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Chromosomal Abnormalities

From DNA Damage to Chromosome Aberrations

Edited by Sonia Soloneski

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IntechOpen Book Series

Genetics

Volume 9

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

Sonia Soloneski has a Ph.D. in Natural Sciences and serves as Professor of Molecular Cell Biology at the School of Natural Sciences and Museum of La Plata, National University of La Plata, Argentina. She is a member of the National Scientific and Technological Research Council (CONICET) of Argentina in the Genetic Toxicology field, the Latin American Association of Environmental Mutagenesis, Teratogenesis and Carcinogenesis (ALAMCTA), the Argentinean Society of Biology (SAB) and the Society of Environmental Toxicology and Chemistry (SETAC). She has authored more than 450 contributions in the field, including scientific publications in peer-reviewed journals and research communications. She has served as a review member for more than 50 scientific international journals. She has been a plenary speaker at scientific conferences and a member of scientific committees. She is a specialist in issues related to Genetic Toxicology, Mutagenesis, and Ecotoxicology.

Contents

Preface

Genome replication is a crucial biological process that ensures the preservation of genetic information. The genetic information in the nucleus has been shown to be intimately associated with speciation, and its variability is responsible for the evolutionary process. While some chromosomal abnormalities are deleterious, others can confer adaptive advantages or contribute to the process of speciation. Additionally, genetic information can be used to aid in the diagnosis and prognosis of various human disorders and diseases. Eukaryotic cells have developed numerous strategies to maintain and preserve their genome. However, perturbations in genome replication represent significant challenges for eukaryotic organisms. Thus, this process must be highly regulated to ensure it only occurs once per cell cycle, which will preserve genome integrity and promote faithful genome transmission from the parental cell to the two daughter cells. DNA instability is a process that leads to cells with unbalanced genomes containing both numerical and/or structural abnormalities. For over a century, cytogenetic studies of chromosomal aberrations in plants and animals have been crucial for understanding the mechanisms of genetic diversity, evolution, disease, and developmental abnormalities. In humans, however, the loss of genome stability has long been and continues to be, recognised as one of the most important aspects of carcinogenesis. The pathways responsible for DNA repair and DNA damage signalling are essential for maintaining genomic stability; any disruption or dysregulation of these pathways can lead to the onset and progression of tumours. Understanding the structural and molecular bases of chromosomal abnormalities remains a fundamental challenge in cell biology, particularly in the field of molecular cytogenetics.

This volume presents a comprehensive exploration of chromosomal abnormalities and their significant implications in biology, medicine, and evolution. Across the eight chapters, this book investigates the key mechanisms of DNA damage and repair, the cytogenetic consequences of employing ionising radiation and several environmental contaminants, and the genomic instability induced by chemotherapeutic agents. It also addresses the evolutionary significance of whole-genome duplication in plants, the epidemiology of chromosomal anomalies in human populations, and the essential role of genetic counselling in rare inherited disorders. By combining classical cytogenetics with modern molecular insights, this book provides readers with a multidisciplinary perspective on genome integrity, disease mechanisms, and therapeutic innovation.

This single volume comprises eight high-quality chapters that describe the implications of generating chromosomal abnormalities in genetic material. The first chapter contributes to our understanding of how several DNA repair pathways contribute to the maintenance of genomic integrity, focusing on the molecular architecture of major DNA repair pathways, their relevance to genome maintenance, and the genetic syndromes that result from inherited defects in these pathways. The second chapter

examines the molecular basis of DNA damage and repair, a critical component of oncogenesis, which may lead to promising targeted therapeutics in the oncologic field. It highlights that a comprehensive understanding of the pathways implicated in the oncogenic process enhances therapeutic strategies, particularly when combined with approaches tailored to each patient's unique genomic profile. The third chapter provides a detailed overview of how radiation energy deposition within chromatin domains initiates a cascade of events—from the generation of specific DNA damage patterns to the repair processes that can ultimately lead to the formation of chromosomal aberrations. The authors introduce several aspects, including how different types of ionising radiation induce chromatin damage, how chromatin responds to irradiation both at individual DNA double-strand break sites and as an interconnected system during the repair process, and how DNA repair mechanisms contribute to the formation of chromosomal aberrations with an emphasis on chromosomal translocations because these are a clinically significant class of aberrations associated with the development of leukaemia and solid tumours. The fourth chapter examines how emerging contaminants, such as nanomaterials, can induce DNA damage through various mechanisms, including oxidative stress, inflammatory processes, and mitochondrial dysfunction. In this chapter, the consequences of such type of damage, including the disruption of cellular integrity, genotoxicity, and induction of chromosomal aberrations, are also discussed. The fifth chapter illustrates how chemotherapeutic agents, including doxorubicin, cisplatin, etoposide, busulfan, and temozolomide, induce senescence through DNA damage response pathways involving the p53, p21, and p16INK4a pathways. Several agents trigger genomic instability, leading to chromosomal aberrations and metabolic reprogramming, which can paradoxically promote tumorigenesis. This chapter highlights the role of chemotherapy-induced senescence in cancer therapy, emphasising the need for a deeper understanding of its molecular mechanisms and the development of targeted interventions to harness its benefits while minimising its detrimental consequences. The sixth chapter provides an overview of whole-genome duplication as a key driver of plant evolution, leading to polyploidy and altered gene expression.

Furthermore, the authors highlight that these evolutionary outcomes are shaped by hybridisation and reproductive isolation, which are investigated through cytogenetic and molecular techniques. The seventh chapter provides a comprehensive overview of the diversity of numerical and structural anomalies based on the analysis of 40,320 karyotypes from the general Colombian population. The authors employ classical cytogenetic techniques and image analysis software to perform karyotypes, identifying seventeen prevalent anomalies and the variability of existing rearrangements within each. The book concludes with a chapter exploring the critical role of genetic counselling in managing mucopolysaccharidoses, a group of rare inherited metabolic disorders. Furthermore, the authors highlight that genetic counselling provides essential support from diagnosis through long-term care, empowering patients with the knowledge, support, and tools necessary to make informed decisions.

This book highlights the importance of various types of chromosomal aberrations and their profound impact on genomic stability, disease development, evolutionary processes, and clinical practice. In doing so, this book builds an understanding of the origins and implications of the different types of chromosomal abnormalities and provides guidance for future investigations.

The editor of *Chromosomal Abnormalities – From DNA Damage to Chromosome Aberrations* is vastly grateful to all the contributing authors for generously sharing their knowledge and insights throughout this book project. They have made an extensive effort to arrange the information included in every valuable chapter. The publication of this book is of high importance for researchers, scientists, biologists, geneticists, teachers, and advanced-level students, as it provides a valuable resource for understanding the origin and implications of chromosomal aberrations and guiding future investigations.

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Chapter 1

DNA Repair Mechanisms and Associated Genetic Cancer Syndromes

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Abstract

Preservation of genomic DNA is fundamental to maintenance of life. The frequency of replication errors is approximately 10^{-10} per base of DNA per cell division. The damaged DNA is repaired by a complex, intricate, interconnected network of various DNA repair mechanisms, chiefly base excision repair (BER), mismatch repair (MMR), nucleotide excision repair (NER), Proofreading Repair, Homologous recombination (HR) and Nonhomologous End-Joining (NHEJ). Mutations in genes controlling these result in hereditary cancer syndromes such as Lynch syndrome, Hereditary breast and ovarian cancer (HBOC), *MUTYH*-associated polyposis (MAP), xeroderma pigmentosum, polymerase proofreading-associated polyposis, etc. There has been growing evidence supporting the potential of exploiting defects in DNA repair as therapeutic targets for cancer management in these syndromes. Deficiency in DNA repair mechanisms renders these tumours with increased sensitivity to platinum agents. Novel therapies like poly (ADP-ribose) polymerase (PARP) inhibitors in patients with homologous DNA repair deficiency (BRCA mutant) and immunotherapy for Lynch syndrome-associated and POLE mutant cancers.

Keywords: DNA repair, cancer predisposition, hereditary cancers, targeted therapy, base excision repair (BER), mismatch repair (MMR), nucleotide excision repair (NER), proofreading repair, homologous recombination (HR), nonhomologous end-joining (NHEJ)

1. Introduction

DNA repair encompasses a diverse array of highly coordinated molecular biological processes responsible for the recognition and removal of damaged DNA segments to maintain genomic integrity. These processes involve the initial sensing of DNA damage, the recruitment of specialised repair proteins to the sites of damage, and a series of coordinated enzymatic steps that re-establish the DNA's structural and functional fidelity. The critical importance of DNA repair in human health was first discovered in *xeroderma pigmentosum,* which provided clear evidence of a direct link between defective DNA repair mechanisms and increased susceptibility to cancers [1].

DNA repair mechanism	Damage repaired	Cell cycle	Accuracy
Base excision Repair (BER)	Small base modifications (e.g. 8-oxoG)	G1/S	High
Nucleotide excision repair (NER)	Bulky adducts, UV lesions	Throughout	High
Homologous recombination (HR)	Double-strand breaks	S/G2	Very High
Nonhomologous end-Joining (NHEJ)	Double-strand breaks	G0/G1	Error-Prone
Mismatch repair (MMR)	Base mismatches, indels	S phase	High
Proofreading repair	Mispairing during replication	S phase	Very High

Table 1.
Various DNA repair mechanisms.

DNA is constantly exposed to a variety of exogenous hazards and endogenous toxic agents that compromise genome stability. Endogenously, errors during DNA replication and byproducts of normal cellular metabolism—such as reactive oxygen species (ROS)—can compromise genome stability. Exogenous agents include ultraviolet (UV) and ionising radiation, environmental chemicals (polycyclic aromatic hydrocarbons, benzene), and a wide range of chemotherapeutic agents. These agents inflict various types of DNA lesions, including single-nucleotide alterations, helix-distorting adducts and dimers, single-strand breaks (SSBs), and double-strand breaks (DSBs) [2]. Many of these lesions are highly cytotoxic if left unrepaired and require specific DNA repair pathways. This chapter explores the molecular architecture of major DNA repair pathways, their relevance to genome maintenance, and the genetic syndromes—often with profound cancer predisposition—that result from inherited defects in these pathways. Different repair pathways are highlighted in **Table 1** and are discussed subsequently in detail.

2. Base excision repair (BER)

DNA is composed of fundamental building blocks called nucleotides, which are composed of a 5-carbon sugar (deoxyribose), a phosphate group, and a nitrogenous base. The four nitrogenous bases found in DNA are adenine (A), cytosine (C), guanine (G), and thymine (T), with A pairing with T and C pairing with G. Adenine and guanine are purines, characterised by a double-ring structure. Cytosine and thymine are pyrimidines, which have a single-ring structure. BER corrects modifications in these nitrogenous bases resulting from oxidation, deamination or alkylation. The modified bases do not distort the DNA helix structure but are prone to mispairing; e.g. 8-oxoguanine (modified G due to ROS) can undergo mismatch pairing with A, which on subsequent DNA replication causes a G:C base pair to be mutated to T:A [1–5].

The BER pathway is initiated with recognition of modified bases by DNA glycosylases, which cleave the N-glycosidic bond of the damaged base, creating an abasic site, also called an apurinic/apyrimidinic (AP) site (AP site). DNA glycosylases are categorised as either monofunctional or bifunctional. Monofunctional DNA glycosylases such as uracil-N glycosylase (UNG) manifest DNA glycosylase activity solely, removing damaged bases and generating an AP site, which is further cleaved by an AP endonuclease. Bifunctional glycosylases such as endonuclease III-like 1 (NTH1) and endonuclease VIII-like 1-3 (NEIL1-3) glycosylases also possess AP lyase activity apart from glycosylase activity, enabling them to directly cleave the DNA backbone at the AP site and introduce an SSB without requiring an AP endonuclease. After the commencement of BER by a DNA glycosylase, subsequent processing can proceed by 'short patch' BER or by long-patch BER pathways. 'Short patch' BER, also known as 'single-nucleotide BER', in which a single nucleotide is excised and subsequently replaced and ligated, whereas long-patch BER involves the removal of a DNA segment spanning 2–10 nucleotides, which is then resynthesised and ligated [1–5].

