergistically activate developmental gene programs.

The essential role of BRG1 in cardiac development is underscored by the embryonic lethality of *Smarca4*-null mouse embryos, which exhibit severe cardiac defects, including hypoplastic myocardium and the absence of the interventricular septum [43]. Mechanistically, BRG1 contributes to defining the fetal cardiac gene expression program by promoting, on one hand, the expression of fetal myosin heavy chain (β -MHC) and, on the other hand, the suppression of the adult isoform (α -MHC). Although BRG1 expression is typically absent in adult hearts, it is reactivated in response to pathological stress, such as pressure overload. This reexpression contributes to defining a fetal-like gene expression profile in stressed cardiomyocytes. Therefore, the BAF complex, through its ATPase subunit BRG1, is not only necessary for development but also for the maladaptive reprogramming of cardiomyocytes in diseased states [44, 45].

3.4 ncRNAs and cardiac development

Beyond DNA methylation, histone modifications, and chromatin remodeling, ncRNAs have emerged as a significant layer of epigenetic regulation in cardiac development. Increasing evidence highlights the importance of several lncRNAs in both heart formation and the maintenance of cardiac function. One such lncRNA, Myheart (*Mhrt*), has been implicated in having a protective role in the heart by repressing hypertrophic gene expression following pressure overload. Under stress conditions, *Mhrt* expression is upregulated and acts as a molecular decoy: it directly binds to the chromatin remodeler BRG1, sequestering it from the promoter of the β -MHC gene. This interaction disrupts BRG1's ability to activate the maladaptive fetal gene program [46].

Another key lncRNA, Upperhand (*UpH*), is essential for cardiac development through its regulation of HAND2, a transcription factor crucial for right ventricle formation. *UpH* has been shown to maintain the super-enhancer region upstream of

HAND2, which is marked by the active histone modification H3K27ac. Disruption of *Uph* transcription leads to a loss of HAND2 expression, highlighting its role in the epigenetic control of transcription factors involved in heart development [47].

The lncRNA *Fendrr* (*fetal-lethal noncoding development regulatory RNA*) has also been identified as vital for cardiac development, particularly in regulating cardiomyocyte proliferation during embryogenesis. It exerts its effects through a dual mechanistic approach: it acts by inhibiting the HMT of the polycomb repressive complex 2 (PRC2), preventing the deposition of the repressive H3K27me3 mark at target promoters. Concurrently, *Fendrr* facilitates gene activation by recruiting trithorax group (TrxG)/MLL complexes, which trimethylate lysine 4 on histone H3 (H3K4me3) [48]. Similarly, Braveheart (*Bvht*) is another developmentally important lncRNA. It supports cardiac lineage specification by preventing PRC2 from binding to MESP1 (mesoderm posterior homolog 1), a gene essential for initiating cardiomyocyte differentiation [49].

4. Epigenetic mechanisms of heart disease

CVD, particularly when it progresses to HF or sudden cardiac death, remains the leading contributor to global morbidity and mortality. A range of pathological conditions (cardiomyopathies, hypertension, MI, and aortic stenosis) is recognized as major precursors to HF [1]. Once the disease is initiated, the heart often undergoes pathological remodeling, a process marked by hypertrophy of cardiomyocytes, fibrotic tissue deposition, and inflammatory responses.

This remodeling process begins in response to extracellular stress signals, which trigger inflammation and result in significant alterations to the extracellular matrix (ECM). These changes include increased ECM deposition and elevated levels of pro-inflammatory mediators, such as IL-6, TNF- α , and C-reactive protein (CRP) [50]. Chronic exposure to these inflammatory cues contributes to the development of fibrosis and cardiomyocyte hypertrophy, ultimately impairing heart function due to increased tissue stiffness and reduced contractility [13]. Fibrosis, often originating from aberrant inflammatory or stress responses, is considered a central pathological event that can lead to both structural and functional decline in various organs [51].

At the cellular level, cardiac remodeling involves heightened protein synthesis and enlarged cardiomyocyte size, which compromise the integrity of sarcomeric architecture and contribute to metabolic and mitochondrial dysfunction [52]. Molecularly, the process is characterized by a reversion to fetal-like gene expression, including increased expression of *Acta1* (α -skeletal actin), *Myh7* (β -myosin heavy chain), and natriuretic peptides *NppA* and *NppB*, disrupting the normal homeostatic gene programs of adult cardiomyocytes [13].

Despite advances in pharmacological therapies – such as angiotensin II receptor blockers, diuretics, and beta-blockers – these treatments often fail to reverse cardiac damage and are not curative [53]. A major challenge lies in the incomplete understanding of the molecular and cellular changes that drive HF, alongside the limited regenerative capacity of adult cardiac tissue [54]. A deeper understanding of the baseline transcriptional networks that maintain cardiomyocyte function may open new avenues for therapies aimed at reversing disease-associated gene expression profiles.

These transcriptional states are regulated by the epigenome, which encompasses DNA and histone modifications, as well as ncRNA-mediated mechanisms [55]. Alterations in epigenetic regulation have been strongly associated with the onset and progression of CVD and HF, with changes noted in DNA methylation, hydroxymethylation, histone modification patterns, chromatin architecture, and ncRNA expression [56]. Moreover, disruptions in these epigenetic processes – often in a cell or tissue-specific manner – have been linked to numerous risk factors for CVD and HF, including diabetes, hypertension, stroke, atherosclerosis, MI, and obesity [57]. A summary of epigenetic factors involved in CVD can be outlined in **Table 2**.

4.1 DNA methylation and heart disease

DNA methylation has emerged as a critical factor in the development of CVDs, although its specific role in HF remains incompletely understood. Numerous works have demonstrated that DNA methylation profiles are altered across various disease-promoting pathways, often leading to observable phenotypic changes. Recent epigenome-wide association studies (EWAS) have helped identify new therapeutic targets and have deepened our understanding of the molecular mechanisms underlying CVD and HF. One EWAS conducted in the CATHGEN cohort revealed six novel methylation sites associated with all-cause mortality, including castor zinc

Category	Key mechanisms/factors	Context/timing	Functional outcome/notes
DNA methylation in heart disease	Altered methylation at CpG sites (e.g., CASZ1), fetal-like reprogramming in cardiomyocytes, prenatal exposure effects, DNMTs (DNMT1, DNMT3A/B), TET enzymes (5-hmC), RNA methylation (METTL3, FTO, m6A)	HF development, pressure overload (TAC), aging	Modulates gene expression in hypertrophy and HF; can be protective or maladaptive; fetal-like methylation may be reactivated in disease; therapeutic targets identified via EWAS; RNA methylation regulates the hypertrophic response and contractility
Histone modifications in heart disease	HMTs: G9a/EHMT2; HDMs: JMJD2A; acetylation: HATs (p300), HDACs (HDAC4, 5, 9)	Cardiac hypertrophy, HF progression	JMJD2A deletion protects against hypertrophy; G9a maintains cardiomyocyte homeostasis; p300 overexpression drives hypertrophy; HDAC4/5/9 inhibit MEF2C, protecting against hypertrophy and HF
Noncoding RNAs (ncRNAs) in heart disease	lncRNAs: <i>Mhrt</i> , <i>Chaer</i> , H19, <i>Chrf</i> , <i>Sarrah</i>	Cardiac hypertrophy, HF, cardiac aging	<i>Mhrt</i> inhibits BRG1, preventing hypertrophy; <i>Chaer</i> inhibits PRC2-mediated repression, promoting hypertrophy; H19 suppresses NFAT, protecting against maladaptive hypertrophy; <i>Chrf</i> regulates miR-489, influencing hypertrophy; <i>Sarrah</i> , downregulated with aging, prevents cardiomyocyte apoptosis

Table 2.
Summary of epigenetic mechanisms and factors involved in cardiovascular disease.

finger 1 (CASZ1), a key regulatory gene linked to adverse outcomes in CVD patients [58].

Animal models, particularly those using transverse aortic constriction (TAC) to simulate cardiac hypertrophy and HF, have been instrumental in exploring how DNA methylation contributes to gene expression changes during disease progression. These studies revealed that DNA methylation deposition patterns are significantly altered in hypertrophic and failing hearts; however, the direct impact on gene expression remains uncertain. One notable finding is that after three weeks of TAC, cardiomyocyte DNA methylation patterns begin to resemble those seen during fetal development, though the functional consequences of this reprogramming are not yet fully understood [59]. Similar fetal-like methylation signatures have also been observed in the aging heart, though their implications for age-associated HF require further investigation [60].

There is currently no consensus regarding the functional outcomes of DNA methylation alterations in HF. Some studies have linked hypermethylation of CpG islands in specific promoter regions with increased expression of genes involved in end-stage cardiomyopathies [61]. Conversely, other research indicates that aberrant DNA methylation can repress critical genes involved in maladaptive pathways, such as those associated with dilated cardiomyopathy [62]. Additionally, evidence suggests that prenatal tobacco exposure can lead to lasting changes in DNA methylation, particularly in repetitive elements like Alu Yb8, potentially elevating the risk of developing CVD later in life [63]. Altered methylation patterns in end-stage HF patients have also been correlated with changes in the expression of angiogenic factors such as PECAM1 [61]. Moreover, some studies suggest that aberrant DNA methylation may impair the heart's ability to respond to physiological stress [64].

As established, DNA methylation in mammals is primarily mediated by DNMTs. DNMT1 plays a critical role in maintaining methylation patterns during the DNA replication process, while DNMT3A and DNMT3B are responsible for *de novo* methylation, especially during embryonic development [65]. Deleting DNMT3B specifically in cardiomyocytes leads to impaired cardiac function and worsened HF under stress conditions [66]. Interestingly, constitutive deletion of both DNMT3A and DNMT3B does not appear to produce an overt cardiac phenotype in mice [67]. On the other hand, pharmacological inhibition of DNMTs using compounds like 5-aza-2-deoxycytidine, 5-azacytidine, or RG108 has shown protective effects, preventing HF onset in animal models [68].

Another important epigenetic layer is DNA hydroxymethylation, a process catalyzed by TET enzymes, which convert 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC). This mark is often enriched in promoter and enhancer regions and is associated with active transcription [69]. While TET enzyme expression remains stable in pressure overload models, redistribution of 5-hmC has been observed during cardiac hypertrophy, particularly in gene bodies and enhancers, leading to increased transcription of fetal cardiac genes such as *Nppa*, *Nppb*, and *Myh7* [69].

RNA methylation – especially N6-methyladenosine (m6A) – has also emerged as a critical regulator of cardiac homeostasis and disease. Similar to DNA methylation, RNA methylation is reversible and dynamically regulated. In the heart, METTL3 (an m6A “writer”) and FTO (an m6A “eraser”) have been identified as key regulators. Notably, blocking METTL3 blunts the hypertrophic response, while

reduced FTO expression in failing hearts leads to diminished cardiomyocyte contractility [70]. The dynamic interplay between these enzymes highlights the broader role of RNA methylation in modulating cardiac function and its potential relevance in HF.

4.2 Histone modifications and heart disease

Several HMTs and HDMTs have been implicated in the regulation of gene expression during cardiac hypertrophy. One notable HDM, lysine demethylase 4A (JMJD2A), demethylates the repressive marks H3K9me3 and H3K26me3, thereby modulating genes involved in the hypertrophic response. Studies using JMJD2A knockout mice demonstrate that the absence of this enzyme confers resistance to pressure overload-induced cardiac hypertrophy [71].

In contrast, the HMT G9a (EHMT2) is essential for maintaining cardiomyocyte homeostasis. G9a regulates cardiomyocyte function by catalyzing the methylation of H3K9me2 and interacting with PRC2's catalytic subunit, EZH2. Importantly, G9a exerts an antihypertrophic effect in healthy cardiomyocytes by repressing the transcriptional activity of myocyte enhancer factor 2C (MEF2C). At baseline, this repression helps keep fetal gene expression silenced in the adult heart [13].

Another critical histone modification involved in cardiac development and disease is acetylation, which generally promotes transcriptional activation, unlike methylation. The balance of histone acetylation is regulated by HATs and HDACs. The HAT p300 has been extensively studied for its role in cardiac development; its deletion results in embryonic lethality accompanied by severe cardiac malformations. Conversely, overexpression of p300 is linked to cardiac hypertrophy, as it acetylates transcription factors such as GATA4 and MEF2C, enhancing their binding to promoters of fetal genes that drive the hypertrophic program [72].

Among the HDACs, HDAC4 (a class II HDAC) represses MEF2C activity through deacetylation, thereby reversing hypertrophic changes [73]. Other class II HDACs, including HDAC5 and HDAC9, similarly inhibit MEF2C function and attenuate the hypertrophic response, underscoring their protective role against cardiac hypertrophy and HF [74].