In short patch BER, following base removal, apurinic/apyrimidinic (AP) endonucleases cleave the phosphodiester backbone at the AP site to generate an SSB. In humans, there are two AP endonucleases: APE1 and APE2, with APE1 being the predominant enzyme involved in BER. The single-strand break is further processed by polynucleotide kinase-phosphatase (PNKP), which prepares the DNA ends for ligation. The phosphatase domain of PNKP removes 3′ phosphate, while its kinase domain phosphorylates 5′ hydroxyl ends, thereby producing ligatable DNA ends. DNA polymerase β then synthesises new DNA by using the undamaged strand as a template, ensuring accurate repair. DNA ligase I or III completes the repair by catalysing the formation of a phosphodiester bond between adjacent nucleotides, thereby sealing the nick [1–5].

Long-patch BER, which occurs primarily in actively dividing cells, involves multiple proteins such as DNA polymerase δ/ε, Proliferating Cell Nuclear Antigen (PCNA), the flap endonuclease FEN1, and DNA ligase I to fill large 2–10 nucleotide gaps [5].

2.1 *MUTYH*-associated polyposis (MAP)

MAP is caused by autosomal recessive inheritance of biallelic germline mutations in the *MUTYH* gene, located on chromosome 1p34.1. This gene encodes a DNA glycosylase that plays a key role in the BER pathway. The MUTYH protein specifically excises misincorporated adenine residues opposite 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxoguanine)—a common oxidative DNA lesion generated by reactive oxygen species (ROS). Failure of this repair mechanism leads to increased G > T transversions [6]. This led to the acquisition of somatic *APC* mutations, leading to the development of tens to a few hundred adenomatous polyps in the colon. Nearly two-thirds of patients with MAP eventually develop colorectal carcinoma, often characterised by uncommon somatic mutations such as *KRAS* p.G12C and *PIK3CA* p.Q546K. MAP accounts for approximately 0.3–0.8% of all colorectal cancer cases. The duodenum is another site where nearly 20% of cases increased adenoma and risk of duodenal adenocarcinoma [7–9]. There is some evidence of an increased risk for breast, urinary bladder, endometrial cancer and skin cancers [9].

2.2 *NTHL1*-related tumour syndrome

This syndrome is associated with autosomal recessive inheritance of biallelic germline mutations in the *NTHL1* gene at 16p13. The *NTHL1* gene encodes a DNA glycosylase that is crucial for repairing oxidised pyrimidines such as 5-hydroxycytosine and 5-hydroxyuracil, which mispair with adenine. Loss of function of this protein results in the accumulation of mutations, particularly C > T transitions, contributing to a characteristic mutational signature (e.g., COSMIC Signature 30). Clinically, the syndrome is associated with a predisposition to adenomatous colorectal polyps and a significantly increased risk of colorectal cancer. There is also growing evidence supporting an increased risk of breast cancer, often presenting as bilateral and multifocal, and at a younger age. Other cancers reported in individuals with biallelic *NTHL1* mutations include endometrial cancer, urothelial carcinoma, head and neck squamous cell carcinoma, basal cell carcinoma, meningioma, and various haematologic malignancies. The tumour spectrum and penetrance are still under exploration [10, 11].

2.3 *MBD4*-associated neoplasia syndrome

MBD4-associated neoplasia syndrome is a rare autosomal recessive disorder caused by biallelic germline mutations in the *MBD4* gene, located at 3q21.3. *MBD4* encodes a DNA glycosylase involved in the BER pathway, with a critical role in correcting G:T mismatches that result from the spontaneous deamination of 5-methylcytosine (mCpG) to thymine. Failure to repair these mismatches leads to characteristic C > T transitions at CpG dinucleotides, contributing to a mutational signature (SBS1) in which more than 60% of single-nucleotide variants (SNVs) are mCpG>TpG transitions. The accumulation of these transitions results in a hypermutator phenotype [11, 12]. Evidence from mouse models supports *MBD4*'s role in mitigating methylation-associated DNA damage [13]. It causes increased predisposition to gastrointestinal polyposis, colorectal carcinoma, acute myeloid leukaemia (AML) and, at a lower frequency, uveal melanoma and schwannomas [11, 12]. Given the high tumour mutation

	***MUTYH*-associated polyposis (MAP)**	***NTHL1*-related tumour syndrome**	***MBD4*-associated neoplasia syndrome**
Inheritance	Autosomal recessive	Autosomal recessive	Autosomal recessive
Gene	*MUTYH* at 1p34.1	*NTHL1* at 16p13.3	*MBD4* at 3q21.3
DNA lesion repaired	8-oxoguanine	5-hydroxycytosine/uracil	T/G mismatches from deaminated mCpG
Mutation signature	C > A transversions (signature 18)	C > A transitions (signature 30)	C > T transitions at CpG sites
Colorectal Polyposis	Multiple (<100)	Variable number of adenomatous polyps	Variable number of adenomatous polyps
Malignancies	Colorectal, duodenal, gastric, ovarian cancer	Colorectal cancer, breast cancer, endometrial cancer	Colorectal cancer, acute myeloid leukaemia, uveal melanoma, schwannoma
Age of onset	40–50s	Often early	Early to mid-adulthood

Table 2.
Different base excision repair-associated genetic syndromes.

burden (TMB) associated with MBD4 deficiency, affected tumours may be sensitive to immune checkpoint inhibitors, offering a potential avenue for immunotherapy [11]. Various BER-associated genetic syndromes are summarised in **Table 2**.

3. Nucleotide excision repairs (NER)

In contrast to BER, the NER pathway repairs bulky helix-distorting lesions spanning over several tens of base pairs in the genome. These DNA lesions are encountered in damage caused by metabolic byproducts of chemical carcinogens such as polycyclic aromatic hydrocarbons (PAHs) present in tobacco smoke, UV-induced cyclobutane pyrimidine dimers (CPDs), 6–4 photoproducts (6–4PPs), and DNA adducts generated by chemotherapeutic agents like cisplatin. Ironically, while chemotherapeutic agents induce such lesions to damage DNA and kill cancer cells, highly mutable tumour cells can develop chemoresistance through upregulation of the NER pathway [14–16].

NER is a complex multi-step process that involves recognition of damage, unwinding of the double helix at the DNA lesion site, single-strand incision at both sides of the lesion, removal of a short oligonucleotide (typically removes ~24–32 nucleotides) around the offending lesion, DNA polymerase replaces the gap using an undamaged single-strand DNA template to synthesise a short complementary sequence and ligation of the remaining single-stranded nick by DNA ligase to restore DNA to its original form. NER operates via two distinct sub-pathways: global genome NER (GG-NER), which is responsible for removing and repairing DNA damage across the entire genome. The other sub-pathway is transcription-coupled nucleotide excision repair (TC-NER), which specifically targets and removes lesions from the template strands of actively transcribed genes [15, 16].

XPC and *DDB2* encode for proteins that recognise bulky lesions produced by UV radiation. ERCC3 and ERCC2 are helicases that open the double helix of DNA at the site of damage. DNA damage is recognised by the *XPA* gene product and the *XPC* gene product in complex with HR23B. The XPC-HR23B complex is only required for GG-NER. In the case of TC-NER, where RNA polymerase is recruited at the site of damage, the transcription complex unwinds the DNA, and XPA is able to bind without assistance from the XPC-HR23B complex. EERC4 and EERC5 are endonucleases that cut the damaged DNA strand at 5′ and 3′ sites, respectively. After lesion removal, Replication Protein A (RPA) stabilises the single-stranded DNA. Replication Factor C (RFC) subsequently facilitates loading of Proliferating Cell Nuclear Antigen (PCNA) at the DNA damage site, which recruits DNA polymerases δ and ε to synthesise the new strand. Finally, DNA Ligase I seals the nick to complete the repair [11, 14–16].

3.1 Xeroderma pigmentosum

it is an autosomal recessive disorder due to biallelic germline variants in seven NER genes: *XPA on 9q22.23, ERCC3 (XPB) on 2q14.3, XPC on 3p25.1*, *ERCC2(XPD) on 19q13.32, DDB2(XPE) on 11p11.2, ERCC4(XPF) on 16p13.12* and *ERCC5(XPG) on 13q33.1,* leading to defective NER and hypersensitivity to UV radiation [11].

Patients characteristically show severe photosensitivity and numerous cutaneous freckles like hyperpigmented macules before the age of 2 years, often accompanied by neurodevelopmental delay. Affected individuals are at high risk of developing skin

cancers such as basal cell carcinoma, squamous cell carcinoma, and melanoma at an unusually young age, particularly on sun-exposed areas. Individuals with xeroderma pigmentosum often experience a reduced life expectancy, with many not surviving beyond their third decade of life; however, those with milder phenotypes may have significantly longer survival [11, 17].

3.2 Cockayne syndrome

Cockayne syndrome is a rare autosomal recessive disorder resulting from mutations in the *ERCC6* (also known as CSB) or *ERCC8* (CSA) genes, which are essential components of the TCR sub-pathway of NER. These mutations impair the ability to repair DNA damage following UV light exposure, particularly in actively transcribed regions of the genome. Individuals with this disorder display unique facial characteristics, including sunken eyes, a beaked nose, and prominent ears. Other clinical features include cachectic dwarfism, cognitive impairment, skin and hair thinning, failure to thrive, short stature with a stooped standing posture, microcephaly, and progressive neurological decline owing to demyelination. Additional symptoms include retinal degeneration with pigmented retinopathy and optic atrophy, kyphoscoliosis, neuromotor dysfunction, impaired vision, hearing, and increased sensitivity to the sun. In contrast to xeroderma pigmentosum, individuals with Cockayne syndrome do not demonstrate an elevated risk of developing cancer [18].

4. Homologous recombination (HR)

Double-strand breaks (DSBs) are among the most severe and challenging forms of DNA damage to repair. They pose significant challenges to genomic stability due to the simultaneous disruption of both DNA strands. If left unrepaired or repaired inaccurately, DSBs can lead to severe genomic consequences, including extensive loss of genetic material, chromosomal rearrangements, genomic instability, or cell death. Although infrequent under normal physiological conditions, DSBs can arise endogenously, most commonly through the collapse of replication forks when DNA polymerases encounter unrepaired base lesions. Exogenous sources, such as ionising radiation, are also potent inducers of DSBs. Chemotherapy and radiotherapy intentionally induce DSBs to exploit their lethality in rapidly dividing cancer cells [19, 20]. There are two main pathways to repair DSBs: nonhomologous end-joining (NHEJ) and homologous recombination repair (HR).

NHEJ functions throughout the cell cycle but is predominantly active during the G0 and G1 phases, prior to DNA replication. In contrast, HR is primarily restricted to the S and G2 phases, when a sister chromatid is available to serve as a homologous template. NHEJ directly ligates broken DNA ends without the need for sequence homology, making it a faster but inherently error-prone process, often resulting in insertions or deletions at the repair site. In comparison, HR is a high-fidelity, template-directed repair mechanism that ensures accurate restoration of the DNA sequence but involves a more complex and tightly regulated series of molecular events [20].

HR can be conceptually divided into three stages: presynapsis, synapsis, and postsynapsis. During presynapsis, the DSB ends are recognised, and the 5′ ends are removed and processed to generate 3′-OH single-stranded DNA (ssDNA) tails. This resection is mediated by the MRE11-RAD50-NBS1 (MRN) complex, which senses

DSB and recruits EXO1 or DNA2/BLM helicase to commence 5′–3′ DNA resection. The resulting ssDNA is rapidly coated by Replication Protein A (RPA) to prevent further dsDNA resection. ATR then localises to the ssDNA ends, arresting the cell cycle for HR to proceed. Compelling evidence suggests that BRCA1 also functions in 5′–3′ resection of DSBs to generate 3′ ssDNA overhangs and later recruits PALB2. Following resection, BRCA1 recruits PALB2, which serves as a molecular bridge to BRCA2. BRCA2 is essential for the replacement of RPA with RAD51, enabling the formation of the RAD51-ssDNA nucleoprotein filament. This rate-limiting step is critical for initiating homology search and strand invasion during the subsequent synapsis phase [21–24].

In synapsis, the Rad51-bound single-stranded DNA invades a homologous DNA strand, forming a displacement loop (D-loop). In postsynapsis, the repair proceeds through one of three distinct pathways: (A) In synthesis-dependent strand annealing (SDSA), the invading DNA strand is displaced after limited synthesis and pairs with the second end of DSB. This process results in localised gene conversion without crossover and may include repeated cycles of strand invasion, synthesis, and disengagement. (B) In break-induced replication, the displacement loop (D-loop) is assembled into a replication fork that enables replication of the entire distal part of the chromosome, often leading to loss of heterozygosity (LOH). (C) In double-strand break repair (DSBR), both ends of the DSB are engaged, either via independent strand invasion or through capture of the second end, leading to the formation of a double Holliday junction. This intermediate structure can be resolved in two primary ways: either by resolvases, which cleave the junction to produce either crossover or non-crossover products, or through a dissolution pathway. Dissolution pathway involves BLM-mediated branch migration and TOPOIIIα-dependent dissolution of a hemi-catenane, thus exclusively yielding non-crossover products [21–24].

4.1 *BRCA1/2*-associated hereditary breast and ovarian cancer syndrome

Autosomal dominant inheritance of germline mutations in the *BRCA1* on 17q21.31 or *BRCA2* gene on 13q13.1. *BRCA1* has 23 coding exons and encodes a protein with 1863 amino acids. *BRCA2* has 27 coding exons and encodes a protein with 3418 amino acids. Individuals are at risk of biallelic inactivation through somatic inactivation of the second wild-type allele. Thus, individuals have an increased lifetime risk of ovarian, breast, prostate and pancreatic cancers. Due to their critical role in HR DNA repair, *BRCA1*- and *BRCA2*-deficient cells exhibit a vulnerability to synthetic lethality when exposed to poly (ADP-ribose) polymerase (PARP) inhibitors, which has become a cornerstone of targeted therapy in BRCA-associated cancers [11].

4.2 PALB2-related cancer predisposition syndrome

It can be associated with an autosomal dominant pattern of inheritance of *PALB2* on 16q12.2, increasing predisposition to breast, ovarian and pancreatic cancers, while an autosomal recessive pattern of inheritance can cause childhood malignancies such as AML, Wilms tumour, medulloblastoma and neuroblastoma [11].

4.3 RAD51-cancer predisposition syndrome

Autosomal dominant inheritance in *RAD51C* on 17q22 and *RAD51D* on 17q12 is associated with an increased risk of developing certain cancers, ovarian and breast cancer [11].

4.4 Fanconi anaemia

It is a genetically and clinically heterogenous disorder due to germline pathogenic variants in the BRCA-associated dsDNA repair pathway, resulting in chromosomal breakage and hypersensitivity to DNA cross-linking agents. It causes early-onset bone marrow failure and a high predisposition to cancer [11].

5. Nonhomologous end-joining (NHEJ)

It is one of the two major pathways responsible for the repair of DSBs. NHEJ is an error-prone mechanism that repairs DSBs by directly ligating DNA ends without requiring sequence homology. It is active throughout the cell cycle, thus, it is the primary repair mechanism in post-mitotic and non-replicating cells. It is crucial in V(D)J recombination during immune development [25–27]. The NHEJ process has various coordinated steps. The repair process begins with the recognition of DNA ends by the Ku70/Ku80 heterodimer, which binds tightly to the DSB. This heterodimer protects the DNA termini from degradation and acts as a platform to recruit additional repair machinery. Ku subsequently recruits the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), forming the DNA-PK holoenzyme. DNA-PKcs belongs to the phosphatidylinositol-3 kinase-related kinase (PIKK) family, which also includes ATM and ATR, kinases central to the broader DNA damage response. Once DNA-PK is assembled, it mediates the bridging and stabilisation of the broken DNA ends, holding them in proximity to ensure proper alignment for subsequent repair steps. If the DNA ends are incompatible or damaged, they undergo end processing. This involves the action of Artemis, a nuclease activated by DNA-PKcs, which trims DNA overhangs and resolves hairpin loops. Additional enzymes such as polynucleotide kinase-phosphatase (PNKP) are responsible for restoring correct chemical termini—specifically, a 5′ phosphate and a 3′ hydroxyl group—required for ligation. DNA polymerases μ (Pol μ) and λ (Pol λ) may also be recruited to fill in short gaps or extend overhangs, generating ligatable ends. The final step in NHEJ is ligation, carried out by the XRCC4-Ligase IV complex, which seals the DNA ends to restore chromosomal integrity [25–27].

5.1 Ataxia-telangiectasia syndrome

A rare autosomal recessive disorder due to pathogenic variants in the *ATM* gene at 11q22.3, thus increasing sensitivity to ionising radiations and agents causing DSBs. Affected individuals show cerebellar degeneration, progressive ataxia, oculocutaneous telangiectasia, immunodeficiency, susceptibility to bronchopulmonary disease and hematolymphoid tumours, particularly acute lymphoblastic leukaemia, T-cell prolymphocytic leukaemia and diffuse large B cell lymphoma [11]. Due to hypersensitivity to radiation in affected individuals, radiotherapy is contraindicated [28].

5.2 CHEK2-related hereditary breast cancer predisposition syndrome

caused due to autosomal dominant inheritance of pathogenic variants in the *CHEK2* gene at 22q12.1. Affected individuals show increased lifetime risk of a wide range of cancers, such as breast, gastric, colon, thyroid, kidney, pancreas and prostate, as well as sarcoma and hematolymphoid malignancies [11]. Penetrance is

variable; hence, not all carriers develop cancer, and risks depend on mutation type and family history. It is considered a moderate risk compared to high penetrance BRCA genes [29].

6. Proofreading repair

The proofreading repair mechanism is an intrinsic function of DNA polymerases that enhances the fidelity of DNA replication. During DNA synthesis, DNA polymerases add nucleotides complementary to the template strand. However, occasional errors such as the incorporation of incorrect nucleotides can occur. To prevent mutations, many DNA polymerases possess a 3′ to 5′ exonuclease activity, which enables them to detect and remove mispaired bases immediately. When an incorrect nucleotide is added, the polymerase detects a distortion in the DNA helix. The enzyme then shifts the newly synthesised strand from its polymerase active site to its exonuclease site, where the mismatched nucleotide is excised. After removal, the strand is repositioned back into the polymerase active site, allowing synthesis to resume correctly. Proofreading is mainly associated with DNA polymerases δ and ε. This proofreading mechanism significantly improves replication accuracy, reducing the error rate from approximately 1 in 10^5 nucleotides to 1 in 10^7 nucleotides. This proofreading mechanism, when combined with post-replication mismatch repair (MMR) systems, the overall fidelity increase to one error per 10^9–10^{10} nucleotides [11, 29–33].

6.1 Polymerase proofreading-associated polyposis (PPAP)

Polymerase proofreading-associated polyposis (PPAP) is a rare, autosomal dominant cancer syndrome caused by germline missense pathogenic variants in the proofreading domains of DNA polymerase ε (*POLE* at 12q24.33) and DNA polymerase δ (*POLD1* at 19q13.33) syndrome shows numerous colorectal adenomatous polyps and an increased risk of endometrial and colon cancer. Cancer shows an ultramutated phenotype with an exceedingly high number of single-nucleotide substitutions due to defective proofreading variants. Unlike other constitutional DNA repair disorders, this syndrome does not require inactivation of both alleles; i.e. only a single mutated allele of DNA polymerase ε or δ is sufficient for disease manifestation [11]. p.Leu424Val (L424V) is the most commonly identified PPAP-causing mutation in DNA polymerase ε; other important reported pathogenic mutations are p.Ser297Phe (S297F) and p.Ser459Phe (S459F). p.Ser478Asn (S478N) is recognised as the most prevalent POLD1 mutation associated with PPAP; other less frequent pathogenic variants include PPAP p.Leu474Pro (L474P) and p.Asp316Asn (D316N) [33–34].

7. Mismatch repairs (MMR)

Recognise and correct base mismatch pairing of A-G and T-C, small insertions and deletions caused during DNA replication, S phase of the cell cycle. The mismatch repair (MMR) system involves a series of sequential steps for the recognition, excision, and resynthesis of the mismatch site in DNA. There are principally four major MMR proteins—MLH1, PMS2, MSH2 and MSH6—encoded by corresponding MMR gene *MLH1* (mut L homologue 1) at 3p22.2, *PMS2* (post-meiotic segregation increased 2) at 7p22.2, *MSH2* (mut S homologue 2) at 2p21 and *MSH6* (mut S

homologue 6) at 2p16.3, respectively. Each MMR protein has a unique function in repairing replication errors. MSH2 and MSH6 proteins form the mismatch recognition complex, MutSα complex, which is responsible for the recognition of mismatched sites, usually single bases or small insertions/deletions (ID) (1–2 nucleotides long), and initiation of repair by forming a sliding clamp around DNA double strands. They form a tetramer with the MutLα complex, a heterodimer complex of MLH1 and PMS2 proteins. MutLα cleaves the DNA strand near both ends of a mismatched nucleotide. Both MLH1 and PMS2 have an ATP-binding domain and require ATP molecules for the endonuclease function. Additional proteins such as proliferating cell nuclear antigen (PCNA), replication factor C (RFC) and exonuclease 1 (Exo 1) are recruited to remove the newly synthesised daughter strand and resynthesise the correct strand. DNA polymerase (Polδ or Polε), along with ligase, helps in resynthesis. Other complexes found in humans include the MutSβ (MSH2-MSH3) heterodimer, which can recognise larger insertions/deletions of up to 16 nucleotides [35, 36].

Apart from correcting bp mismatch errors, these proteins are responsible for maintaining the length of microsatellites. Microsatellites are short tandem repeats, ranging from 1 to 10 nucleotides in length, and are typically repeated 5–50 times. They are found in both coding and non-coding regions of the genome. They are prone to errors during DNA replication due to slippage of DNA polymerase, leading to insertion of new bp. MMR proteins repair the microsatellite repeat number and return it to the original value. However, if the MMR mechanism is impaired due to mutations in the MMR gene, the insertion errors in microsatellites remain unrepaired, and the number of microsatellite repeats increases. This alteration in microsatellite repeat numbers is referred to as microsatellite instability [11, 35, 36].

7.1 Lynch syndrome (LS)

Lynch syndrome (LS) is a cancer-predisposing syndrome inherited in an autosomal dominant manner resulting from a loss of function of one of four different proteins (MLH1, MSH2, MSH6 and PMS2). The *EPCAM*, which encodes a cell adhesion molecule, is not an MMR gene but can result in LS. Deletions at the 3′ end of the *EPCAM* gene, which is 17 kb upstream of *MSH2*, eliminate its transcription termination signal. This results in transcriptional read-through of *MSH2*, causing DNA methylation at its promoter and chromatin modifications that trigger epigenetic silencing of *MSH2*. Thus, an abnormal *EPCAM* gene at the position adjacent to the *MSH2* gene also inhibits MSH2 expression [35–37].

MSH2 and *MLH1* mutations are the most frequent due to their obligatory functions in the MMR system. The most common types of mutations in these genes are nonsense mutations, where a single base substitution introduces a premature stop codon, leading to a truncated and typically nonfunctional protein. Additionally, missense mutations—where a single nucleotide change results in the substitution of one amino acid for another—can also occur [35].