4.3 ncRNAs and heart disease

The investigation of lncRNAs in cardiac hypertrophy and HF is still in its early stages. High-throughput sequencing technologies have uncovered thousands of lncRNAs whose expression is dysregulated in cardiac diseases such as HF, yet most remain poorly characterized. Gaining insight into the diverse regulatory mechanisms of these transcripts in cardiac pathophysiology will be crucial for developing innovative therapeutic approaches [75].

One of the earliest studied lncRNAs in the heart is myosin heavy chain-associated RNA transcript (*Mhrt*), which overlaps with the *Myh7* gene locus. *Mhrt* binds directly to BRG1, providing protection against hypertrophic growth. Overexpression of *Mhrt* in mice subjected to TAC improved cardiac function by regulating the transcriptional switch between *Myh6* and *Myh7*, a key transcriptional change that has been described in the cardiomyocyte hypertrophic response [46].

Another significant lncRNA involved in cardiac hypertrophy is the cardiac hypertrophy-associated epigenetic regulator (*Chaer*). *Chaer* interacts with the catalytic subunit of the PRC2 complex, EZH2, and inhibits the deposition of the repressive mark H3K27me3 at the promoters of several hypertrophic genes, thus promoting hypertrophy. Mice lacking *Chaer* exhibit improved cardiac function and reduced hypertrophy following pressure overload [14].

The lncRNA *H19* also plays a role in cardiomyocyte hypertrophy and apoptosis. Its expression increases during the early hypertrophic phase of HF but declines in the decompensated heart. *H19* interacts with PRC2 and suppresses the prohypertrophic transcription factor NFAT (nuclear factor of activated T cells). Mice deficient in *H19* develop more severe hypertrophy under pressure overload, while *H19* overexpression enhances cardiac function in models of afterload-induced HF [76].

Additionally, the lncRNA *Chrf* regulates miR-489; repression of miR-489 promotes maladaptive cardiac hypertrophy, illustrating another layer of complex epigenetic regulation [77].

In the context of cardiac aging, the lncRNA OXCT1 antisense RNA 1 (*Sarrah*) has been identified as a key noncoding transcript. *Sarrah* is downregulated during aging and plays an antiapoptotic role in cardiomyocytes, which is necessary for their survival [78]. Studies on the epigenetic mechanisms underlying cardiac aging will be critical for the development of therapeutic tools for the improvement of lifestyle in the aged population.

5. Conclusions

In the context of CVD and HF, it is critical to consider the influence of aging on cardiovascular health. Aging is associated with numerous functional changes in the heart, including diastolic and systolic dysfunction, arrhythmias, and atrial fibrillation. The increased prevalence of CVDs in older populations is linked to chronic inflammation, oxidative stress, reactive oxygen species (ROS) production, apoptosis, and myocardial deterioration [50]. Recent work has shown that aging hearts undergo a metabolic shift: cardiomyocytes increase glycolysis while reducing oxidative phosphorylation, which contributes to age-associated decline in cardiac function. By integrating epigenomic, transcriptomic, and metabolomic data across young and old mice, this study identified that a set of conserved enhancers related to the glycolysis pathway becomes activated during the aging process. This effect was shown to be a result of pseudohypoxia, wherein hypoxia-related responses are induced even under normal oxygen levels, mediated via the histone acetyltransferase p300/CBP. Importantly, pharmacologic inhibition of p300/CBP before the onset of cardiac aging reversed this metabolic remodeling, leading to a more aerobic metabolic profile, better heart contractility, and an attenuation of age-related cardiac deterioration [79]. This work is crucial in the understanding of the underlying epigenetic mechanisms altered during the aging process.

In addition to their role in disease mechanisms, epigenetic factors also show promise as diagnostic biomarkers. Biomarkers are measurable indicators that can reflect disease states or how a patient responds to treatment. Among these, miRNAs stand out due to their stability, ease of detection in various body fluids, and strong conservation across species, which makes it easier to translate findings from animal

studies to humans. As a result, miRNAs are being actively explored as potential predictive biomarkers in CVD [80].

In addition, it is important to consider the significance of different environmental cues and their effect on cardiovascular health. Although significant advances have been made in nanotoxicology, the epigenetic mechanisms underlying nanoparticle (NP)-induced toxicity remain poorly understood. A recent study found that exposure to iron-based magnetic NPs alters the distribution of histone modification H3K27ac, leading to changes in enhancer activity and gene expression, highlighting epigenetic reprogramming as a key contributor to nanotoxicity [81]. These findings could have important implications for CVD. The fact that toxicants can modulate enhancer activity through epigenetic mechanisms – coupled with the role of enhancers in driving the transcriptional changes underlying cardiac hypertrophy and the metabolic shift that causes the impairment of cardiac function in aging – suggests that environmental toxicants may adversely affect heart function via an epigenetic pathway.

Acronyms and abbreviations

3'UTR	3' untranslated region
5mC	5-methylcytosine
α -MHC	alpha-myosin heavy chain (adult isoform)
β -MHC	beta-myosin heavy chain (fetal isoform)
BAF	BRG1/BRM-associated factor (chromatin-remodeling complex)
BMP	bone morphogenetic protein
BVHT	Braveheart
CHAER	cardiac hypertrophy-associated epigenetic regulator
CMCs	cardiomyocytes
CRP	C-reactive protein
CVD	cardiovascular disease
DNMT	DNA methyltransferase
DOT1L	disruptor of telomeric silencing 1-like
ECM	extracellular matrix
EMT2 (G9a)	euchromatic histone lysine methyltransferase 2
EWAS	epigenome-wide association studies
EZH2	enhancer of zeste homolog 2
FENDRR	fetal-lethal noncoding developmental regulatory RNA
FGF8	fibroblast growth factor 8
FTO	fat mass and obesity-associated protein (m6A RNA demethylase)
GATA	GATA-binding transcription factors
HAT	histone acetyltransferase
HAND	heart and neural crest derivatives expressed proteins
HDAC	histone deacetylase
HDMT	histone demethylase
HF	heart failure
HMT	histone methyltransferase
IL-6	interleukin-6

JMJD2A	Jumonji domain-containing protein 2A (histone demethylase)
lncRNA	long noncoding RNA
m6A	N6-methyladenosine
MBP	methyl-CpG-binding protein
MEF2C	myocyte enhancer factor 2C
MESP1	mesoderm posterior bHLH transcription factor 1
METTL3	methyltransferase-like 3 (m6A RNA methyltransferase)
MHRT	Myheart
NANOG	NANOG homeobox transcription factor
ncRNA	noncoding RNA
NFAT	nuclear factor of activated T-cells
NOTCH	Notch signaling pathway
NKX2.5	NK2 homeobox 5
OCT	octamer-binding transcription factors
PRC2	polycomb repressive complex 2
PTM	posttranslational modification
ROS	reactive oxygen species
SARRAH	senescence-associated lncRNA regulating heart regeneration
SET	SET domain-containing histone methyltransferases
SMYD1	SET and MYND domain-containing protein 1 (histone methyltransferase)
SRF	serum response factor
TAC	transverse aortic constriction
TBX5	T-box transcription factor 5
TET	ten-eleven translocation (methylcytosine dioxygenases)
TGF- β	transforming growth factor beta
TNF	tumor necrosis factor
UPH	Upperhand
VSMC	vascular smooth muscle cell
WHSC1	Wolf-Hirschhorn syndrome candidate 1 (histone methyltransferase)
WNT	Wnt signaling pathway (family of glycoproteins)

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Conflict of interest

The authors declare no conflict of interest.

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References

- [1] Muka T, Imo D, Jaspers L, Colpani V, Chaker L, van der Lee SJ, et al. The global impact of noncommunicable diseases on healthcare spending and national income: A systematic review. *European Journal of Epidemiology*. 2015;**30**:251–277
- [2] McMurray JJV. Systolic heart failure. *New England Journal of Medicine*. 2010;**362**(3):228–238
- [3] Papait R, Condorelli G. Epigenetics in heart failure. *Annals of the New York Academy of Sciences*. 2010;**1188**:159–164
- [4] Papait R, Corrado N, Rusconi F, Serio S, Latronico MVG. It's time for an epigenomics roadmap of heart failure. *Current Genomics*. 2015;**16**(4):237–244
- [5] Schleithoff C, Voelter-Mahlknecht S, Dahmke IN, Mahlke U. On the epigenetics of vascular regulation and disease. *Clinical Epigenetics*. 2012;4
- [6] Bonasio R, Tu SJ, Reinberg D. Molecular signals of epigenetic states. *Science*. 2010;**330**(6004):612–616
- [7] Cantone I, Fisher AG. Epigenetic programming and reprogramming during development. *Nature Structural & Molecular Biology*. 2013;**20**(3):282–289
- [8] Bergman Y, Cedar H. DNA methylation dynamics in health and disease. *Nature Structural & Molecular Biology*. 2013;**20**(3):274–281
- [9] Berdasco M, Esteller M. Genetic syndromes caused by mutations in epigenetic genes. *Human Genetics*. 2013;**132**(4):359–383
- [10] Papait R, Greco C, Kunderfranco P, Latronico MVG, Condorelli G. Epigenetics: A new mechanism of regulation of heart failure? *Basic Research in Cardiology*. 2013;**108**(4)
- [11] Jones PA. Functions of DNA methylation: Islands, start sites, gene bodies and beyond. *Nature Reviews Genetics*. 2012;**13**(7):484–492
- [12] Dykes IM, Emanuelli C. Transcriptional and post-transcriptional gene regulation by long non-coding RNA. *Genomics, Proteomics & Bioinformatics*. 2017;**15**(3):177–186
- [13] Papait R, Serio S, Pagiatakis C, Rusconi F, Carullo P, Mazzola M, et al. Histone methyltransferase G9a is required for cardiomyocyte homeostasis and hypertrophy. *Circulation*. 2017;**136**:1233–1246
- [14] Wang Z, Zhang XJ, Ji YX, Zhang P, Deng KQ, Gong J, et al. The long noncoding RNA Chaer defines an epigenetic checkpoint in cardiac hypertrophy. *Nature Medicine*. 2016;**22**:1131–1139
- [15] Muka T, Koromani F, Portilla E, O'Connor A, Bramer WM, Troup J, et al. The role of epigenetic modifications in cardiovascular disease: A systematic review. *International Journal of Cardiology*. 2016;**212**:174–183
- [16] Cheng Z, Zhang Y. Role of mammalian DNA methyltransferases in development. *Annual Review of Biochemistry*. 2020;**89**:135–158
- [17] Sasaki H, Matsui Y. Epigenetic events in mammalian germ-cell development: Reprogramming and

- beyond. *Nature Reviews Genetics*. 2008;**9**(2):129–140
- [18] Lan Y, Banks KM, Pan H, Verma N, Dixon GR, Zhou T, et al. Stage-specific regulation of DNA methylation by TET enzymes during human cardiac differentiation. *Cell Reports*. 2021;**37**(10):110095
- [19] Onodera A, González-Avalos E, Lio CW, *et al.* Roles of TET and TDG in DNA demethylation in proliferating and non-proliferating immune cells. *Genome Biology*. 2021;**22**:186
- [20] Sokolova V, Sarkar S, Tan D. Histone variants and chromatin structure, update of advances. *Computational and Structural Biotechnology Journal*. 2022;**5**(21):299–311
- [21] Turner BM. Defining an epigenetic code. *Nature Cell Biology*. 2007;**9**(1):2–6
- [22] Mellert HS, McMahon SB. Biochemical pathways that regulate acetyltransferase and deacetylase activity in mammalian cells. *Trends in Biochemical Sciences*. 2009;**34**(11):571–578
- [23] Gray ZH, Honer MA, Ghatalia P, Shi Y, Whetstine JR. 20 years of histone lysine demethylases: From discovery to the clinic and beyond. *Cell*. 2025;**188**(87):1747–1783
- [24] Ng SS, Yue WW, Oppermann U, Klose RJ. Dynamic protein methylation in chromatin biology. *Cellular and Molecular Life Sciences*. 2009;**66**(3):407–422
- [25] van Weerd JH, Koshiba-Takeuchi K, Kwon C, Takeuchi JK. Epigenetic factors and cardiac development. *Cardiovascular Research*. 2011;**91**(2):203–211
- [26] Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, et al. Landscape of transcription in human cells. *Nature*. 2012;**489**:101–108
- [27] Latronico MVG, Condorelli G. MicroRNAs and cardiac pathology. *Nature Reviews Cardiology*. 2009;**6**(6):418–429
- [28] Amaral PP, Mattick JS. Noncoding RNA in development. *Mammalian Genome : Official Journal of the International Mammalian Genome Society*. 2008;**19**(7–8):454–492
- [29] Kesharwani V, Shahshahan HR, Mishra PK. Cardiac transcriptome profiling of diabetic Akita mice using microarray and next generation sequencing. *PLoS One*. 2017;**12**(8)
- [30] Harvey RP. Patterning the vertebrate heart. *Nature Reviews Genetics*. 2002;**3**(7):544–556
- [31] Srivastava D, Olson EN. A genetic blueprint for cardiac development. *Nature*. 2000;**407**(6801):221–226
- [32] Olson EN. Gene regulatory networks in the evolution and development of the heart. *Science*. 2006;**313**(5795):1922–1927
- [33] Srivastava D, Olson EN. Knowing in your heart what’s right. *Trends in Cell Biology*. 1997;**7**(11):447–453
- [34] Lin Q, Schwarz J, Bucana C, Olson EN. Control of mouse cardiac morphogenesis and myogenesis by transcription factor MEF2C. *Science*. 1997;**276**(5317):1404–1407
- [35] Owens GK, Kumar MS, Wamhoff BR. Molecular regulation of vascular smooth muscle cell

differentiation in development and disease. *Physiological Reviews*. 2004;**84**(3):767–801