Individuals with LS have a heightened risk of developing not only CRC but also a range of extracolonic malignancies such as endometrial, ovarian, stomach, small intestine, urinary tract, pancreatic and brain. Muir-Torre syndrome, a variant of LS, follows an autosomal dominant inheritance pattern. It is characterised by the presence of visceral malignancies similar to those seen in LS, along with distinctive cutaneous manifestations. These include sebaceous neoplasms—such as sebaceous adenoma, sebaceoma, and sebaceous carcinoma—as well as keratoacanthomas. The National Comprehensive Cancer Network (NCCN) recommends universal screening

for both colorectal and endometrial cancers. This is typically done through microsatellite instability (MSI) testing and/or immunohistochemical (IHC) staining for mismatch repair (MMR) proteins to identify individuals at risk for LS and potential candidates for immunotherapy. For MSI, a panel of the five consensus mononucleotide repeats—BAT-25, BAT-26, NR-21, NR-24 and NR-27 (MONO-27)—is used; a tumour will be called MSI if at least three markers out of five are unstable. In IHC, protein expression of MMR proteins is evaluated. Absence of IHC staining in the nucleus of tumour cells but retained expression in normal cells such as lymphocytes, endothelial cells, and stromal cells strongly supports the presence of MMR gene inactivation. Due to the autosomal pattern of inheritance, non-tumour cells in LS have one normal allele that shows retained MMR protein expression serving as an internal control (**Figure 1**) [35–41].

7.2 Constitutional mismatch repair deficiency (CMMRD)

Constitutional mismatch repair deficiency (CMMRD) is an autosomal recessive pattern of inheritance that results in germline biallelic mismatch repair gene abnormalities. On performing MMR IHC, there is no internal control; due to biallelic inactivation, all cells show an MMR-deficient pattern. It causes a broad range of tumours in

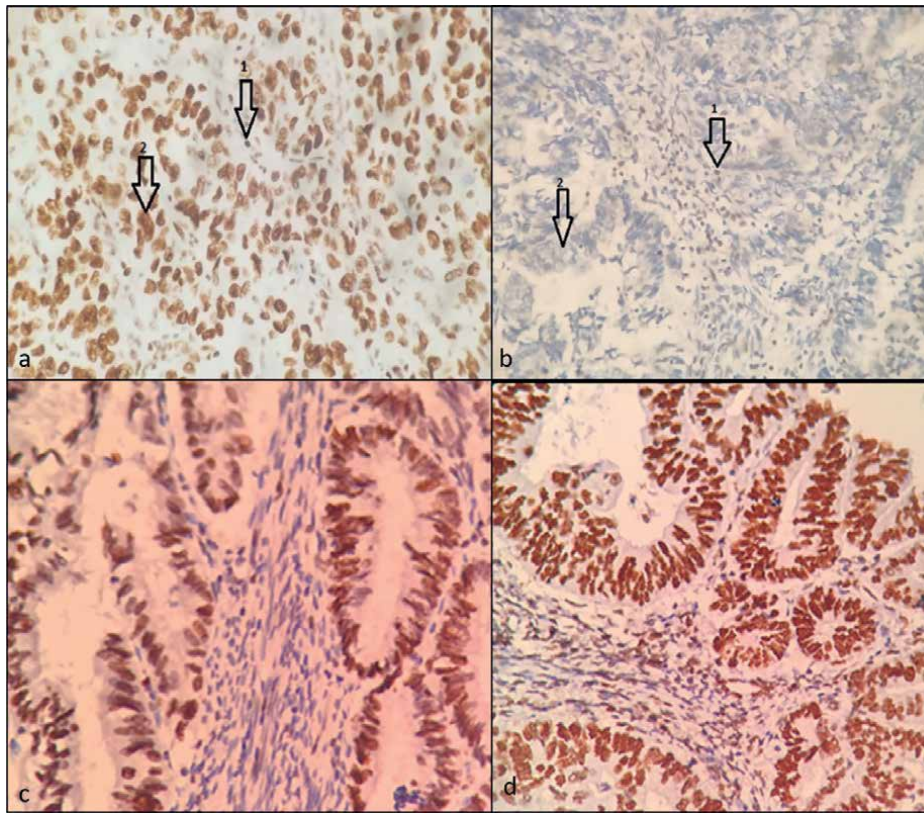

Figure 1.
MMR protein evaluation by immunohistochemistry in endometrial carcinoma in a case of Lynch syndrome (a) retained MLH1 expression (b) loss of PMS2 expression (c) retained MSH2 expression (d) retained MSH6 expression.

childhood, adolescence and early adulthood, including haematological malignancies, brain tumours, colorectal, endometrial, etc. [11].

8. Conclusion

DNA repair mechanisms are critical for maintaining genomic integrity and preventing carcinogenesis. A detailed understanding of these pathways and how their dysfunction due to mutations in key regulatory genes contributes to hereditary cancer syndromes has provided valuable insights into tumour biology. By recognising patterns of inheritance and phenotypic manifestations, at-risk individuals can be identified effectively, enabling earlier intervention in the disease course. Ultimately, leveraging DNA repair insights in clinical settings holds significant promise for improving patient outcomes and advancing cancer care by utilising therapies that target these repair deficiencies, acting synergistically with specific genetic mutations.

Disclosure

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Conflict of interest

None.

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Chapter 2

DNA Repair and Therapeutic Uses in Oncologic Field

Carolina Algara-Ramírez and Lorenza Saenger-Rivas

Abstract

DNA repair mechanisms and DNA damage response are paramount for maintaining genomic stability and preventing carcinogenesis. The impending or deregulation of these pathways may result in tumor progression. Cancer cells normally exhibit compromised DNA repair functions, making them more dependent on other mechanisms, which contributes to making them susceptible to therapeutic interventions, such as targeting DNA damage response pathways and offering more precise and personalized therapies. Therefore, an in-depth understanding of these processes of halting reaction against harm and restoration of DNA is crucial for developing tailored treatment to each mutagenic profile of cancer cells. This article explores the molecular basis of DNA damage and repair as a piece of oncogenesis that may lead to promising targeted therapeutics in the oncologic field.

Keywords: DNA damage response (DDR), DNA repair, cancer, genomic instability, targeted therapy, mutagenesis

1. Introduction

From the inception of oncology, a primary objective of cancer treatment has been to target the mechanisms involved in deoxyribonucleic acid (DNA) damage and repair. The pathways responsible for DNA repair and DNA damage signaling are crucial for maintaining genomic stability through mechanisms such as cell growth, division and death. Any disruption or dysregulation of these pathways can result in the progression of carcinogenesis and the development of tumors. The discovery of the first oncogene dates to the 1970s: the Rat sarcoma (RAS) gene. This milestone led to the foundation of the role of the oncogene: promoting uncontrolled cell growth [1]. Conventional knowledge of oncology has led to the development of four main cancer treatments: (i) surgery, (ii) chemotherapy, (iii) radiotherapy, and (iv) immunotherapy.

Our current understanding of oncology, however, shows that certain cellular lineages demonstrate an elevated susceptibility to malignant transformation, as they exhibit a reduced capacity for DNA repair and an insufficient response to DNA damage when compared to their normal counterparts. Additionally, these cells have the ability to activate mutagenic pathways that promote oncogenesis. Consequently, neoplastic cells

typically rely substantially on a specific subset of repair mechanisms, making them more susceptible to the inhibition of DNA damage response (DDR) compared to normal cells, which maintain comprehensive DNA repair and DDR abilities. Mismatch repair (MMR) deficiency is another area of interest, particularly in the context of immunotherapy. Tumors that present defective MMRs show high mutation rates, making them more susceptible to immune checkpoint inhibitors. Normally, human cancer cells suffer around 1000 to 20,000 point mutations including insertions, deletions, and rearrangements [2].

A profound understanding of these pathways has the potential to improve therapeutic approaches and create consciousness in the medical community regarding genotoxicity in the environment. In 1980, the reconnaissance of tumor suppressor genes initiated investigation into cancer genetics, leading to other genes like tumor protein 53 (TP53), phosphatase and TENsin homolog (PTEN), and adenomatous polyposis coli (APC) which are key to genomic consistency and signaling cancer development. We intend to investigate the mechanisms of DNA repair within the oncological research field and examine therapeutic strategies that target these pathways to enhance and tailor cancer treatment.

For instance, poly (ADP-ribose) polymerase 1 (PARP) inhibitors have demonstrated effectiveness in various types of solid tumors [3]. Moreover, alpha-targeted therapy (ATT) can provide powerful and localized radiation specifically to cancerous cells and the tumor microenvironment, thereby managing cancer while reducing toxicity. This approach offers an innovative perspective in oncology, as demonstrated in prostate cancer, where promising outcomes concerning survival and safety have been observed [4]. Another signaling pathway that has inspired targeted therapies (mTOR inhibitors) is PI3K/AKT/mTOR, which is only responsible for controlling cell growth, survival and metabolism. These examples serve to illustrate the plethora of novelty therapies which have the potential to dramatically alter individuals, families, communities and the entire healthcare system overall [1]. Immune checkpoint inhibitors, therefore, have led to lasting survival benefits [1]. Cancer therapy revolves around the concept of synthetic lethality, where defects in specific DNA repair pathways render cancer cells particularly vulnerable to targeted inhibitors aforementioned. These inhibitors can not only be used as monotherapy but combined with existing treatments like chemotherapy and radiotherapy to enhance therapeutic efficacy.

2. DNA damage, repair mechanisms and damage response (DDR)

2.1 DNA damage

DNA is generally susceptible to damage by multiple factors, both endogenous and exogenous. These factors include, but are not limited to, single-strand DNA breaks (SSBs), double-strand DNA breaks (DSBs), DNA-protein crosslink (DPC), bulky adducts and base mismatch. Nuclear DNA (nDNA) repair mechanisms are divided into the following principal pathways: (1) direct reversal, primarily addressing the damage caused by alkylating agents, (2) base excision repair (BER), targeting SSBs and non-bulky impaired DNA bases, (3) nucleotide excision repair (NER), correcting bulky, helix-disrupting DNA damage, (4) MMR, responsible for the correction of insertion/deletion loops (IDLs) and base–base mismatches, (5) recombinational repair, which is further divided into homologous recombination repair (HRR) and non-homologous end joining (NHEJ), predominantly addressing DSBs, (6) alternative nonhomologous end joining (alt-NHEJ, MMEJ), engaged in the repair of DSBs, and (7) translesion synthesis (TLS), which is

predominantly considered a mechanism for DNA damage tolerance [5]. DNA replication stress (RS) is also a major cause of genome stability because of activation of oncogenes like Ras, Myc, and Cyclin E that initiates aberrant replication, RNA:DNA hybrids (R-Loops), replication-transcription collisions, and defective nucleotide metabolism, and DDR malfunction that makes DNA synthesis slowdown because of irreparable lesions on the genetic information [6].

While each of these factors are repaired differently, that is, DSBs can be repaired using either error-prone or non-homologous end-joining (NHEJ) and less error-prone or homologous recombination (HR).

Several investigations have identified novel anticancer strategies by focusing on mitochondrial DNA (mtDNA) repair pathways to uphold genetic integrity, safeguard mtDNA from oxidative harm, and enhance cellular viability. The repair mechanism notably involves the BER pathway, which mainly targets damage induced by reactive oxygen species and may substantially contribute cellular resilience against oncological therapeutic agents [6].

2.2 Overview of DNA repair

DNA repair constitutes a vital mechanism that safeguards genomic integrity and averts the onset of diseases, including malignancies. Numerous repair pathways exist, such as base excision repair and HR, which are essential for rectifying damage to genetic material inflicted by external factors or inaccuracies during replication. Malfunction of these pathways can result in the accumulation of mutations, facilitating tumor advancement and the emergence of treatment-resistant cancer cells. Furthermore, DNA damage may arise from an array of intrinsic and extrinsic influences, encompassing genotoxic agents as oxidative stress, ultraviolet (UV) and ionizing radiation, chemical exposure, misincorporated ribonucleotide, defects on DNA repair pathways or abnormal structures, cellular metabolism and contact with mutagenic substances (**Figure 1**) [7].

Each healthy cell utilizes a spectrum of DNA repair mechanisms to uphold genomic stability, including, among others:

- BER: Repairs SSBs, that do not interfere in the structure, by removing damaged bases from the helix creating apurinic or apyrimidinic sites. This calls different repair factors to work on filling the gap.

- NER: Eliminates single-strand errors that distort the helix configuration. It divides into global genome NER (GG-NER) and transcription-coupled NER (TC-NER) which helps generally and on active genes, respectively. Both, having the DNA-dependent ATPase/helicase transcription factor IIH (TFIIH) that corroborates the lesion. Then, the faulty strand is cut and filled again correctly.

- Double-strand break repair (DSBR): Restores damage to both strands where no accurate template is left to guide the genetic coding. It has developed two different mechanisms: NHEJ and HRR.

- NHEJ: Ligates DNA by making an excision of the DSB and gluing the new ends, concluding on changing the sequence inevitably.

- HRR: Restores sequence by duplicating sister chromatids. It frequently happens in S and G2 phases where daughter DNA is closest. Breast and ovarian cancer normally lose this function on BRCA1 and BRCA2 [2].

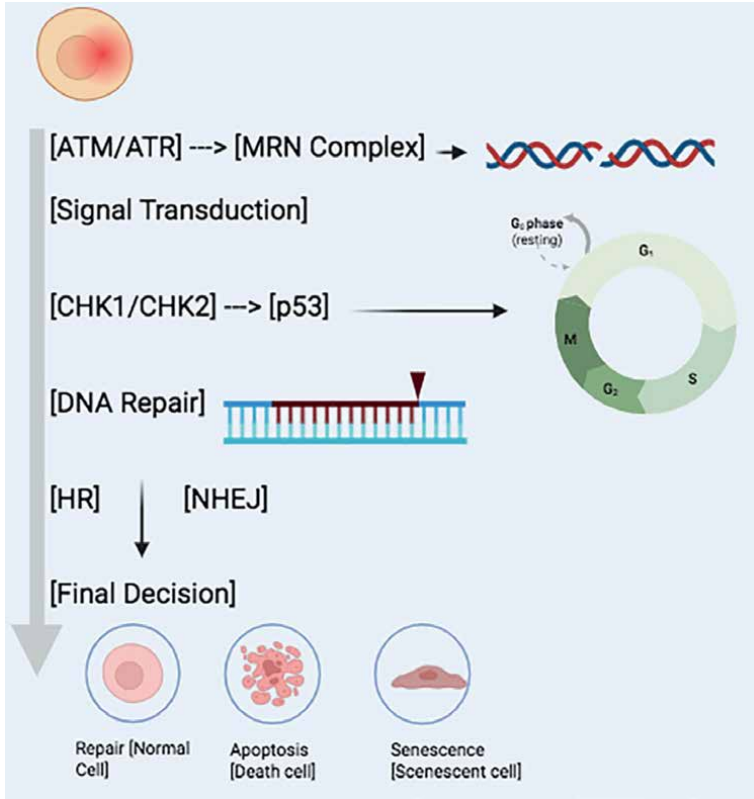

Figure 1.
Overview of the DNA damage response (DDR) signaling pathways.

- MMR: Rectifies inaccuracies that arise in DNA during replication such as erroneous base pairs or minor insertions and deletions. It functions as a "proofreading system" for DNA, ensuring the accurate duplication of genetic information and averting harmful mutations within cells [2].

Each of these pathways fulfills a crucial function in detecting and restoring specific varieties of DNA damage. Cells incapable of efficiently repairing DNA damage are increasingly susceptible to the accumulation of mutations, a defining characteristic of malignant cells [8, 9]. In fact, several studies have shown that the identification of specific genetic signatures, as evidenced in the construction of a genetic profile for serous ovarian carcinoma, has a predictive accuracy of 98.6% to differentiate between benign and malignant neoplasms, highlighting the potential clinical application of these investigations in personalized chemotherapy [10, 11]. Therefore, understanding the mechanisms of DNA repair is not only relevant for cellular health but also for the advancement in precision oncology.

2.3 The role of DDR in tumorigenesis

The DDR pathway detects and responds to DNA damage by activating repair mechanisms or, when the damage is too severe, initiating cell death. As previously stated, DNA repair is a vital process that allows cells to correct damage to their genetic material, thus

contributing to genomic stability [1]. The inactivation of loss of tumor suppressing functions leads to changes or deletions that allow unchecked cell growth [7]. The aforementioned is compounded when certain oncogenic mutations, such as those in p53 or breast cancer gene 1 and 2 (BRCA1/2), compromise DNA repair, enhancing tumorigenic potential [12, 13]. The aforementioned, together with the fact that oncogenes encode proteins for its proliferation and survival via point mutations, gene amplifications or chromosomal rearrangements highlight essential proteins that are frequently overexpressed in cancer cells: PARP, DNA-PKcs, BRCA 1/2, ATM, ATR, and Chk1/2 [7].

3. Targeting DNA repair mechanisms in cancer therapy

3.1 DDR inhibition as a therapeutic strategy

Research into therapeutic applications in oncology has gained great relevance and traction, particularly in the context of targeting DDR pathways. As previously stated, since cancer cells are more reliant on specific repair mechanisms, inhibiting these pathways can selectively kill tumor cells while sparing normal cells. Several DDR inhibitors, such as PARP inhibitors, are already in clinical use or undergoing clinical trials. PARP inhibitors assist in DNA repair and deactivate the ribosomes that cancer cells require to proliferate. They are exploring the concept of synthetic lethality, where a defect in one repair pathway (e.g., BRCA mutations) leads to a reliance on an alternative pathway, providing an innovative approach when combined with radiation to improve therapeutic efficacy [1, 6, 10]. We can say that cancer cells that emerge from a faulty DNA repair mechanism become addicted to other fixing pathways in order to survive and further develop [14]. In conclusion, PARP inhibitors also help sensitize to chemotherapy and radiotherapy, thereby augmenting efficacy to the treatment. Additionally, multi-kinase inhibitors can supplement extra sensitivity to PARP inhibitors by targeting VEGFR, PDGFR, and FGFR which induce hypoxic environments and halt angiogenesis (**Figure 2**) [15].

3.2 PARP inhibitors and other targeted therapies

PARP inhibitors target PARP enzymes, as indicated by the name, which participate in BER and HRR. They promote synthetic lethality by impeding the repair of SSBs, resulting in the accumulation of DSBs and subsequent cell demise. Conversely, these inhibitors have demonstrated the capacity to modify the immune response by augmenting tumor neoantigen presentation and improving the effectiveness of immune checkpoint inhibitors [2, 16, 17].

Targeted therapies represent a remarkable progression in oncologic treatment, with Targeted Alpha Therapy (TαT) illustrating how radiotherapeutic strategies can be optimized to achieve enhanced tumor specificity. This therapeutic modality relies alpha-emitting radionuclides such as actinium-225 (^{225}Ac) or bismuth-213 (^{213}Bi) conjugated to molecules that selectively attach to specific targets expressed on the surfaces of cancer cells. Upon administration, these radiopharmaceuticals concentrate in the tumor and emit high-energy, short-range alpha particles that induce fatal damage in malignant cells while minimizing injury to adjacent healthy tissues.

This methodology has exhibited particularly encouraging outcomes in the management of metastatic castration-resistant prostate cancer (mCRPC), notably through the implementation of conjugates that target prostate-specific membrane antigen (PSMA).

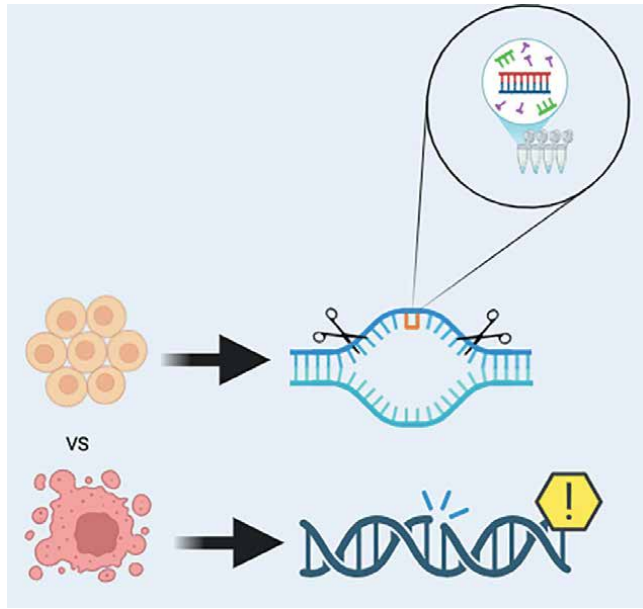

Figure 2.
Schematic representation of DNA damage repair mechanisms in normal cells versus cancer cells.

Recent investigations have confirmed that TαT not only enhances overall survival but also provides a superior tolerability profile when compared to traditional therapies, owing to its elevated biological specificity and reduced systemic toxicity. As a result, TαT is emerging as an invaluable instrument in precision oncology, broadening the therapeutic landscape for advanced and treatment-resistant tumors [4].

Everolimus and temsirolimus, mTOR antagonists, diminish neoplastic cell proliferation and trigger apoptosis. They have also shown the capacity to influence the immune response by modulating T-cell activity and cytokine synthesis, potentially augmenting the antitumor response (**Table 1**) [18, 19].

Ataxia telangiectasia Rad3-related (ATR) inhibitors: One of the main mediators of the RS response is ATR and it belongs to the phosphoinositide 3-kinase-related kinase (PIKK) family. It is in charge of stabilizing the replication fork, lowering origin firing, slowing down replication, and activating the G2/M cell cycle checkpoint [20].

CHK1 inhibitors: The cell cycle checkpoint proteins ataxia-telangiectasia-mutated-and-Rad3-related kinase (ATR) and its major downstream effector checkpoint kinase 1 (CHK1) prevent the entry of cells with damaged or incompletely replicated DNA into mitosis when the cells are challenged by DNA damaging agents, such as radiation therapy (RT) or chemotherapeutic drugs, that are the major modalities to treat cancer, ATR and/or CHK1 suppress RS en by inhibiting excess origin firing, particularly in cells with activated oncogenes [21].

Ataxia telangiectasia-mutated (ATM) kinases inhibitors are activated by DSBs, and cells that are deficient in ATM experience abnormal DNA repair. Activated ATM phosphorylates p53 at serine 15 to activate it and phosphorylates MDM2 to prevent its inhibitory binding to p53. ATM also phosphorylates and activates CHK2, which phosphorylates p53 at another activating site (serine 20). p53 induces p21 to inhibit CDK2/cyclin E to induce arrest at the G1 phase of the cell cycle [18].