[36] Majesky M. Development of coronary vessels. *The FASEB Journal*. 2015;**29**

[37] Li Y, Du J, Deng S, *et al.* The molecular mechanisms of cardiac development and related diseases. *Signal Transduction and Targeted Therapy*. 2024;**9**:368

[38] Sim CB, Ziemann M, Kaspi A, Hari Krishnan KN, Ooi J, Khurana I, *et al.* Dynamic changes in the cardiac methylome during postnatal development. *The FASEB Journal*. 2015;**29**:1329–1343

[39] Felician G, Collesi C, Lusic M, Martinelli V, Dal Ferro M, Zentilin L, *et al.* Epigenetic modification at Notch responsive promoters blunts efficacy of inducing Notch pathway reactivation after myocardial infarction. *Circulation Research*. 2014;**115**(7):636–49

[40] Nimura K, Ura K, Shiratori H, Kaneda Y. A histone H3 lysine 36 trimethyl-transferase Whsc1 links transcription factors to Wolf-Hirschhorn syndrome. *Journal of Gene Medicine*. 2010;**12**(12):1051

[41] Gottlieb PD, Pierce SA, Sims RJ, Yamagishi H, Weihe EK, Harriss JV, *et al.* Bop encodes a muscle-restricted protein containing MYND and SET domains and is essential for cardiac differentiation and morphogenesis. *Nature Genetics*. 2002;**31**:25–32

[42] Cattaneo P, Kunderfranco P, Greco C, Guffanti A, Stirparo GG, Rusconi F, *et al.* DOT1L-mediated H3K79me2 modification

critically regulates gene expression during cardiomyocyte differentiation. *Cell Death & Differentiation*. 2016;**23**:555–564

[43] Takeuchi JK, Lou X, Alexander JM, Sugizaki H, Delgado-Olguín P, Holloway AK, *et al.* Chromatin remodelling complex dosage modulates transcription factor function in heart development. *Nature Communications*. 2011;**2**:187

[44] Takeuchi JK, Lickert H, Bisgrove BW, Sun X, Yamamoto M, Chawengsaksophak K, *et al.* Baf60c is a nuclear Notch signaling component required for the establishment of left-right asymmetry. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;**104**:846–851

[45] Li J, Zy. M, Cui Y, *et al.* Cardiac-specific deletion of BRG1 ameliorates ventricular arrhythmia in mice with myocardial infarction. *Acta Pharmacologica Sinica*. 2024;**45**:517–530

[46] Han P, Li W, CH L, Yang J, Shang C, Nuernberg ST, *et al.* A long noncoding RNA protects the heart from pathological hypertrophy. *Nature*. 2014;**514**:102–106

[47] KM A, DM A, McAnally JR, Shelton JM, Bassel-Duby R, and Olson EN. Transcription of the non-coding RNA upperhand controls Hand2 expression and heart development. *Nature*. 2016;**539**(7629):433–436

[48] Grote P, Wittler L, Hendrix D, Koch F, Wahrlich S, Beisaw A, *et al.* The tissue-specific lncRNA Fendrr is an essential regulator of heart and body wall development in the mouse. *Developmental Cell*. 2013;**24**:206–214

- [49] Klattenhoff CA, Scheuermann JC, Surface LE, Bradley RK, Fields PA, Steinhäuser ML, et al. Braveheart, a long noncoding RNA required for cardiovascular lineage commitment. *Cell*. 2013; **152**:570–583
- [50] Curtis AB, Karki R, Hattoum A, Sharma UC. Arrhythmias in patients ≥80 years of age: Pathophysiology, management, and outcomes. *Journal of the American College of Cardiology*. 2018;**71**(18):2041–2057
- [51] Yang Z, Jiang S, Shang J, Jiang Y, Dai Y, Xu B, et al. LncRNA: Shedding light on mechanisms and opportunities in fibrosis and aging. *Ageing Research Reviews*. 2019;**52**:17–31
- [52] Kehat I, Molkentin JD. Molecular pathways underlying cardiac remodeling during pathophysiological stimulation. *Circulation*. 2010;**122** (25):2727–2735
- [53] McMurray JJ. Clinical practice. Systolic heart failure. *New England Journal of Medicine*. 2010;**362**(3): 228–238
- [54] Porrello ER, Mahmoud AI, Simpson E, Hill JA, JA R, Olson EN, et al. Transient regenerative potential of the neonatal mouse heart. *Science*. 2011;**331**:1078–1080
- [55] Papait R, Serio S, Condorelli G. Role of the epigenome in heart failure. *Physiological Reviews*. 2020;**100** (4):1753–1777
- [56] Zhang W, Song M, Qu J, Liu GH. Epigenetic modifications in cardiovascular aging and diseases. *Circulation Research*. 2018;**123**(7): 773–786
- [57] Rodgers JL, Jones J, Bolleddu SI, Vanthenapalli S, Rodgers LE, Shah K, et al. Cardiovascular risks associated with gender and aging. *Journal of Cardiovascular Development and Disease*. 2019;**6**(2)
- [58] Abdulrahim JW, Kwee LC, Grass E, Siegler IC, Williams R, Karra R, et al. Epigenome-wide association study for all-cause mortality in a cardiovascular cohort identifies differential methylation in castor zinc finger 1 (CASZ1). *Journal of the American Heart Association*. 2019;**8**(21)
- [59] Gilsbach R, Preissl S, Grüning BA, Schnick T, Burger L, Benes V, et al. Dynamic DNA methylation orchestrates cardiomyocyte development, maturation and disease. *Nature Communications*. 2014;**5**:5288
- [60] Pagiatakis C, Musolino E, Gornati R, Bernardini G, Papait R. Epigenetics of aging and disease: A brief overview. *Aging Clinical and Experimental Research* 2019;**737**–745
- [61] Movassagh M, Choy MK, Goddard M, Bennett MR, Down TA, Foo RS. Differential DNA methylation correlates with differential expression of angiogenic factors in human heart failure. *PLoS One*. 2010;**5**(1)
- [62] Haas J, Frese KS, Park YJ, Keller A, Vogel B, Lindroth AM, et al. Alterations in cardiac DNA methylation in human dilated cardiomyopathy. *EMBO Molecular Medicine*. 2013;**5**:413–429
- [63] Xu R, Hong X, Zhang B, et al. DNA methylation mediates the effect of maternal smoking on offspring birthweight: A birth cohort study of multi-ethnic US mother–newborn pairs. *Clinical Epigenetics*. 2021;**13**:47

- [64] Fatima N, Schooley JF, Claycomb WC, Flagg TP. Promoter DNA methylation regulates murine SUR1 (Abcc8) and SUR2 (Abcc9) expression in HL-1 cardiomyocytes. *PLoS One*. 2012;7(7):e41533
- [65] Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases DNMT3A and DNMT3B are essential for de novo methylation and mammalian development. *Cell*. 1999;99(3):247–257
- [66] Vujic A, Robinson EL, Ito M, Haider S, Ackers-Johnson M, See K, et al. Experimental heart failure modelled by the cardiomyocyte-specific loss of an epigenome modifier, DNMT3B. *Journal of Molecular and Cellular Cardiology*. 2015;82:174–183
- [67] Nührenberg TG, Hammann N, Schnick T, Preißl S, Witten A, Stoll M, et al. Cardiac myocyte de novo DNA methyltransferases 3a/3b are dispensable for cardiac function and remodeling after chronic pressure overload in mice. *PLoS One*. 2015;10(6):e0131019
- [68] Stenzig J, Schneeberger Y, Löser A, Peters BS, Schaefer A, Zhao RR, et al. Pharmacological inhibition of DNA methylation attenuates pressure overload-induced cardiac hypertrophy in rats. *Journal of Molecular and Cellular Cardiology*. 2018;120:53–63
- [69] Greco CM, Kunderfranco P, Rubino M, Larcher V, Carullo P, Anselmo A, et al. DNA hydroxymethylation controls cardiomyocyte gene expression in development and hypertrophy. *Nature Communications*. 2016;7:12418
- [70] Pagiatakis C, Condorelli G. The RNA methylome blackboard. *Circulation*. 2019;139(4):546–548
- [71] Zhang QJ, Chen HZ, Wang L, Liu DP, Hill JA, Liu ZP. The histone trimethyllysine demethylase JMJD2A promotes cardiac hypertrophy in response to hypertrophic stimuli in mice. *Journal of Clinical Investigation*. 2011;121(6):2447–2456
- [72] Miyamoto S, Kawamura T, Morimoto T, Ono K, Wada H, Kawase Y, et al. Histone acetyltransferase activity of p300 is required for the promotion of left ventricular remodeling after myocardial infarction in adult mice in vivo. *Circulation*. 2006;113:679–690
- [73] Backs J, Worst BC, Lehmann LH, Patrick DM, Jebessa Z, Kreusser MM, et al. Selective repression of MEF2 activity by PKA-dependent proteolysis of HDAC4. *Journal of Cell Biology*. 2011;195:403–415
- [74] Chang S, McKinsey TA, Zhang CL, Richardson JA, JA H, Olson EN. Histone deacetylases 5 and 9 govern responsiveness of the heart to a subset of stress signals and play redundant roles in heart development. *Molecular and Cellular Biology*. 2004;24(19):8467–8476
- [75] Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, et al. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. *Nature*. 2009;458:223–227
- [76] Pagiatakis C, Hall IF, Condorelli G. Long non-coding RNA H19 : A new avenue for RNA therapeutics in cardiac hypertrophy? *European Heart Journal*. 2020;41(36):3475–3476
- [77] Wang K, Liu F, Zhou LY, Long B, Yuan SM, Wang Y, et al. The long noncoding RNA CHRF regulates cardiac

hypertrophy by targeting miR-489.
Circulation Research. 2014;**114**:
1377–1388

[78] Trembinski DJ, Bink DI, Theodorou K, Sommer J, Fischer A, van Bergen A, et al. Aging-regulated antiapoptotic long non-coding RNA Sarrah augments recovery from acute myocardial infarction. *Nature Communications*. 2020;**11**:2039

[79] Serio S, Pagiatakis C, Musolino E, Felicetta A, Carullo P, Laura Frances J, Papa L, Rozzi G, Salvarani N, Miragoli M, Gornati R, Bernardini G, Condorelli G, Papait R. Cardiac aging is promoted by pseudohypoxia increasing p300-induced glycolysis. *Circulation Research*. 2023;**133**(8):687–703

[80] Matsumoto S, Sakata Y, Suna S, Nakatani D, Usami M, Hara M, et al. Circulating p53-responsive microRNAs are predictive indicators of heart failure after acute myocardial infarction. *Circulation Research*. 2013;**113**:322–326

[81] Gamberoni F, Borgese M, Pagiatakis C, Armenia I, Grazù V, Gornati R, Serio S, Papait R, Bernardini G. Iron oxide nanoparticles with and without cobalt functionalization provoke changes in the transcription profile via epigenetic modulation of enhancer activity. *Nano Letters*. 2023;**23**(19):9151–9159

Lactylation-Driven Epigenetic Modulation: A New Frontier in Vascular Aging and Associated Disorders

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Abstract

Vascular aging is a fundamental process driving age-related cardiovascular and cerebrovascular diseases, characterized by endothelial dysfunction, vascular smooth muscle cell (VSMC) senescence, and extracellular matrix (ECM) remodeling. Recent studies have unveiled protein lactylation, a novel post-translational modification where lactate covalently binds to lysine residues, as a critical metabolic-epigenetic mechanism linking glycolytic flux to vascular pathophysiology. Under conditions such as hypoxia, hyperglycemia, or oxidative stress, accumulated lactate is converted to lactoyl-CoA and transferred by enzymes like p300/CBP to modify histones and non-histone proteins, thereby regulating gene expression and cellular functions. This article comprehensively summarizes the dual roles of lactylation in vascular aging and associated disorders. Lactylation promotes endothelial inflammation, VSMC osteogenic transition, and ECM calcification through specific targets such as H3K18la, NF- κ B, and metabolic enzymes, thereby accelerating atherosclerosis, diabetic vasculopathy, pulmonary hypertension, and aortic aneurysms. Conversely, in certain contexts, it supports reparative macrophage polarization and plaque stability. The interplay between lactylation and other modifications further fine-tunes vascular homeostasis. Emerging therapeutic strategies, including small-molecule inhibitors of lactylation writers p300/CBP, lactate-lowering agents, and natural compounds, show promise in preclinical models. However, challenges remain in specificity, detection, and clinical translation. Future research should integrate multi-omics approaches and spatial technologies to elucidate cell-specific lactylation networks, facilitating the development of targeted interventions against vascular aging and its complications.