Therapy	Target	Mechanism of Action	Current Status	Indications	Clinical Trials
Olaparib (Lynparza)	PARP (poly(ADP-ribose) polymerase)	Inhibits DNA repair by blocking PARP, leading to accumulation of DNA damage in BRCA-mutated cancers	Approved for ovarian cancer; in trials for other cancers	Ovarian, breast, prostate, pancreatic cancer	Ongoing (Phase III)
Veliparib	PARP	Inhibits PARP enzymes, preventing repair of single-strand DNA breaks. Leads to cell death in tumors with defective repair mechanisms	Clinical trials	Breast, ovarian, non-small cell lung cancer (NSCLC)	Ongoing (Phase II/III)
Rucaparib (Rubraca)	PARP	Similar to olaparib, inhibits PARP and prevents repair of DNA damage in cells with BRCA mutations	Approved for ovarian cancer, clinical trials for others	Ovarian, prostate, breast cancer	Ongoing (Phase II)
Talazoparib (Talzenna)	PARP	PARP inhibitor that prevents DNA repair and leads to accumulation of DNA damage	Approved for breast cancer with BRCA mutations	Breast cancer (HER2-negative, BRCA-mutated)	Ongoing (Phase III)
Ceralasertib	ATR (Ataxia-Telangiectasia Mutated and Rad3-related)	ATR inhibitor that impairs DNA damage repair mechanisms and enhances cancer cell sensitivity to chemotherapy	Clinical trials	Solid tumors, breast, lung, ovarian cancer	Ongoing (Phase I/II)
AZD1775	WEE1 Kinase	Inhibits WEE1 kinase, preventing DNA repair during cell division, resulting in the accumulation of DNA damage	Clinical trials	Ovarian, breast, and colorectal cancers	Ongoing (Phase II/III)
Atezolizumab (Tecentriq)	PD-L1 (Programmed Death-Ligand 1)	Inhibits the PD-L1 checkpoint, enhancing immune response against tumor cells, particularly those with DDR defects	Approved for various cancers	Lung cancer, bladder cancer, triple-negative breast cancer	Approved and ongoing (Phase III)
Pembrolizumab (Keytruda)	PD-1 (programmed death 1)	PD-1 inhibitor that enhances immune response, used in combination with other DDR-targeted therapies	Approved for various cancers	Lung cancer, melanoma, head and neck cancers	Ongoing (Phase III)

Therapy	Target	Mechanism of Action	Current Status	Indications	Clinical Trials
M458	DNA-PK (DNA-dependent protein kinase)	Inhibits DNA-PK, leading to impaired DNA repair and increased tumor cell death	Clinical trials	Solid tumors, breast, ovarian cancer	Ongoing (Phase I/II)

Table 1.
Summary of DDR-targeted therapies in clinical development.

WEE1 inhibitors: The tyrosine kinase Wee1 is a dual specificity kinase that can phosphorylate CDK1 at Tyr15 and inhibit [20]. One key mechanism of WEE1 activation is through the ATR pathway as part of response to replication stress. Inhibition of WEE1 abrogates the G2/M cell-cycle checkpoint, and cells transit into mitosis with damaged or under-replicated DNA [20].

DNA-PK inhibitors: DNA-PK is a fundamental component in NHEJ and is a serine/threonine kinase. It has been shown that the inhibition of it in cancer cells by its own has modest influence on viability and/or proliferation, nonetheless, combining it with radiation and chemotherapy it has demonstrated improved cytotoxicity and antitumor synergic efficacy [20].

Other therapies like programmed death ligand 1 (PD-L1), inhibitors and programmed death 1 (PD-1) inhibitors, are being investigated as targeted therapies. These are classes of immune checkpoint inhibitors and play an important role in tumor immune evasion. If combined, they lead to suppression of T-cell activity; therefore, by inhibiting them, we can enhance the system's ability to find and attack cancer cells or, as the case may be, restore their activity. Some examples of these therapies are atezolizumab and durvalumab [22, 23].

3.3 Clinical applications and challenges

Clinical applications have also shown promise regarding the function of DNA repair inhibitors by introducing treatment methodologies that capitalize on the susceptibilities of malignant cells. Genetic markers and genome mapping are important for stratifying patients, classifying them according to their cancer developing risk, early detection and clinical recommendations such as prophylactic surgeries or chemoprevention strategies. PARP inhibitors, notably talazoparib, rucaparib, niraparib and olaparib (that count with FDA approval), are of particular importance in obstructing the repair of damaged DNA, which triggers apoptosis in tumors that already manifest deficiencies in repair mechanisms: synthetic lethality. Editing technologies like ribonucleic acid interference (RNAi) and clustered regularly interspaced short palindromic repeats (CRISPR) have made synthetic lethality a priority in their therapeutic research [14]. These agents have demonstrated efficacy in managing cancers harboring BRCA mutations, such as ovarian and breast neoplasms.

Additional therapies aimed at DDR, including inhibitors of ATM kinase, ataxia telangiectasia Rad3-related ATR, DNA-dependent protein kinase (DNA-PK), Chk 1/2, and Wee 1 inhibitors are also under investigation. ATM controls cell cycle progression, transcriptional regulation, chromatin remodeling and apoptosis which make ATM protein inhibition a viable way to suppress tumor growth [24]. On the other hand, ATR can identify stalled replication forks and induce responses, therefore

guaranteeing the genomic integrity of cells. Cancer cells are more dependent on ATR signaling thus making it an excellent target in cancer therapy [24]. Inhibiting ATR, therefore, elevates the sensitivity of cancer cells to genotoxicity and can induce apoptosis. CHK1 mediates the repair process and delays the cell cycle progression, delaying this process and allowing cells to be repaired. Consequently, inhibition of CHK1 may result in cell death by avoiding restarting the stalled replication forks [24]. The use of these targets need to be thought of in the context of the cancer cells being treated, the type of DNA damage that we are facing as every inhibitor engages differently.

Moreover, the incorporation of genetic profiles and biomarkers in the formulation of customized treatment regimens is enhancing therapeutic outcomes, stressing the significance of precision medicine in contemporary oncology [11]. This methodology not only addresses the unique attributes of each tumor but also increases treatment effectiveness by tailoring to the biological idiosyncrasies of each patient. Future treatments will also take advantage of artificial synthetic lethality, which targets DDR in tumors that do not have DNA repair deficiencies [14]. Despite encouraging findings, challenges remain, such as the emergence of resistance mechanisms that enable tumor cells to adapt and evade DDR inhibition. Synthetic rescue is when inhibiting repair mechanisms result in further mutations providing protection against these new therapies [14]. Other challenges still remain: affording access to these treatments for everyone, as well as the recency of these findings are not perfect yet so combination therapies targeting multiple DDR pathways are being explored to surmount these obstacles [25, 26].

Although cells have developed sophisticated systems to preserve genomic integrity, these mechanisms are far from flawless. Telomere attrition, while initially acting as a tumor-suppressive factor, can instigate significant instability if cell cycle checkpoints are compromised. Centrosome amplification and partial dysfunction of the spindle assembly checkpoint permit cancer cells to withstand chromosomal missegregation, thus contributing to aneuploidy. Epigenetic alterations, although reversible, can unpredictably silence tumor suppressor genes or activate oncogenes, and their therapeutic manipulation entails considerable risks. Mitochondrial DNA mutations are challenging to interpret and may facilitate tumor proliferation through modified metabolism and immune evasion. Ultimately, DNA repair pathways themselves can be undermined in cancer, with error-prone repairs exacerbating further instability and conferring treatment resistance. Overall, while these mechanisms serve a protective role, they also introduce susceptibilities that cancer cells can evolve to exploit and survive [24].

3.4 Recurring challenges in drug development of DNA damage response inhibitors and other anticancer therapeutics

In the context of cancer treatment, several critical factors must be considered to optimize therapeutic outcomes. The therapeutic window refers to the optimal range of a drug dosage that achieves the desired therapeutic effect while minimizing adverse side effects. This window is not fixed and can vary significantly depending on the severity of the disease and the overall health status of the patient. Determining the appropriate dose and schedule of a drug is equally crucial, as both the quantity administered and the frequency of administration directly influence the drug's efficacy and potential toxicity. This is particularly important in combination therapies, where interactions between drugs can amplify effects or increase the risk of complications.

Another vital aspect is target engagement, which describes the degree to which a drug binds to and modulates its intended molecular target. High target engagement is essential for therapeutic effectiveness and for limiting off-target effects that could harm healthy tissues. Patient selection also plays a pivotal role in precision oncology, where identifying individuals most likely to benefit from a specific therapy—based on cancer type, stage, and detailed molecular profiling—can significantly improve treatment success rates. Finally, therapy resistance remains a major challenge in oncology. Tumors often evolve mechanisms to evade the effects of treatment, rendering initially effective drugs less useful over time. A deep understanding of these resistance pathways is essential for developing strategies to overcome or delay resistance, ultimately leading to more durable responses in patients [20].

4. Genomic instability and mutagenesis in cancer cells

4.1 Mutagenic pathways in oncogenesis

Cancerous cells often exhibit an elevated degree of genomic instability, a phenomenon that can be attributed to intrinsic deficiencies within the elaborate systems responsible for DNA repair. This instability acts as a pivotal driving force behind the gradual accumulation of mutations, which ultimately enable the intricate process of tumorigenesis, whereby normal cells evolve into malignant ones. The presence of defective DNA repair pathways, including but not limited to, those involved in cellular mechanisms that uphold genomic integrity, substantially contributes to the mutagenic environment that typifies cancer cells, thereby amplifying their malignant potential. Ultimately, this interplay between genomic instability and dysfunctional DNA repair mechanisms emphasizes the complex relationship between genetic anomalies and the advancement of cancer, shedding light on the multifaceted nature of tumor development and the challenges faced in the strive for effective cancer treatments.

The occurrence of mutations within essential genes responsible for DNA repair mechanisms can significantly facilitate the gradual accumulation of mutations within both tumor suppressor genes and oncogenes, thereby promoting the overall progression and evolution of cancer pathology. For instance, the functional loss of the BRCA1/2 genes, which are critical in the repair of double-strand DNA breaks via homologous recombination, results in a profound inability to adequately repair such vital genomic damage, ultimately leading to the accumulation of additional mutations that significantly propel the intricate process of tumorigenesis [25, 26].

4.2 The impact of genomic instability on cancer progression

The occurrence of genomic instability is a vital element that promotes the swift evolution of tumors, thereby allowing neoplastic cells to rapidly get used to diverse therapeutic stresses, which encompass, but are not limited to, modalities such as chemotherapy and radiotherapy, ultimately enabling these malignant cells to avoid the impacts of such interventions. As a result of the gradual accrual of mutations, cancer cells are adept at acquiring resistance to established conventional therapeutic approaches, which consequently convolutes the design of effective treatment methodologies and significantly contributes to an overall adverse prognosis for patients. It is, therefore, of the utmost importance to attain a thorough comprehension of the

mutagenic pathways that underlie cancer progression, as this insight is crucial for the formulation of more potent and efficient therapeutic approaches that specifically address the fundamental causes of genomic instability [27, 28].

5. Conclusion

The strategic manipulation of DNA repair pathways within the framework of cancer treatment has arisen as an exceptionally promising route that could substantially magnify the overall effectiveness of therapeutic outcomes for individuals afflicted by malignancies. By leveraging the intrinsic vulnerabilities found in cancerous cells, particularly those exemplified by compromised DNA repair mechanisms, healthcare practitioners are presented with the opportunity to selectively obliterate tumor cells while concurrently reducing the collateral harm inflicted on surrounding normal tissues, thereby safeguarding healthy cellular structures. The continued and thorough investigation of DDR pathways, along with inquiries into genomic instability and the processes of mutagenesis, possesses significant potential to transform the cancer treatment paradigm, thereby making therapeutic strategies not only more effective but also more accurately customized to the distinct genetic characteristics inherent to each individual tumor. Ultimately, such progress could herald a new era of personalized medicine in oncology, in which treatment regimens are meticulously crafted to correspond with the specific molecular profiles of patients' tumors, thereby enhancing prognoses and quality of life. As we advance in precision medicine, it remains essential to continue exploring the molecular universe of DNA repair and its interplay with oncology therapies, thereby ensuring a holistic approach in the battle against this devastating illness.

In conclusion, the strategic exploitation of DDR pathway defects has redefined the landscape of cancer treatment. While significant progress has been made, fully realizing the potential of this approach requires continued research into the molecular mechanisms of resistance, the identification of novel biomarkers, and the development of combination regimens tailored to each patient's unique genomic profile. As our understanding of DDR biology deepens, so too will our ability to deliver personalized, durable, and effective therapies to cancer patients worldwide.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 3

Deciphering Chromosomal Translocation Mechanisms: The Influence of Radiation Type and Chromatin Architecture

Martin Falk, Michael Hausmann, Myriam Schäfer, Iva Falková, Jiří Toufar and Lucie Toufarová

Abstract

Microscopy has revolutionized our understanding of (radiation-induced) DNA damage, DNA repair, chromosomal aberration (CHA) formation, and the spatiotemporal coordination of these processes. Advances in microscopy, computational methods, and molecular biophysics have enabled the investigation of these mechanisms down to the nanoscopic—single-molecule—level in intact cells. A key milestone in our understanding of radiation-induced DNA damage, repair, and misrepair was the discovery that chromatin architecture plays a fundamental and multifaceted role in all these processes. In this chapter, we examine how radiation energy deposition within chromatin domains initiates a cascade of events, from the generation of specific DNA damage patterns to the repair processes that can ultimately result in CHA formation. We address three critical questions: (a) how different types of ionizing radiation (IR) induce chromatin damage, (b) how chromatin responds to irradiation at individual DNA double-strand break (DSB) sites and as an interconnected system during repair, and (c) how DNA repair mechanisms contribute to the formation of CHAs. Special emphasis is placed on chromosomal translocations (CHTs), a clinically significant class of CHAs associated with the development of leukemia and solid tumors.

Keywords: chromosomal translocations, chromatin architecture, DNA double-strand breaks (DSBs), photon radiation, densely ionizing particle radiation, radiation-induced DNA damage at microscale and nanoscale, DNA damage repair mechanisms, local and systemic chromatin responses to irradiation, microscopy techniques, single-molecule localization microscopy (SMLM)

1. Introduction

Our understanding of DNA damage, DNA repair, chromosomal aberrations (CHAs), and their formation has been revolutionized by advancements in microscopy [1–24], computational methods [25, 26], and molecular biophysics techniques. These innovations have enabled the investigation of these processes at the single-molecule

level within intact cells. Chromosomal aberrations (CHAs) are structural or numeric rearrangements of genetic material that arise spontaneously due to essential cellular processes or as a consequence of exposure to environmental factors that induce DNA lesions, particularly single- or double-strand breaks (DSBs) [27, 28]. While certain therapeutic and warfare chemicals, such as mustard gas, can efficiently induce DSBs [29, 30], ionizing radiation (IR) is the most potent agent in this regard. Moreover, IR generates additional types of DNA damage at DSB sites, complicating repair and increasing the likelihood of CHA formation [27, 28].

CHAs can have severe biological consequences, leading either to cell death during mitosis or to carcinogenesis. In the case of mitotic catastrophe, aberrant chromosomes—such as dicentric chromosomes—prevent the proper segregation of genetic material, ultimately blocking the formation of viable daughter cells [31]. While mitotic cell death is the predominant outcome following formation of some types of CHA, certain CHAs, such as stable (balanced) chromosomal translocations (CHTs), can persist through successive cell divisions [32], contributing to cancer initiation and progression [33]. CHAs are thus hallmarks of cancer cells, not only driving tumorigenesis but also exacerbating genomic instability during later stages of cancer development [34]. However, chromosomal rearrangements are not exclusively deleterious—they also play essential physiological roles, such as facilitating immunoglobulin gene reorganization for antibody production [35, 36], and have been integral to species evolution [37, 38]. In addition, the presence of dicentric chromosomes, ring chromosomes, and micronuclei (a byproduct of CHAs) serves as a cornerstone of radiation biodosimetry, enabling dose estimation in individuals exposed to radiation when physical dosimetry data are unavailable [39, 40].

Understanding the mechanisms underlying CHA formation is therefore a critical task in modern (radio)biology and medicine. This chapter focuses on the mechanism of formation of chromosomal translocations (CHT) and, to a lesser extent, deletions induced by IR. Although CHTs arise from a seemingly straightforward process—the erroneous rejoining of DNA ends—the molecular pathways governing their formation remain incompletely understood. It is well established that CHTs arise from defective DSB repair [41–43], making it a central focus of CHT research.

In human cells, DSBs are primarily repaired *via* two major pathways [44, 45]: non-homologous end-joining (NHEJ) [46] and homologous recombination (HR) [47–49], along with several backup mechanisms of yet unclear classification (e.g., alternative NHEJ [alt-NHEJ] and microhomology-mediated end-joining [MMEJ]) [50, 51]. These pathways exhibit fundamental differences in repair dynamics, efficiency, and fidelity. NHEJ is a rapid but error-prone process capable of repairing multiple DSBs throughout the cell cycle. In contrast, HR is a highly precise but slower mechanism that is largely restricted to late S/G2 phases and repairs only specific subsets of DSBs [27, 28, 52]. Therefore, the selection of a particular repair pathway at each DSB site represents a critical determinant of CHT formation (**Figure 1**) [5, 53]. Despite significant advances, the molecular basis of this decision-making process remains elusive, although multiple factors influencing pathway choice at both nuclear and local DSB levels have been identified [5, 52, 54–60].

A second major factor influencing CHT formation is the intrinsic nature of DSBs and the microdosimetric distribution of these breaks within the cell nucleus (**Figure 1**) [13, 61, 62]. While the chemical nature of some DNA ends permits direct rejoining, others require processing before DSB repair can occur [63]. Moreover, the complexity and multiplicity of DSBs impose still discussed constraints on the applicability of NHEJ, HR, and alternative repair pathways [64–67]. Complex DSBs involve additional DNA lesions at the same break

site, whereas multiple DSBs refer to clusters of breaks occurring in close proximity [27, 28]. The formation of both complex and multiple DSBs significantly enhances IR-induced cell lethality and increases the risk of CHT formation [13, 68–73].

Different types of IR and other DSB-inducing agents generate distinct DSB profiles both in terms of their nature and nuclear distribution [5, 13, 70], fundamentally affecting the risk, nature, and extent of CHT formation (**Figures 1** and **2**). As discussed later, high-LET (linear energy transfer) radiation types are particularly efficient in generation of complex and multiple DSBs, clustered along the particle flight path [13, 74, 75].

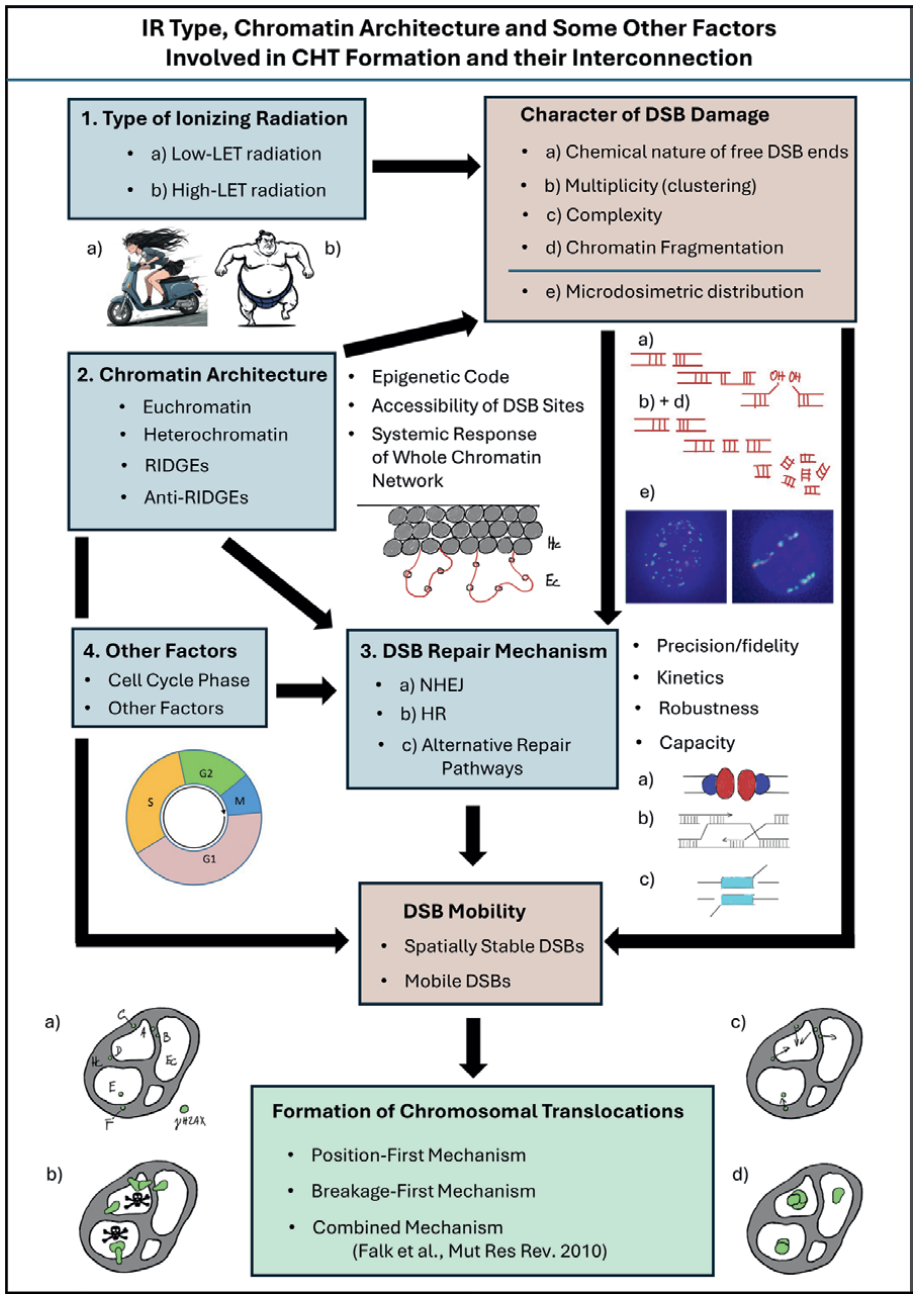

Figure 1.
IR type, chromatin architecture and some other factors involved in CHT formation and their interconnection. The type of ionizing radiation (1) and chromatin architecture (2) are two critical factors that influence the mechanisms of double-strand break (DSB) repair (3). Additionally, other factors (4), such as the phase of the cell cycle, also affect DSB repair. The specific combination of the type of IR and chromatin architecture determines the nature of DSB damage (top right red box) across various parameters, including the chemical nature of DSB DNA ends, DSB multiplicity, DSB complexity, and the spatial distribution of DSBs within the cell nucleus. Chromatin architecture plays a role in selecting particular repair mechanisms at individual DSB sites by influencing DSB accessibility to repair proteins and the formation of repair complexes. The differing repair mechanisms vary significantly in multiple aspects of the repair process and its outcomes, including repair kinetics, robustness, capacity, and fidelity. The characteristics of DSBs, the surrounding chromatin architecture, and the chosen repair mechanism at individual DSBs dictate the mobility of DSBs—most DSBs are spatially stable, while DSBs in heterochromatic regions can be highly mobile due to local decondensation of damaged chromatin domains. This mobility is a critical factor in the formation of chromosomal translocations. The potential effects of DSB mobility and the two primary hypotheses regarding chromosomal translocation formation are outlined in the bottom panel: (a) DNA double-strand breaks A to F are created by ionizing radiation at specified sites within the cell nucleus. Due to the statistically nonrandom architecture of the genome, certain chromosomes and chromosomal loci are more frequently in proximity to one another. Consequently, if DSBs remain relatively immobile (only following Brownian motion), translocations can occur only between damaged loci that were already close together due to the nonrandom chromatin landscape prior to irradiation. Thus, the highest probability of chromosomal translocation formation occurs between loci A and B, corresponding to the Position-First Hypothesis. (b) However, heterochromatic domains that experience DSBs must undergo decondensation to facilitate repair progression, which is associated with increased DSB mobility and their protrusion into nearby compartments with lower chromatin density. This movement also enables DSB interactions over longer distances. Additionally, the chromatin architecture surrounding DSB sites significantly alters the likelihood of chromosomal translocation formation based on spatial distances (see **Figure 4** *for a more detailed explanation). This model, proposed by Falk et al. [1, 3], encompasses elements of both the Position-First and Breakage-First Hypotheses. (c) Alternatively, it is also possible that all DSBs can exhibit high mobility, as observed after exposure to high linear energy transfer (LET) radiation. This places enhanced DSB movement as a primary factor in the mechanism of chromosomal translocation formation, supporting the Breakage-First Hypothesis. Some researchers extend this hypothesis to suggest a "directional" movement of DSBs into "repair factories," where multiple DSBs are repaired simultaneously (d). Although supportive data for repair factories have not been found at the microscopic level, clustering of DSBs cannot be excluded at the nanoscale.*

Understanding of CHT generation by different types of IR become particularly relevant in the context of hadron therapy [76–79] and space radiation exposure, where different IR types pose unique challenges for genome stability—an issue of growing importance for planned manned missions to the Moon and Mars (**Figure 2**) [80, 81].