Keywords: lactylation, vascular aging, epigenetic modulation, metabolic reprogramming, vascular disease

1. Introduction

Vascular aging is a cornerstone of age-related morbidity, underpinning pathologies ranging from atherosclerosis and hypertension to Alzheimer's disease and renal failure [1, 2]. As arteries lose elasticity and endothelial function declines, blood vessels become prone to stiffening, reduced nitric oxide bioavailability, and chronic inflammation. These changes impair tissue perfusion, elevate cardiac workload, and drive end-organ damage [3–5]. Notably, vascular aging is not a passive process but an active maladaptation fueled by oxidative stress, mitochondrial dysfunction, and the senescence-associated secretory phenotype (SASP), where senescent cells release pro-inflammatory cytokines and proteases [6]. For example, senescent vascular smooth muscle cells (VSMCs) secrete matrix metalloproteinases (MMPs) that degrade elastin, accelerating arterial stiffening [7–9]. The systemic consequences are profound: microvascular rarefaction in the brain exacerbates cognitive decline [10], while coronary artery remodeling increases ischemic heart disease risk [11]. Thus, vascular aging is both a biomarker and a driver of multisystem decline, making it a critical target for interventions aimed at extending healthspan.

Post-translational modifications (PTMs) dynamically regulate cellular aging by modulating protein stability, localization, and interaction networks [12, 13]. Acetylation, phosphorylation, and ubiquitination are well-established PTMs that govern processes such as DNA repair, metabolic flux, and stress responses. Among diverse cell types, vascular cells are uniquely sensitive to PTM-dependent signaling because of their constant exposure to hemodynamic forces, fluctuating oxygen levels, and metabolic stress [14]. Moreover, maladaptive PTM patterns can amplify endothelial dysfunction and smooth muscle phenotypic switching, both hallmarks of vascular aging [15]. In vascular cells, PTMs act as molecular switches: SIRT1-mediated deacetylation of endothelial nitric oxide synthase (eNOS) maintains vascular tone, while phosphorylation of AMP-activated protein kinase (AMPK) coordinates energy homeostasis during hypoxia [16, 17]. Aging disrupts PTM equilibrium – hyperacetylation of histones due to declining sirtuin activity promotes pro-inflammatory gene expression, while aberrant ubiquitination impairs proteostasis, leading to toxic protein aggregation [18]. Intriguingly, PTMs also serve as metabolic sensors. For instance, O-GlcNAcylation, a nutrient-sensitive modification, links hyperglycemia to endothelial dysfunction in diabetes [19]. These findings position PTMs as both mediators of aging and therapeutic levers, exemplified by NAD⁺ boosters that restore sirtuin activity to ameliorate vascular senescence.

The discovery of protein lactylation by Zhang et al. unveiled a paradigm in which lactate, a glycolysis byproduct, directly modifies lysine residues, integrating metabolic states with epigenetic regulation [20]. Under hypoxic or hyperglycemic conditions, lactate accumulation drives lactoyl-CoA synthesis, enabling enzymes like p300/CBP to catalyze lactylation on histones (e.g., H3K18la) and non-histone targets [21–23]. Equally important, lactylation operates as a bona fide epigenetic mechanism. Lactate-derived lactoyl-CoA enables writer enzymes – including p300/CBP and the ACSS2–KAT2A axis – to deposit histone lactylation at active chromatin, thereby tuning transcriptional programs in a context-dependent manner [20, 24]. Counterbalancing this, class I histone deacetylases (HDAC1–3) function as delactylases that erase histone lactylation and reset chromatin states [25]. Additional reports nominate aminoacyl-tRNA synthetases (AARS1/2) as global

lysine lactyltransferases that sense L-lactate and regulate innate immune signaling [26, 27]. Crosstalk with other epigenetic marks (e.g., acetylation and methylation) further integrates metabolic status with enhancer activity, chromatin accessibility, and gene silencing [28]. Highlighting these writer/eraser systems and their interactions strengthens the conceptual framework of lactylation as a central epigenetic regulator in diseases.

Lactylation has emerged as a critical post-translational modification implicated in a broad spectrum of diseases. Recent studies have highlighted its regulatory roles in cancer [29], neurodegeneration [30], infection [31], fibrosis [32], and metabolic complications (**Table 1**), underscoring its wide-reaching impact on cellular physiology and pathology. These findings collectively establish lactylation as a fundamental epigenetic and metabolic signal that transcends organ systems and disease categories. Building on this broad relevance, the subsequent sections focus specifically on how lactylation interfaces with vascular cell biology and contributes to the processes of vascular aging.

In vascular aging, lactylation exerts dual roles: it sustains inflammation by enhancing NF- κ B signaling in macrophages [30, 33], thereby destabilizing atherosclerotic plaques, while in diabetic vasculature, it disrupts endothelial tight junctions by lactylating occludin, exacerbating microvascular leakage [31, 34–37]. Lactylation also competes with acetylation at shared lysine sites, creating a dynamic interplay that fine-tunes gene expression [25]. Recent work demonstrates that lumican promotes calcific aortic valve disease by driving histone H3 lactylation at specific sites (H3K14la and H3K9la), which facilitates the expression of osteogenic genes such as Runx2 and BMP2 and ultimately leads to aortic valve calcification [38]. Additionally, studies in pulmonary fibrosis reveal that lactate derived from lung myofibroblasts induces histone lactylation in macrophages via p300, promoting a profibrotic phenotype, a mechanism highlighting lactylation's role in intercellular metabolic signaling [32]. These insights, combined with advancements in lactylation-specific inhibitors and CRISPR-based epigenetic editing, underscore its potential as a therapeutic target [25, 39]. Lactylation highlights its crucial role in the metabolic regulation of age-related processes, thereby offering innovative strategies for improving vascular dysfunction.

This chapter outlines the role of lactylation in vascular aging processes: (i) biochemical foundations, encompassing the sources of lactate/lactoyl-CoA, enzymatic writers and erasers, and interactions with other chromatin marks; (ii) cellular and pathway modules, detailing how lactylation reprograms endothelial cells, vascular smooth muscle cells, and immune cells to influence inflammation, oxidative stress, senescence, extracellular matrix remodeling, and calcification; and (iii) disease applications, including atherosclerosis, hypertension, aneurysm, diabetic vasculopathy, and other aging-related conditions. Building on this framework, we synthesize translational opportunities alongside key challenges and conclude with forward-looking perspectives on therapeutic targeting and research priorities.

2. Literature search and selection

We conducted a narrative search of PubMed, Web of Science, and Scopus covering 2019–September 2025, supplemented with seminal pre-2019 lactylation reports. Core queries combined terms such as “lactylation” OR “lactoyl-lysine” with

Disease	Molecular target/ Pathway	Evidence type	Key lactylation finding	Functional consequence	Therapeutic note	Ref
Vascular disease – Atherosclerosis	ASF1A–p300–H3K18la → SNAI1 (EndMT)	ECs, Mouse, Human plaques	Endothelial H3K18la drives EndMT and plaque progression	Plaque progression ↑	Glycolysis or ASF1A inhibition lowers H3K18la & disease burden	[40, 41]
Vascular disease – Medial calcification (VSMC)	NR4A3 → glycolysis ↑ → H3K18la	VSMC, Mouse	Glycolytic flux elevates H3K18la; osteogenic reprogramming	Medial calcification ↑	Target NR4A3/glycolysis miti- gates calcification	[42]
Cancer/Tumor microen- vironment (TME)	H3K18la at immune effec- tor loci (CD8 + T cells)	Preclinical, Human samples	Remodels TME; sustains CD8 + T-cell metabolism & function	Immune evasion/ tumor growth ↑	Potential synergy with immu- notherapy via lactate modulation	[43]
Neurodegeneration/ Alzheimer's	Microglial H3K18la → NF- κB amplification	Mouse, Human brain	Amplifies inflammatory signal- ing; accelerates brain aging	Neuroinflammation ↑	p300/HDAC axis as target; bio- marker potential	[30]
Infection/Sepsis	HMGB1 lactylation/acety- lation → exosomal release	Mouse, Clinical samples	Lactate drives HMGB1 release	Hyperinflammation ↑	Target lactate production/export to blunt cytokine storm	[31]
Pulmonary Fibrosis	Myofibroblast lactate → macrophage H3 lactylation	Mouse, Cell models	Induces profibrotic macrophage program	Fibrosis progression ↑	p300 inhibition reduces profibro- tic phenotype	[32]
Skeletal Muscle Aging	Histone lactylation at DNA repair/proteostasis loci	Mouse	Restoring lactylation rescues aging defects	Function & proteosta- sis ↑	Exercise/hypoxia paradigms modulate lactate–lactylation axis	[44]
Diabetic Kidney Disease	ACSF2–K182 lactylation (mitochondrial)	Mouse, Human biopsies	K1a impairs mitochondrial function; tubular injury	DKD progression ↑	Reduce lactate flux/lactylation as therapeutic strategy	[45]

Table 1.
Representative evidence of lactylation in vascular and extra-vascular diseases.

vascular keywords (endothelium, VSMC, aging, calcification, atherosclerosis, hypertension) and, for scope broadening, oncology/neurology/infection/fibrosis/metabolism. We included original experimental/clinical studies and high-quality reviews in English and excluded abstracts without full text or non-peer-reviewed content. Screening proceeded from titles/abstracts to full text by two authors, with disagreements resolved by consensus. Because this is a narrative review, we did not perform a quantitative synthesis or risk-of-bias scoring; however, we prioritized mechanistically informative studies and those providing in vivo or human evidence to support the claims summarized in this chapter.

3. Basics of protein lactylation

Protein lactylation, a novel PTM, involves the covalent attachment of lactate to lysine residues, forming lactoyl-lysine (Kla) adducts [46–48]. Mechanistically, lactate is converted to lactoyl-CoA via lactate dehydrogenase (LDH) or acyl-CoA synthetases (ACSS1/2), followed by enzymatic transfer of the lactoyl group to target proteins by lysine lactoyltransferases such as p300/CBP [49, 50]. Histone lactylation alters chromatin structure to regulate gene transcription, notably driving immune cell polarization and stress adaptation [51]. Non-histone lactylation further modulates metabolic enzymes and signaling pathways, implicating this PTM in aging, cancer, and inflammatory diseases. Detection of lactylation relies on advanced techniques, including high-resolution mass spectrometry for site-specific mapping, pan-/site-specific antibodies for immunoblotting and imaging [43], and isotopic tracing to track metabolic sources [52]. However, challenges persist, such as antibody cross-reactivity with similar acylations and low modification stoichiometry, requiring refined enrichment protocols.

Lactylation dynamically interacts with classical PTMs, creating a regulatory network that fine-tunes cellular responses. It competes with acetylation for shared lysine residues, with enzymes like p300/CBP balancing these modifications in a context-dependent manner [53]. For instance, hypoxia-induced lactate accumulation shifts the equilibrium toward lactylation, suppressing pro-inflammatory genes while promoting tissue repair [20, 54]. Synergistically, lactylation primes chromatin for methylation by recruiting DNA methyltransferases, as observed in cancer-related epigenetic silencing [55, 56]. Preliminary evidence also suggests lactylation may stabilize proteins by inhibiting ubiquitination-mediated degradation, though mechanisms remain elusive [57]. These interactions underscore lactylation's role as a metabolic-epigenetic rheostat, integrating glycolytic flux with transcriptional and post-translational programs. Future research must address technical limitations, elucidate non-histone targets, and explore therapeutic modulation of lactylation enzymes to combat diseases like atherosclerosis and diabetic vasculopathy. By unraveling its crosstalk with other PTMs, lactylation research promises to redefine our understanding of metabolic control in health and disease. (Table 2)

4. Mechanisms of vascular aging

Vascular aging, a hallmark of age-related cardiovascular and cerebrovascular diseases, is driven by multifaceted molecular and structural alterations in

Class	Representative	Role in lactylation	Illustrative vascular relevance	Refs
Writer	p300/CBP	Catalyzes histone lactylation using lactoyl-CoA	Endothelial inflammation; VSMC reprogramming	[25, 47]
Writer (coupled)	ACSS2–KAT2A	Generates lactoyl-CoA and couples with KAT2A to install histone lactylation	Links metabolic flux to chromatin in disease	[24]
Putative writer	AARS1/2	Sense L-lactate and act as global lysine lactyltransferases	Innate immunity/inflammatory tone	[26]
Eraser	HDAC1–3	Delactylases that remove histone lactylation	Resets pathological lactylation programs	[58]
Transport/supply	MCT1/MCT4	Modulate lactate pools that feed lactylation	MCT4 inhibition promotes a reparative macrophage phenotype	[59]
Crosstalk	Acetylation/Methylation	Interdependent regulation of enhancers and repression complexes	Impacts chromatin accessibility and gene silencing	[60]

Table 2.
Key components and relevance of epigenetic regulation of lactylation.

endothelial cells (ECs), VSMCs, and the extracellular matrix (ECM). Endothelial dysfunction initiates this cascade, characterized by diminished nitric oxide (NO) bioavailability due to reduced eNOS activity and elevated oxidative stress from reactive oxygen species (ROS). Age-related mitochondrial dysfunction and NAD⁺ depletion further impair EC barrier integrity, promoting endothelial-to-mesenchymal transition (EndMT) and pro-inflammatory signaling, which exacerbate leukocyte adhesion and vascular permeability [61]. VSMC senescence, a key contributor to arterial stiffening, arises from telomere attrition, DNA damage accumulation, and the SASP, which releases cytokines that drive ECM remodeling and calcification [62]. Phenotypic switching of VSMCs, from contractile to synthetic states, fuels neointimal hyperplasia and vascular fibrosis, while diminished autophagy and heightened endoplasmic reticulum stress accelerate apoptosis [63, 64]. ECM remodeling, a hallmark of vascular aging, involves collagen deposition, elastin fragmentation, and glycosaminoglycan accumulation, mediated by imbalanced MMPs and their inhibitors TIMPs. This structural degradation reduces arterial compliance, elevates pulse wave velocity, and impairs mechanotransduction, perpetuating a vicious cycle of hemodynamic stress and cellular damage [65].

Beyond these core mechanisms, epigenetic dysregulation and chronic low-grade inflammation amplify vascular aging [66, 67]. Age-related shifts in microbiome-derived metabolites and circadian rhythm disruption further perturb vascular homeostasis. Emerging evidence implicates mitochondrial transfer between ECs and VSMCs, as well as non-coding RNA networks, in modulating senescence pathways [68, 69]. Together, these interconnected processes, spanning cellular dysfunction, ECM degradation, and systemic metabolic dysregulation, underlie the progression of atherosclerosis, hypertension, and aneurysmal disease. Targeting these axes, particularly via NAD⁺ boosters, senolytics, and ECM-stabilizing therapies, holds promise for mitigating age-related vascular decline and extending cardiovascular healthspan [70].

5. Role of lactylation in vascular aging

Vascular aging, characterized by progressive endothelial impairment, smooth muscle senescence, and ECM stiffening, is intricately regulated by metabolic-epigenetic crosstalk, with protein lactylation emerging as a pivotal molecular switch [71]. Lactylation, the covalent attachment of lactate to lysine residues, directly links glycolytic reprogramming to transcriptional and post-translational dysregulation, driving age-related vascular pathologies through mechanisms spanning cellular energetics, inflammatory signaling, and chromatin remodeling [72].

5.1 Metabolic reprogramming and lactate-driven lactylation

Aged vascular cells undergo a metabolic shift toward aerobic glycolysis, fueled by mitochondrial dysfunction, NAD⁺ depletion, and hypoxia-inducible factor 1 α (HIF-1 α) activation [73]. This results in excessive lactate production, while impaired lactate clearance via downregulated monocarboxylate transporters (MCTs) elevates intracellular lactate pools [74, 75]. Lactate is enzymatically converted to lactoyl-CoA by acyl-CoA synthetases (ACSS1/2) and subsequently transferred to target proteins by lysine lactoyltransferases such as p300/CBP [31]. In ECs, hyperglycemia and oxidative stress further amplify this process, creating a feedforward loop that sustains lactylation. For instance, in diabetic vasculopathy, elevated glucose flux through the polyol pathway increases lactate synthesis, promoting lactylation of ECM proteins like collagen IV, which exacerbates arterial stiffness [76, 77].

5.2 Epigenetic and non-epigenetic regulatory networks

Lactylation has emerged as a key modulator of aging processes through both epigenetic and non-epigenetic mechanisms. In cellular senescence and tissue aging, lactylation serves a dual role: it can antagonize age-related functional decline in some contexts, yet promote pathological aging in others, governed by cell type-specific and metabolic microenvironments [44, 78, 79]. In aged skeletal muscle, a marked decline in histone lactylation is associated with reduced expression of genes critical for DNA repair and proteostasis. Hypoxia or exercise can restore glycolytic flux and lactyl-CoA levels, increasing histone lactylation at promoters of genes involved in cell cycle and maintenance pathways, thereby ameliorating muscle aging and preserving function [44]. Similarly, in the heart, lactylation interacts with other metabolic-dependent epigenetic marks, particularly methylation, to regulate myocardial aging. This crosstalk influences inflammatory responses, mitochondrial function, and metabolic homeostasis, positioning dual epigenetic therapy as a promising strategy to delay age-related cardiac decline [80]. Notably, lactylation extends beyond histones. Non-histone lactylation is increasingly recognized as a pivotal regulator in aging. Proteins involved in neuronal excitability, inflammation, and cellular communication undergo lactylation, impacting CNS homeostasis and contributing to neurodegenerative processes. Microglial lactylation, for instance, influences neuroinflammation and neuronal survival, suggesting broader implications for brain aging and related diseases [81, 82].

5.3 Lactylation in vascular cell dysfunction: Molecular targets and pathways

Lactylation directly disrupts vascular cell homeostasis by modifying critical proteins [26]. In ECs, lactylation of histone H3 at lysine 18 (H3K18la) recruits bromodomain-containing protein 4 (BRD4) to activate pro-inflammatory genes (e.g., IL-6, VCAM-1), while lactylation of the transcription factor NF- κ B p65 enhances its nuclear retention, perpetuating endothelial activation and leukocyte adhesion [83]. Concurrently, lactate-mediated inhibition of SIRT1 deacetylase activity reduces eNOS phosphorylation, diminishing NO bioavailability and promoting oxidative stress [84]. In VSMCs, lactylation drives phenotypic switching, calcification, and apoptosis. H3K18 lactylation upregulates CHI3L1 expression, promoting osteogenic transdifferentiation of VSMCs [85]. The NR4A3 enhances glycolysis and lactate production, further increasing histone lactylation levels, which mediate medial arterial calcification [42]. Moreover, deficiency of the E3 ubiquitin ligase March2 disrupts K33-linked polyubiquitination of pyruvate kinase M2 (PKM2), activation of a p53-dependent apoptotic transcriptional program, exacerbates VSMC apoptosis [86].

6. Lactylation in vascular aging-associated diseases

Vascular aging is a central driver of age-related cardiovascular diseases (CVDs), with protein lactylation emerging as a critical metabolic-epigenetic regulator linking glycolytic flux to disease progression [87]. This post-translational modification, catalyzed by lactate-derived lactoyl-CoA, modulates cellular responses in ECs, VSMCs, and immune cells, contributing to atherosclerosis, hypertension, diabetic vasculopathy, vascular calcification, and abdominal aortic aneurysm (AAA). Below, we dissect lactylation's role in these pathologies through molecular and cellular mechanisms.

6.1 Atherosclerosis

Atherosclerosis is driven by lactylation-dependent reprogramming of both vascular endothelial and immune cells, linking metabolic dysregulation to epigenetic inflammation. In ECs, lipid peroxidation and aerobic glycolysis induce lactate accumulation, promoting P300/ASF1A-mediated histone H3K18 lactylation (H3K18la). This modification activates transcription of EndMT genes, disrupting endothelial integrity and accelerating plaque formation. Genetic or pharmacological inhibition of ASF1A or glycolysis attenuates H3K18la and mitigates EndMT-induced atherogenesis [40]. Concurrently, lactylation critically modulates immune cell behavior within plaques. In macrophages, inhibition of the lactate exporter MCT4 increases intracellular lactate, elevating H3K18la levels, which activate anti-inflammatory and reparative genes, favoring an M2 phenotype and enhancing plaque stability [88]. Exercise induces a protective mechanism via MeCP2 lactylation at K271 in macrophages, which interacts with H3K36me3 to repress the transcription factor RUNX1, promoting M2 polarization and increasing collagen content to stabilize atherosclerotic lesions [89]. Beyond histones, lactylation also regulates immune metabolism by shaping macrophage polarization, T-cell differentiation, and B-cell activity, highlighting its role as a metabolic checkpoint in inflammatory plaque

progression [90]. Thus, lactylation exerts dual roles in atherosclerosis: it exacerbates disease via EndMT in the endothelium, yet confers protection through immunomodulatory reprogramming in macrophages, positioning it as a cell-type-specific therapeutic target.

6.2 Hypertension and pulmonary arterial hypertension

Lactylation plays a critical role in the pathogenesis of both systemic hypertension and pulmonary arterial hypertension (PAH) by modulating VSMC function, metabolic reprogramming, and immune-inflammatory responses. In systemic hypertension, angiotensin II signaling promotes glycolytic switching in VSMCs, resulting in lactate accumulation and subsequent lactylation of contractile proteins such as caldesmon and myosin light chain kinase. This impairs actomyosin dynamics, enhances calcium sensitivity, and sustains vasoconstriction, contributing to arterial stiffening. In PAH, hypoxia serves as a key driver of metabolic reprogramming. HIF-mediated upregulation of glycolytic enzymes enhances lactate production in pulmonary arterial smooth muscle cells (PASMCs). This lactate induces histone lactylation, particularly at H3K18, which promotes the expression of pro-proliferative genes such as *PGF*, *Gbe1*, and *Ythdf2*, accelerating PASMC proliferation and pulmonary vascular remodeling. Inhibition of glycolysis with oxamate attenuates this lactylation-driven pathological process, confirming its metabolic-epigenetic basis [91]. Beyond proliferation, lactylation also induces cellular senescence in PASMCs via prelamin A accumulation. In hypoxic PH models, prelamin A upregulation, dependent on histone lactylation, triggers senescence-associated IL-6 secretion, which paradoxically promotes further proliferation and vascular remodeling. Suppression of lactylation reduces prelamin A levels and ameliorates these effects, highlighting lactylation's role in sustaining a senescent-proinflammatory loop [92]. Lactylation further contributes to PAH by modulating the immune microenvironment and RNA methylation. It influences N6-methyladenosine modifications and immune cell activity, fostering a pro-inflammatory and pro-remodeling milieu that exacerbates disease progression [93, 94]. Thus, lactylation functions as a converging mechanism linking hypoxia, glycolysis, and epigenetic regulation in both systemic and pulmonary hypertension.

6.3 Vascular calcification

Vascular calcification, a pathological process prevalent in aging, diabetes, and chronic kidney disease, is orchestrated by lactylation-induced osteogenic transdifferentiation of VSMCs. Diabetic hyperglycemia and uremic metabolites drive lactate accumulation, promoting extensive lactylation modifications that activate pro-calcific signaling. Specifically, H3K18 lactylation serves as a key epigenetic switch under high glucose conditions. It upregulates *CHI3L1* expression, which, in turn, activates the IL-13–IL-13R α 2–JAK1–STAT3 pathway, accelerating VSMC osteogenic differentiation and arterial medial calcification. Site-specific mutation at H3K18 significantly attenuates this process, confirming its functional necessity [85]. The orphan nuclear receptor NR4A3 further amplifies this cascade by enhancing glycolytic flux and lactate production. NR4A3 directly binds to promoters of glycolytic genes, increasing histone lactylation levels, which subsequently promote transcription of the pro-calcifying gene *Phospho1*. NR4A3 deficiency reduces glycolysis, lactylation, and

calcification, underscoring its role in coupling metabolic reprogramming to epigenetic-driven calcification [41]. These mechanisms are not limited to the arterial wall – aortic valve calcification also depends on lactylation. In valvular interstitial cells (VICs), lactylation at H3K9 and other sites upregulates Runx2, a master osteogenic transcription factor. The natural compound andrographolide (AGP) attenuates calcification by inhibiting p300-mediated H3 lactylation and reducing lactate production via LDHA suppression, highlighting lactylation as a druggable target [95]. Collectively, lactylation functions as a critical metabolic–epigenetic nexus in vascular calcification, linking hyperglycemia, uremia, and hyperlactatemia to osteogenic reprogramming and hydroxyapatite deposition. Targeting lactylation through inhibitors of lactate production, epigenetic writers, or downstream effectors offers novel therapeutic avenues to mitigate vascular and valvular calcification.

6.4 Diabetic vasculopathy

Diabetic vasculopathy, encompassing both micro – and macrovascular damage, is profoundly driven by a hyperglycemia-induced surge in lactate production and subsequent protein lactylation. This metabolic–epigenetic axis disrupts vascular homeostasis across multiple organ systems. In the macrovascular compartment, hyperglycemia promotes arterial medial calcification via specific histone lactylation pathways. Lactate accumulation in the diabetic microenvironment induces H3K18 lactylation in VSMCs, driving osteogenic differentiation of VSMCs and accelerating arterial calcification [85]. Concurrently, in the microvascular compartment, lactylation mediates end-organ damage, particularly in diabetic kidney disease. Global lactylome profiling of renal tissues from diabetic models reveals a marked upregulation of lysine lactylation, with the majority of modified proteins localized to mitochondria. A key mechanistic finding identifies K182 lactylation on ACSF2, a mitochondrial enzyme. This lactylation impairs ACSF2 function, inducing mitochondrial dysfunction, oxidative stress, and promoting renal tubule injury. The upregulation of lactylated ACSF2 is observed in both diabetic mouse models and human diabetic kidney biopsies, positioning it as a key contributor to the progression of diabetic nephropathy [45].

6.5 Aortic dissection and abdominal aortic aneurysm

Aortic dissection (AD) and abdominal aortic aneurysm (AAA) represent life-threatening impairments of vascular structural integrity, with lactylation emerging as a crucial regulator in their pathogenesis through metabolic reprogramming and immune-inflammatory activation. Multi-omics approaches, particularly single-cell and bulk RNA sequencing, have revealed pronounced lactylation activity across immune and vascular cell populations in AAA, correlating strongly with enhanced inflammatory responses and disease severity [96]. In AD, global lactylation profiling identified significant modifications in mitochondrial energy pathways, notably lactylation of ATP synthase subunit a (ATP5F1A) at lysine 531, which impaired mitochondrial ATP production, increased reactive oxygen species, and promoted phenotypic switching of VSMCs, thereby accelerating medial degeneration [97]. The E3 ubiquitin ligase March2 has been identified as a key protective factor in aortic aneurysm/dissection (AAD). Its deficiency exacerbates AAD progression by

disrupting PKM2 ubiquitination and tetramerization, enhancing glycolytic flux and histone H3K18 lactylation. This lactylation drives p53-mediated VSMC apoptosis, a hallmark of AAD pathogenesis. Restoration of March2 or activation of PKM2 with TEPP-46 ameliorates these effects, underscoring the therapeutic potential of targeting lactylation [86].

Lactylation also contributes to immune dysregulation in these conditions. In AAA, elevated lactylation is linked to immune cell infiltration and inflammatory activation, with machine learning algorithms identifying PGK1 and HMGA1 as lactylation-related diagnostic biomarkers exhibiting high accuracy [98]. Similarly, in thoracic AD, monocyte and macrophage populations demonstrate high lactylation activity, augmenting their inflammatory responses and intercellular communication [99]. Genetic studies further support a shared susceptibility between AAA and cardiometabolic traits, particularly in lipid metabolism and inflammation pathways, highlighting lactylation's role in intersecting metabolic and immune mechanisms [100]. Moreover, macrophage-derived inflammatory signaling, such as through ILF3, exacerbates AAA by amplifying NF- κ B activity and suppressing Nrf2-mediated anti-inflammatory responses, pathways potentially modulated by lactylation [101]. Collectively, these findings position lactylation as a central mechanism driving aortic wall deterioration and immune-metabolic dysregulation in AD and AAA, offering promising biomarkers and therapeutic targets for these catastrophic vascular conditions.

7. Therapeutic targeting of lactylation in vascular diseases

Targeting the lactylation axis presents a promising therapeutic strategy for vascular aging-related diseases, with interventions focusing on inhibiting lactate production, modulating epigenetic writers/erasers, and utilizing immunometabolic reprogramming.

7.1 Targeting lactylation enzymes and metabolites

Small-molecule inhibitors directly targeting lactylation regulatory enzymes are under active development. The catalytic p300/CBP inhibitor A-485 competes with acetyl-CoA, potently and selectively suppressing histone lactylation and demonstrating efficacy in tumor models, highlighting its potential for vascular applications where p300 drives pathological lactylation [102]. Similarly, the HDAC3-specific inhibitor RGFP966 attenuates palmitic acid-induced phenotypic switching in VSMCs by restoring lipid metabolism and autophagy, suggesting its utility in ameliorating lactylation-associated vasculopathy [103]. Beyond direct epigenetic targeting, modulating lactate availability is a viable strategy. Dichloroacetate (DCA), a pyruvate dehydrogenase kinase (PDK) inhibitor, reduces lactate accumulation by shifting metabolism toward oxidative phosphorylation. DCA has shown efficacy in abdominal aortic aneurysm (AAA) models by suppressing neutrophil infiltration, NETosis, and VSMC dedifferentiation, thereby preventing aortic dilation [104]. Targeting the newly identified lactyl-CoA synthetase ACSS2 or its interaction with lactyltransferase KAT2A also offers a novel approach to disrupt lactylation at its source [24].

7.2 Immunometabolic and lactylation-targeted therapies

Lactylation modulates immune cell function in the vascular microenvironment, providing opportunities for immunometabolic therapy. Inhibiting lactate exporters like MCT4 reprograms macrophages toward a reparative phenotype by elevating H3K18la and activating anti-inflammatory genes, thereby enhancing plaque stability in atherosclerosis [88]. Combining lactate metabolism inhibition with immunotherapy is a promising frontier. For instance, LDHA inhibition with oxamate reduces lactylation and disrupts the immunosuppressive microenvironment, potentially enhancing the efficacy of CAR-T cell therapy in vascular settings, as demonstrated in tumor models [105]. Furthermore, HIF-1 α inhibitors like digoxin and 2-methoxyestradiol, which suppress glycolytic flux and lactate production, have been shown to attenuate AAA progression by reducing MMP expression [106].

7.3 Natural compounds and traditional medicine

Several natural compounds modulate lactylation with therapeutic effects. AGP alleviates aortic valve calcification by inhibiting p300-mediated H3 lactylation and suppressing LDHA-driven lactate production [95]. Salvianolic acid B (Sal B) downregulates LDHA expression and H3K18la, exerting anti-inflammatory and antifibrotic effects in liver and cardiovascular injury models [107]. Formononetin, an isoflavone, promotes fatty acid β -oxidation via the SIRT1/PGC-1 α /PPAR α pathway, offering a strategy to reduce lactate generation and subsequent lactylation in metabolic diseases [107]. These findings are supported by systematic reviews highlighting the potential of Traditional Chinese Medicine to restore lactylation homeostasis by dual regulation of lactyltransferases and lactate metabolism [108].

7.4 Future directions and clinical translation

While preclinical studies are promising, the clinical translation of lactylation-targeted therapy is still nascent. The MCT1/2 inhibitor AZD3965 is currently in clinical trials for lymphoma (NCT01791595) [109], and its results may inform cardiovascular applications. Future efforts should prioritize the development of more specific lactylation writers/erasers inhibitors and explore combination strategies that simultaneously target metabolic pathways and immune responses. Integrating multi-omics approaches with spatial technologies will be crucial for identifying cell-specific lactylation targets and developing personalized therapeutic regimens for vascular aging diseases.

8. Challenges and future perspectives

The exploration of protein lactylation as a metabolic-epigenetic regulator in vascular aging and associated diseases faces significant technical, translational, and clinical challenges that must be addressed to realize its therapeutic potential. Despite the increasing recognition of lactylation as a pivotal epigenetic

modification involved in vascular aging and associated disorders, several challenges hinder its clinical translation. First, the precise molecular mechanisms regulating lactyl-CoA synthesis, transport, and the site-specific occurrence of lactylation remain poorly understood [25, 110]. Current detection methods lack the sensitivity and specificity required to comprehensively map lactylation sites across the proteome, particularly in low-abundance proteins [111, 112]. Furthermore, the interplay between lactylation and other post-translational modifications, such as acetylation, methylation, and phosphorylation, introduces additional complexity, making it difficult to delineate the specific role of lactylation in vascular pathophysiology [113–115]. The lack of specific inhibitors or activators for lactylation “writers,” “erasers,” and “readers” further impedes functional studies and drug development [20, 25, 114]. Moreover, most existing evidence is derived from preclinical models, and large-scale human studies validating lactylation as a biomarker or therapeutic target in vascular aging remain limited.

Looking forward, future research should prioritize the development of advanced methodologies for precise lactylation profiling, such as high-resolution mass spectrometry and modification-specific antibodies [111, 112]. Interdisciplinary strategies that integrate multi-omics approaches, including epigenomics, metabolomics, and proteomics, will be crucial for elucidating the lactylation network in vascular cells under conditions of aging and stress [116, 117]. The identification of small-molecule modulators that selectively target lactylation-related enzymes could provide innovative therapeutic options for vascular disorders such as atherosclerosis, calcific aortic valve disease, and diabetic vasculopathy. In addition, investigating lactate-lowering interventions, including MCT inhibitors, glycolysis modulators, and lifestyle modifications, may contribute to the regulation of lactylation dynamics and the attenuation of vascular aging [108, 118, 119]. Finally, rigorously designed clinical studies are urgently required to elucidate the association between lactylation levels and vascular health outcomes, ultimately facilitating the development of lactylation-based diagnostics and therapies for age-related cardiovascular diseases.

9. Conclusion

Protein lactylation has emerged as a pivotal metabolic-epigenetic mechanism that intricately links glycolytic flux to vascular aging and its associated pathologies. This novel post-translational modification, driven by lactate accumulation under conditions such as hypoxia, hyperglycemia, and oxidative stress, modulates a wide array of cellular processes, including gene expression, immune responses, mitochondrial function, and extracellular matrix remodeling, through both histone and non-histone targets. In vascular aging, lactylation acts as a double-edged sword: it exacerbates endothelial dysfunction, VSMC senescence, and calcification via pathways involving H3K18la, NF- κ B, and metabolic enzymes, thereby accelerating atherosclerosis, diabetic vasculopathy, pulmonary hypertension, and aortic aneurysms. Conversely, in certain contexts, such as macrophage polarization, lactylation promotes reparative phenotypes and enhances plaque stability. The dynamic interplay between lactylation and other PTMs, such as acetylation and ubiquitination, further fine-tunes vascular

homeostasis, highlighting its role as a metabolic rheostat. Emerging therapeutic strategies, including small-molecule inhibitors of p300/CBP, lactate-lowering agents, natural compounds, and epigenetic editing tools, show promising efficacy in preclinical models by targeting lactylation at metabolic, enzymatic, and transcriptional levels. However, challenges remain in achieving cell-type specificity, improving detection methodologies, and translating these findings into clinical applications. Future research should leverage multi-omics and spatial technologies to elucidate cell-specific lactylation networks and their crosstalk with other metabolic and inflammatory pathways. The development of more specific modulators of lactylation writers, erasers, and readers, combined with lactate-centric interventions, holds great potential for mitigating vascular aging and its complications. Ultimately, a deeper understanding of lactylation's role in vascular biology may pave the way for novel diagnostic biomarkers and targeted therapies, offering hope for extending cardiovascular healthspan in an aging population.

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References

- [1] Sivakumar R, Senghor KA, Vinodhini V, et al. Metformin: An old drug with new tricks—Promising role in vascular aging and cardioprotection. *Drugs & Aging*. 2025;**42**(8):709–731
- [2] Pacinella G, Ciaccio AM, Tuttolomondo A. Endothelial dysfunction and chronic inflammation: The cornerstones of vascular alterations in age-related diseases. *International Journal of Molecular Sciences*. 2022;**23**:15722
- [3] Pierce GL, Coutinho TA, DuBose LE, et al. Is it good to have a stiff aorta with aging? Causes and Consequences. 2022;**37**:154–173
- [4] Townsend RR, Wilkinson IB, Schiffrin EL, et al. Recommendations for improving and standardizing vascular research on arterial stiffness: A scientific statement from the American Heart Association. *Hypertension (Dallas, Tex. 1979)*. 2015;**66**:698–722
- [5] Bautista-Niño PK, Portilla-Fernandez E, Rubio-Beltrán E, et al. Local endothelial DNA repair deficiency causes aging-resembling endothelial-specific dysfunction. *Clinical Science*. 2020;**134**:727–746
- [6] Khavinson V, Linkova N, Dyatlova A, et al. Senescence-associated secretory phenotype of cardiovascular system cells and inflammaging: Perspectives of peptide regulation. *Cells*. 2022;**12**:106
- [7] Lacolley P, Regnault V, APJCr A. Smooth muscle cell and arterial aging: Basic and clinical aspects. *Cardiovascular Research*. 2018;**114**:513–528
- [8] Wang M, Zhang J, Telljohann R, et al. Chronic matrix metalloproteinase inhibition retards age-associated arterial proinflammation and increase in blood pressure. *Hypertension*. 2012;**60**:459–466
- [9] van Der Linden J, Stefens SJ, Heredia-Genestar JM, et al. Ercc1 DNA repair deficiency results in vascular aging characterized by VSMC phenotype switching, ECM remodeling, and an increased stress response. *Aging Cell*. 2024;**23**:e14126
- [10] Tucsek Z, Toth P, Tarantini S, et al. Aging exacerbates obesity-induced cerebrovascular rarefaction, neurovascular uncoupling, and cognitive decline in mice. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*. 2014;**69**:1339–1352
- [11] Heusch G, Libby P, Gersh B, et al. Cardiovascular remodelling in coronary artery disease and heart failure. *The Lancet*. 2014;**383**:1933–1943
- [12] Duan G, DJPcb W. The roles of post-translational modifications in the context of protein interaction networks. *PLoS Computational Biology*. 2015;**11**:e1004049
- [13] Xiao J, Qin X, Chen W, et al. Immunoinflammation and post-translational modifications in the aging process. *Journal of Translational Medicine*. 2025;**23**:910
- [14] Hsieh HJ, Liu CA, Huang B, et al. Shear-induced endothelial

mechanotransduction: The interplay between reactive oxygen species (ROS) and nitric oxide (NO) and the pathophysiological implications. *Journal of Biomedical Science*. 2014;**21**:3

[15] Zhu L, Liu Y-P, Huang Y-T, et al. Cellular and molecular biology of posttranslational modifications in cardiovascular disease. *Biomedicine & Pharmacotherapy*. 2024;**179**:117374. 117374

[16] Chen Z, Peng I-C, Cui X, et al. Shear stress, SIRT1, and vascular homeostasis. *Proceedings of the National Academy of Sciences*. 2010;**107**:10268–10273

[17] Chen Z. *Endothelial Biology/ Homeostasis: The Roles of AMPK and SIRT1 in eNOS Phosphorylation and Deacetylation*: UC Riverside; 2010

[18] Ebert T, Tran N, Schurgers L, et al. Ageing-oxidative stress, PTMs and disease. *Molecular Aspects of Medicine*. 2022;**86**:101099

[19] Peterson SB, Hart G. New insights: A role for O-GlcNAcylation in diabetic complications. *Biology M*. 2016;**51**: 150–161

[20] Zhang D, Tang Z, Huang H, et al. Metabolic regulation of gene expression by histone lactylation. *Nature*. 2019;**574**:575–580

[21] Lin J, Ji Z, Di Z, et al. Overexpression of Tfap2a in mouse oocytes impaired spindle and chromosome organization. *International Journal of Molecular Sciences*. 2022;**23**:14376

[22] Zhang H, Zhao J, Yu J, et al. Lactate metabolism and lactylation in cardiovascular disease: Novel

mechanisms and therapeutic targets. *Frontiers in Cardiovascular Medicine*. 2024;**11**:1489438

[23] Xu Y, Ma X, Ni W, et al. PKM2-driven lactate overproduction triggers endothelial-to-mesenchymal transition in ischemic flap via mediating TWIST1 lactylation. *Advanced Science*. 2024;**11**:2406184

[24] Zhu R, Ye X, Lu X, et al. ACSS2 acts as a lactyl-CoA synthetase and couples KAT2A to function as a lactyltransferase for histone lactylation and tumor immune evasion. *Cell Metabolism*. 2025;**37**:361–376.e367

[25] Moreno-Yruela C, Zhang D, Wei W, et al. Class I histone deacetylases (HDAC1-3) are histone lysine delactylases. *Science Advances*. 2022;**8**: eabi6696. DOI: 10.1126/sciadv.abi6696

[26] Li H, Liu C, Li R, et al. AARS1 and AARS2 sense L-lactate to regulate cGAS as global lysine lactyltransferases. *Nature*. 2024;**634**:1229–1237

[27] Zong Z, Xie F, Wang S, et al. Alanyl-tRNA synthetase, AARS1, is a lactate sensor and lactyltransferase that lactylates p53 and contributes to tumorigenesis. *Cell*. 2024;**187**: 2375–2392.e2333

[28] Zhang K, Jagannath C. Crosstalk between metabolism and epigenetics during macrophage polarization. *Epigenetics & Chromatin*. 2025;**18**:16. DOI: 10.1186/s13072-025-00575-9

[29] Chen J, Huang Z, Chen Y, et al. Lactate and lactylation in cancer. *Signal Transduction and Targeted Therapy*. 2025;**10**:38

[30] Wei L, Yang X, Wang J, et al. H3K18 lactylation of senescent

microglia potentiates brain aging and Alzheimer's disease through the NFκB signaling pathway. *Journal of Neuroinflammation*. 2023;**20**:208

[31] Yang K, Fan M, Wang X, et al. Lactate promotes macrophage HMGB1 lactylation, acetylation, and exosomal release in polymicrobial sepsis. *Cell Death & Differentiation*. 2022;**29**:133–146

[32] Cui H, Xie N, Banerjee S, et al. Lung myofibroblasts promote macrophage profibrotic activity through lactate-induced histone lactylation. *American Journal of Respiratory Cell and Molecular Biology*. 2021;**64**:115–125

[33] Shu M, Lu D, Zhu Z, et al. Insight into the roles of lactylation in macrophages: Functions and clinical implications. *Clinical Science*. 2025;**139**:151–169

[34] Duan Y, Liu D, Yu H, et al. Transcription and post-translational mechanisms: Dual regulation of adiponectin-mediated Occludin expression in diabetes. *Cell & Bioscience*. 2024;**14**:126

[35] Lin J, Zhang X, Ge W, et al. Rnd3 ameliorates diabetic cardiac microvascular injury via facilitating Trim40-mediated Rock1 ubiquitination. *Diabetes*. 2025;**74**:569–584

[36] Fan M, Yang K, Wang X, et al. Lactate impairs vascular permeability by inhibiting HSPA12B expression via GPR81-dependent signaling in sepsis. *Shock*. 2022;**58**:304–312

[37] Chen X, Wang Y, Wang J-N, et al. Lactylation-driven FTO targets CDK2 to aggravate microvascular anomalies in diabetic retinopathy. *EMBO Molecular Medicine*. 2024;**16**:294–318

[38] Huang Y, Wang C, Zhou T, et al. Lumican promotes calcific aortic valve disease through H3 histone lactylation. *European Heart Journal*. 2024;**45**:3871–3885

[39] Thakore PI, Black JB, Hilton IB, et al. Editing the epigenome: Technologies for programmable transcription and epigenetic modulation. *Nature Methods*. 2016;**13**:127–137

[40] Dong M, Zhang Y, Chen M, et al. ASF1A-dependent P300-mediated histone H3 lysine 18 lactylation promotes atherosclerosis by regulating EndMT. *Acta Pharmaceutica Sinica B*. 2024;**14**:3027–3048

[41] Ma W, Jia K, Cheng H, et al. Orphan nuclear receptor NR4A3 promotes vascular calcification via histone lactylation. *Circulation Research*. 2024;**134**:1427–1447

[42] Raychaudhuri D, Singh P, Chakraborty B, et al. Histone lactylation drives CD8(+) T cell metabolism and function. *Nature Immunology*. 2024;**25**:2140–2151

[43] Meng F, He J, Zhang X, et al. Histone lactylation antagonizes senescence and skeletal muscle aging by modulating aging-related pathways. *Advanced Science*. 2025;**12**:e2412747

[44] Chen J, Feng Q, Qiao Y, et al. ACSF2 and lysine lactylation contribute to renal tubule injury in diabetes. *Diabetologia*. 2024;**67**:1429–1443

[45] Jennings EQ. Identifying Regulatory Mechanisms of the Post-Translational Modification. Lactoylls: The University of Arizona; 2023

[46] Zhang D, Gao J, Zhu Z, et al. Lysine l-lactylation is the dominant lactylation

isomer induced by glycolysis. *Nature Chemical Biology*. 2025;**21**:91–99

[47] Zong Z, Ren J, Yang B, et al. Emerging roles of lysine lactyltransferases and lactylation. *Nature Cell Biology*. 2025;**27**:563–574

[48] Sheng X, Lin H, Cole PA, et al. Biochemistry and regulation of histone lysine l-lactylation. *Nature Reviews Molecular Cell Biology*. 2025

[49] Wang X, Guo Y, Fu Y, et al. Acyl post-translational modification of proteins by metabolites in cancer cells. *Cell Death Discovery*. 2025;**11**:247

[50] Zhou J, Ma X, Liu X, et al. The impact of histone lactylation on the tumor microenvironment and metabolic pathways and its potential in cancer therapy. *Genes & Genomics*. 2024;**46**:991–1011

[51] Minami N, Hong D, Stevers N, et al. Imaging biomarkers of TERT or GABPB1 silencing in TERT-positive glioblastoma. *Neuro-Oncology*. 2022;**24**:1898–1910

[52] An W, Kim J, Rgjc R. Ordered cooperative functions of PRMT1, p300, and CARM1 in transcriptional activation by p53. *Cell*. 2004;**117**:735–748

[53] Jiang J, Wang R, Song P, et al. Lactate facilitates pancreatic repair following acute pancreatitis by promoting reparative macrophage polarization. *Cellular and Molecular Gastroenterology and Hepatology*. 2025;**19**:101535. DOI: 10.1016/j.jcmgh.2025.101535

[54] Yang L, Zuo S, Jia R, et al. Lactylation-boosted polycomb repression of KLF4 elicits glycolysis in

retinoblastoma: A positive feedback circuit between histone modifications. *Cancer Letters*. 2025;**625**:217804

[55] Wollmann H, Stroud H, Yelagandula R, et al. The histone H3 variant H3.3 regulates gene body DNA methylation in *Arabidopsis thaliana*. *Genome Biology*. 2017;**18**:94

[56] Hong H, Han H, Wang L, et al. ABCF1-K430-Lactylation promotes HCC malignant progression via transcriptional activation of HIF1 signaling pathway. *Cell Death & Differentiation*. 2025;**32**(4):613–631

[57] Peng X, Du J. Histone and non-histone lactylation: Molecular mechanisms, biological functions, diseases, and therapeutic targets. *Molecular Biomedicine*. 2025;**6**:38. DOI: 10.1186/s43556-025-00275-6

[58] Bao C, Ma Q, Ying X, et al. Histone lactylation in macrophage biology and disease: From plasticity regulation to therapeutic implications. *EBioMedicine*. 2025;**111**:105502. DOI: 10.1016/j.ebiom.2024.105502

[59] Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Research*. 2011;**21**:381–395. DOI: 10.1038/cr.2011.22

[60] Ungvari Z, Tarantini S, Donato AJ, et al. Mechanisms of vascular aging. *Circulation Research*. 2018;**123**:849–867

[61] Gardner SE, Humphry M, Bennett MR, et al. Senescent vascular smooth muscle cells drive inflammation through an interleukin-1 α -dependent senescence-associated secretory phenotype. *Arteriosclerosis*,

Thrombosis, and Vascular Biology. 2015;**35**:1963–1974

[62] Childs BG, Durik M, Baker DJ, et al. Cellular senescence in aging and age-related disease: From mechanisms to therapy. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2015;**21**:1424–1435

[63] Huang L, Ding R, Yan K, et al. The Role of Endoplasmic Reticulum Stress in Fine Particulate Matter-Induced Phenotype Switching of Vascular Smooth Muscle Cells. *Chemical Research in Toxicology*. 2025;**38**: 1072–1081

[64] Lacolley P, Regnault V, Segers P, et al. Vascular smooth muscle cells and arterial stiffening: Relevance in development, aging, and disease. *Physiological Reviews*. 2017;**97**: 1555–1617

[65] Tejero J, Lazure F, APJTIE G, et al. Methylmalonic acid in aging and disease. *Trends in Endocrinology & Metabolism*. 2024;**35**:188–200

[66] Herman AB, Occean JR, Sen P. Epigenetic dysregulation in cardiovascular aging and disease. *The Journal of Cardiovascular Aging*. 2021;**1**:10

[67] Dai ZQ, Lu S, Shen ZT, et al. Intracellular In Situ Assembled DNA Networks Targeting Mitochondria Enable Selective Elimination of Senescent Cells and Improve Cell Viability. *Advanced Healthcare Materials*. 2025:e2501030. DOI: 10.1002/adhm.202501030

[68] Lin K, Luo X, Du C, et al. ANRIL modulates endothelial senescence and angiogenesis through SASP-driven miR146a regulation in age-related

vascular dysfunction. *Mechanisms of Ageing and Development*. 2025;**225**:112058

[69] López-Otín C, Blasco MA, Partridge L, et al. The hallmarks of aging. *Cell*. 2013;**153**:1194–1217

[70] Ouyang J, Wu D, Gan Y, et al. Unraveling the metabolic–epigenetic nexus: A new frontier in cardiovascular disease treatment. *Cell Death & Disease*. 2025;**16**:183

[71] Chen X, Yuan Y, Zhou F, et al. Lactylation: From homeostasis to pathological implications and therapeutic strategies. *MedComm*. 2025;**6**:e70226

[72] Das A, Huang GX, Bonkowski MS, et al. Impairment of an endothelial NAD⁺-H2S signaling network is a reversible cause of vascular aging. *Cell*. 2018;**173**: 74–89.e20

[73] Alique M, Sánchez-López E, Bodega G, et al. Hypoxia-inducible factor-1 α : The master regulator of endothelial cell senescence in vascular aging. *Cells*. 2020;**9**:195

[74] Li Q, Zhao R, Shen Y, et al. Lactylation in Tumor Immune Escape and Immunotherapy: Multifaceted Functions and Therapeutic Strateg. 2025;**8**:0793

[75] Brooks GA, Brown MA, Butz C, et al. Cardiac and skeletal muscle mitochondria have a monocarboxylate transporter MCT1. *Journal of Applied Physiology*. 1999;**87**:1713–1718

[76] Rabkin SW. Collagen type IV as the link between arterial stiffness and dementia. *American Journal of Translational Research*. 2023;**15**: 5961–5971

- [77] Zheng T, Gu YP, Wang JM, et al. Lactate-triggered histone lactylation contributes to podocyte epithelial-mesenchymal transition in diabetic nephropathy in mice. *Chemico-Biological Interactions*. 2025;**408**:111418. DOI: 10.1016/j.cbi.2025.111418
- [78] Chen J, He J, Wang X, et al. Glis1 inhibits RTEC cellular senescence and renal fibrosis by downregulating histone lactylation in DKD. *Life Sciences*. 2025;**361**:123293. DOI: 10.1016/j.lfs.2024.123293
- [79] Jing F, Zhang J, Zhang H, et al. Unlocking the multifaceted molecular functions and diverse disease implications of lactylation. *Biological Reviews*. 2025;**100**:172–189
- [80] Niu QQ, Xi YT, Guo YQ, et al. Lactylation and methylation: Dual epigenetic codes and potential therapeutic targets in myocardial aging. *Ageing Research Reviews*. 2025;**112**:102849. DOI: 10.1016/j.arr.2025.102849
- [81] Yang H, Mo N, Tong L, et al. Microglia lactylation in relation to central nervous system diseases. *Neural Regeneration Research*. 2025;**20**:29–40
- [82] Jiang X, Yang Y, Li X, et al. Lactylation: An innovative approach to disease control. *Aging and Disease*. 2024;**16**:2130–2150
- [83] Shahid S, Pantakani M, Binder L, et al. Small Molecule BRD4 Inhibitors Apabetalone and JQ1 rescues endothelial cells dysfunction, protects monolayer integrity and reduces midkine expression. *Molecules (Basel, Switzerland)*. 2022;**27**(21):7453. DOI: 10.3390/molecules27217453
- [84] Qiu -L-L, Tan -X-X, Yang -J-J, et al. Lactate improves postoperative cognitive function through attenuating oxidative stress and neuroinflammation in aged mice via activating the SIRT1 pathway. *Experimental Neurology*. 2025;**385**:115136
- [85] Zhu Y, J-c C, J-l Z, et al. A new mechanism of arterial calcification in diabetes: Interaction between H3K18 lactylation and CHI3L1. *Clinical Science*. 2025;**139**:115–130
- [86] Li YE, Liu S, Wang L, et al. March2 alleviates aortic aneurysm/dissection by regulating PKM2 polymerization. *Circulation Research*. 2025;**136**:e73–e93
- [87] Hu Y, He Z, Li Z, et al. Lactylation: The novel histone modification influence on gene expression, protein function, and disease. *Clinical Epigenetics*. 2024;**16**:72
- [88] Zhang Y, Jiang H, Dong M, et al. Macrophage MCT4 inhibition activates reparative genes and protects from atherosclerosis by histone H3 lysine 18 lactylation. *Cell Reports*. 2024;**43**(5):114180
- [89] Chen L, Zhang M, Yang X, et al. Methyl-CpG-binding 2 K271 lactylation-mediated M2 macrophage polarization inhibits atherosclerosis. *Theranostics*. 2024;**14**:4256
- [90] Xiong Y, Zhou J, Wang J, et al. How lactate and lactylation shape the immunity system in atherosclerosis. *International Journal of Molecular Medicine*. 2025;**56**:163
- [91] Chen A, Chen Z, Huang B, et al. Hypoxia-induced histone lactylation promotes pulmonary arterial smooth muscle cells proliferation in pulmonary

hypertension. *Molecular and Cellular Biochemistry*. 2025;**480**:5685–5697

[92] Zhang J, Luo M-Y, Li N-P, et al. Histone lactylation-derived prelamins A accelerates pulmonary arterial smooth muscle cells senescence in hypoxia-induced pulmonary hypertension rats. *International Immunopharmacology*. 2025;**159**:114871

[93] S-s Z, Liu J, Q-c W, et al. Role of histone lactylation interference RNA m6A modification and immune microenvironment homeostasis in pulmonary arterial hypertension. *Frontiers in Cell and Developmental Biology*. 2023;**11**:1268646

[94] T-y P, J-m L, X-l Z, et al. The role of lactate metabolism and lactylation in pulmonary arterial hypertension. *Respiratory Research*. 2025;**26**:99

[95] Wang C, Wang S, Wang Z, et al. Andrographolide regulates H3 histone lactylation by interfering with p300 to alleviate aortic valve calcification. *British Journal of Pharmacology*. 2024;**181**:1843–1856

[96] Li J, Huang Z, He J, et al. Integrative analysis of lactylation-associated features in abdominal aortic aneurysm and its immune microenvironment utilizing scRNA-seq and bulk RNA sequencing. *Circulation Journal*. 2025;**89** (12):1857–1870

[97] Yu T, Li X, Wang C, et al. Lactylation of mitochondrial adenosine triphosphate synthase subunit alpha regulates vascular remodeling and Progression of Aortic Dissection. *Research*. 2025;**8**:0799

[98] Ye J, Fu Y, Ke Y, et al. Lactylation associated biomarkers and immune

infiltration in aortic dissection. *Scientific Reports*. 2025;**15**:21536

[99] Peng J, Jiang Z, Song J, et al. Identification of lactylation-related hub genes as novel therapeutic and diagnostic targets for thoracic aortic dissection. *Cell Signal*. 2025:111944

[100] Zheng S, Tsao PS, Pan C. Abdominal aortic aneurysm and cardiometabolic traits share strong genetic susceptibility to lipid metabolism and inflammation. *Nature Communications*. 2024;**15**:5652

[101] Z-y W, Cheng J, Wang Y, et al. Macrophage ILF3 promotes abdominal aortic aneurysm by inducing inflammatory imbalance in male mice. *Nature Communications*. 2024;**15**:7249

[102] Lasko LM, Jakob CG, Edalji RP, et al. Discovery of a selective catalytic p300/CBP inhibitor that targets lineage-specific tumours. *Nature*. 2017;**550**: 128–132

[103] Zhang S, Nie F, Zeng Y, et al. RGFP966 inhibits palmitic acid induced VSMCs phenotypic transition by targeting ATGL. *Biochimica Et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*. 2025;**1870**:159597

[104] Griepke S, Grentzmann A, Tripodi GL, et al. Targeting the pyruvate dehydrogenase kinase/ pyruvate dehydrogenase axis modulates neutrophil and smooth muscle cell pathological responses and prevents abdominal aortic aneurysm formation. *Cardiovascular Research*. 2025;**121**:1269–1281

[105] Sun T, Liu B, Li Y, et al. Oxamate enhances the efficacy of CAR-T therapy against glioblastoma via suppressing ectonucleotidases and CCR8 lactylation.

Journal of Experimental & Clinical Cancer Research. 2023;**42**:253

[106] Tsai S-H, Huang P-H, Hsu Y-J, et al. Inhibition of hypoxia inducible factor-1 α attenuates abdominal aortic aneurysm progression through the down-regulation of matrix metalloproteinases. Scientific Reports. 2016;**6**:28612

[107] Liao J, Xie X, Wang N, et al. Formononetin promotes fatty acid β -oxidation to treat non-alcoholic steatohepatitis through SIRT1/PGC-1 α /PPAR α pathway. Phytomedicine. 2024;**124**:155285

[108] Qiu Y, Shao X. Histone Lactylation in Diseases: Regulation by Traditional Chinese Medicine and therapeutic implications. Drug Design, Development and Therapy. 2025;**19**: 6435–6459

[109] Halford SER, Walter H, McKay P, et al. Phase I expansion study of the first-in-class monocarboxylate transporter 1 (MCT1) inhibitor AZD3965 in patients with diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL). Journal of Clinical Oncology. 2021;**39**:3115–3115

[110] Gaffney DO, Jennings EQ, Anderson CC, et al. Non-enzymatic lysine lactoylation of glycolytic enzymes. Cell Chemical Biology. 2020;**27**:206–213.e206

[111] Wan N, Wang N, Yu S, et al. Cyclic immonium ion of lactyllysine reveals widespread lactylation in the human proteome. Nature Methods. 2022;**19**:854–864

[112] Wang Z, Hao D, Zhao S, et al. Lactate and lactylation: Clinical applications of routine carbon source

and novel modification in human diseases. Molecular & Cellular Proteomics. 2023;**22**:100641

[113] Qu J, Li P, Sun Z. Histone lactylation regulates cancer progression by reshaping the tumor microenvironment. Frontiers in Immunology. 2023;**14**:1284344

[114] Gong H, Zhong H, Cheng L, et al. Post-translational protein lactylation modification in health and diseases: A double-edged sword. Journal of Translational Medicine. 2024;**22**:41

[115] Izzo LT, Wellen KE. Histone lactylation links metabolism and gene regulation. Nature. 2019;**574**:492–493. DOI: 10.1038/d41586-019-03122-1

[116] Liao Z, Chen B, Yang T, et al. Lactylation modification in cardio-cerebral diseases: A state-of-the-art review. Ageing Research Reviews. 2025;**104**:102631

[117] Jing F, Zhu L, Zhang J, et al. Multi-omics reveals lactylation-driven regulatory mechanisms promoting tumor progression in oral squamous cell carcinoma. Genome Biology. 2024;**25**:272

[118] Wu P, Zhu T, Huang Y, et al. Current understanding of the contribution of lactate to the cardiovascular system and its therapeutic relevance. Frontiers in Endocrinology. 2023;**14**:1205442

[119] McNeillis R, Greystoke A, Walton J, et al. A case of malignant hyperlactaemic acidosis appearing upon treatment with the mono-carboxylase transporter 1 inhibitor AZD3965. British Journal of Cancer. 2020;**122**:1141–1145



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Epigenetics is changing the way we think about disease. It helps explain how the same DNA can lead to very different outcomes, and how inflammation, metabolism, environmental exposures, and lifestyle can leave lasting effects on gene activity without altering the genetic code. This edited volume offers a concise, connected overview of key epigenetic processes such as DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs, and shows how they shape immune imbalance, tissue remodeling, vascular and organ aging, host–pathogen interactions, and cancer behavior, including progression and treatment response. Rather than treating each condition in isolation, the book highlights shared regulatory principles and the “rules” that govern epigenetic plasticity across systems. The result is a practical guide to the language of chromatin in health and disease, with clear value for researchers, clinicians, and advanced students seeking a reliable framework for interpreting complex phenotypes and understanding why epigenetic mechanisms have become central to biomarker development, patient stratification, and epigenome-informed therapeutic strategies.

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