A pivotal breakthrough in the study of radiation-induced DNA damage, repair, and misrepair was the discovery that chromatin organization in the cell nucleus is highly nonrandom [82–93] and that this *chromatin architecture* plays a fundamental and multifaceted role across multiple stages of CHT formation [1, 3, 94–101], including radiation energy deposition [101–104], the generation of distinct DNA damage patterns [5, 13, 103, 104], the chromatin response at individual DSBs [1, 3–5, 15, 17, 18, 25] and as an interconnected network (system) [25, 105–108], DSB repair pathway selection [5, 109–111], and ultimately, the emergence of CHTs [1, 3, 94–101]. Thus, chromatin architecture represents the third critical factor in CHT formation. Notably, these three factors—IR type, repair pathway, and chromatin architecture—do not act independently but interact in a highly specific manner, producing combinatorial effects that shape the outcome of CHT formation [5].

2. DNA double-strand break generation: The critical first step in chromosomal translocation formation

2.1 Impact of chromatin architecture and physical characteristics of ionizing radiation on DSB induction and chromosomal translocation formation

This chapter begins by exploring the generation of DNA double-strand breaks (DSBs) by different types of ionizing radiation (IR). Extensive research has established

that various IR types differ significantly in their dose deposition patterns, leading to distinct DNA damage profiles [13, 112, 113]. IR can be categorized into four main types: (i) sparsely ionizing (low-LET) uncharged photonic radiation, including X-rays and gamma rays; (ii) protons; (iii) densely ionizing (high-LET) heavy charged particles; and (iv) neutrons. While protons and neutrons are often classified separately due to their unique physical properties, the DNA damage they induce closely resembles that caused by photonic radiation [114, 115] and high-LET heavy ions [113, 116, 117], respectively.

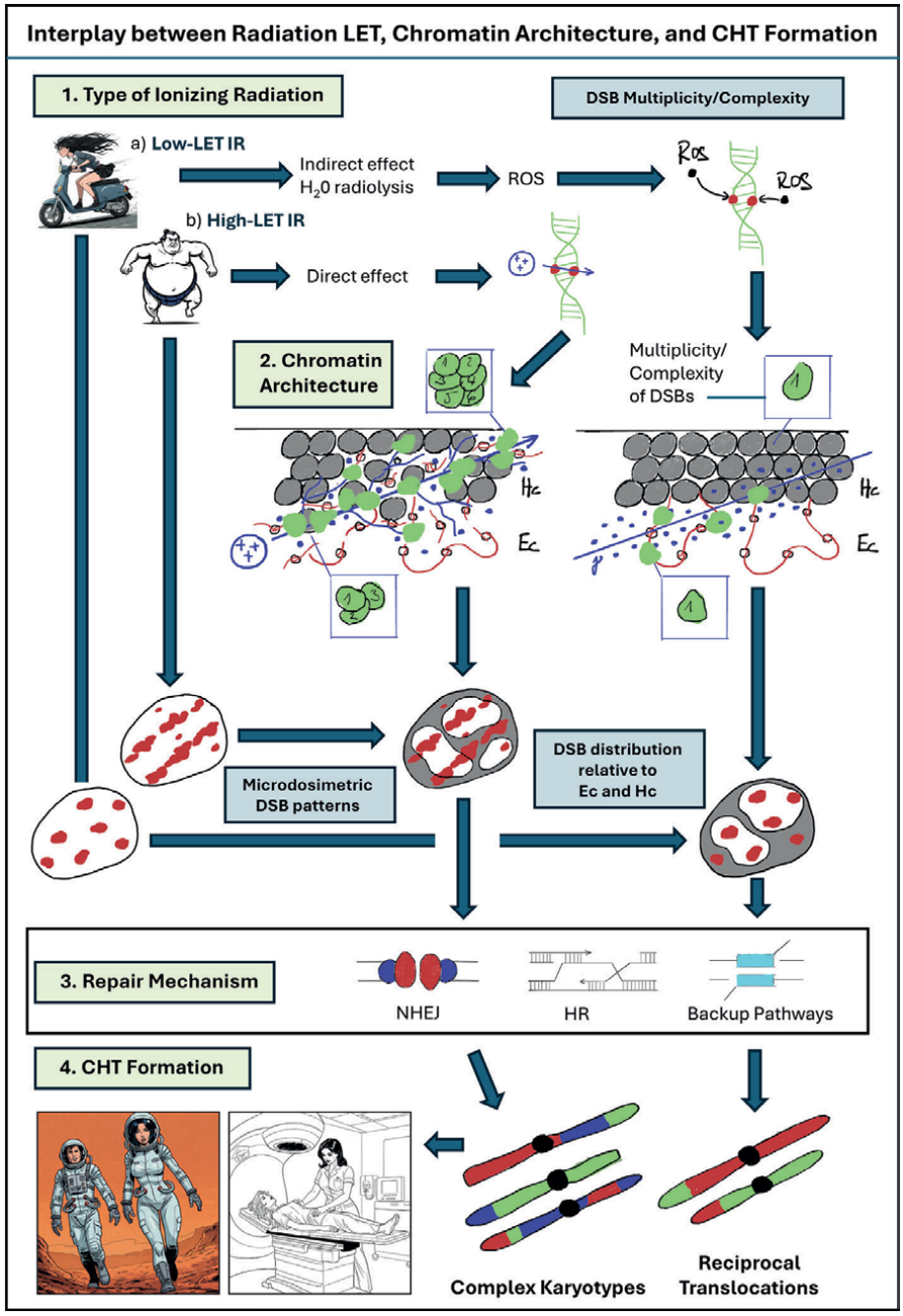

Figure 2.
Interplay between radiation LET, chromatin architecture, and chromosomal translocation formation. (1) Horizontal movement in the figure: Low-LET and high-LET radiation types induce DNA damage through different mechanisms. Low-LET radiation generates double-strand break (DSB) lesions in DNA primarily through the action of reactive oxygen species (ROS) produced by the radiolysis of water. To generate a single DSB, two ROS are required, each attacking one strand of the DNA molecule in close proximity. In contrast, heavily charged particles damage DNA through the direct interaction of the incident particle with the DNA molecule (direct effect). (1) Vertical movement in the figure: The same dose of radiation can be deposited into the cell by only a few high-LET particles as opposed to a significantly larger number of low-LET photons. Photons transfer their energy randomly and entirely at the site of interaction, while heavy charged particles continuously and densely ionize their surrounding environment, primarily along the channel created by the particle. Additionally, the density of ionization increases as the particle slows down and approaches the Bragg peak. Single DSBs (red foci) produced by multiple photons are therefore randomly distributed throughout the cell nucleus with some preference for euchromatin, whereas densely ionizing particles create clusters of DSBs accumulated along their flight path (DSB tracks, shown in red). (2) Differential interaction with chromatin domains: Due to the aforementioned differences in energy deposition mechanisms, both low-LET and high-LET radiations interact differently with heterochromatin and euchromatin. Photon radiation is unable to disrupt chromatin architecture, and the resultant ROS (depicted as small blue dots) primarily attack DNA (red line) that is unprotected by binding proteins (gray spheres) that can partly shield the DNA from ROS. Consequently, more DSBs are generated in euchromatin, which is more hydrated and less protected by binding proteins than heterochromatin. However, the complexity and multiplicity of DSBs (enlarged insets) are similar in both heterochromatin and euchromatin. In contrast, densely ionizing heavy charged particles possess the potential to significantly disrupt chromatin structure, compromising DNA protection in heterochromatin provided by chromatin-binding proteins, thereby leading to chromatin fragmentation. Accordingly, unlike low-LET radiation, the damage in heterochromatin is more severe due to the higher number of DNA targets per unit volume compared to euchromatin. Consequently, DSBs generated in heterochromatin are more complex and numerous than those in euchromatin. Hydration of chromatin and shielding from ROS are less critical factors here because the majority of DSBs are generated directly by the incident radiation particles and secondary electrons. The characteristics of DSBs and their spatial distribution in the nucleus, particularly concerning their clustering and localization within heterochromatin and euchromatin, influence the selection of the repair mechanism for individual DSBs. The type of IR (1), the chromatin architecture (2), the repair mechanism (3), and potentially other factors modulate the mechanisms of DSB repair and the risk of chromosomal translocations (4). Low-LET radiation primarily generates simple reciprocal translocations among various chromosomes, while high-LET radiation induces complex translocations between adjacent chromosomes along the particle's trajectory (interchromosomal translocations) or within a single chromosome (intrachromosomal translocations). It is crucial to study these complex translocations (and karyotypes in general) particularly concerning health risks for astronauts involved in planned interplanetary missions to Mars and for patients undergoing hadron therapy.

Photonic radiation, widely present on Earth and commonly used in cancer radiotherapy, is well recognized even by the general public. In contrast, high-LET heavy ions may seem more exotic, yet their biological relevance is substantial. Radon exposure, for example, is the second leading cause of lung cancer after smoking [118]. Additionally, novel radiotherapy approaches, such as carbon-ion therapy [119], rely on accelerated heavy ions, and future manned space missions—particularly to Mars—will expose astronauts to complex mixed radiation fields, including protons and high-LET heavy ions [120, 121]. Protons are already widely applied in clinical radiotherapy [122], whereas neutrons are primarily encountered in nuclear reactor operations and atomic weapon detonations [123]. The biological impact of these IR types varies significantly, as reflected in their radiation weighting factors (wR), which range from 1 to approximately 20 [27, 124]. These differences in biological effectiveness have been closely linked to the nature and spatial distribution of DSBs induced in DNA [125]. Understanding how different IR types generate specific DSB damage patterns is thus the first critical step toward elucidating the mechanisms of chromosomal translocation (CHT) formation.

Beyond the physical properties of IR (**Figure 2(1)**), the structural organization of DNA within the nucleus plays a crucial role in determining radiation-induced damage (**Figure 2(2)**) [1–5, 125–127]. In eukaryotic cells, DNA is not free-floating but is intricately packaged into chromatin, a hierarchically organized complex of DNA, histones, non-histone proteins [128], and non-coding RNAs (ncRNAs) [129, 130].

While the precise higher-order organization of chromatin beyond the nucleosome level remains incompletely understood, it is well established that DNA is not randomly arranged in numerous hierarchical levels. At the highest levels, chromatin is structured into functional 3D domains containing specific sequence motifs, such as regions of increased gene expression (RIDGEs) and their structural and functional counterparts, anti-RIDGEs [131], or euchromatin (Ec) and heterochromatin (Hc) [132, 133], and is spatially compartmentalized into individual chromosomal territories [86, 134].

The dynamic organization of chromatin is essential for various cellular processes, including gene regulation, replication, and DNA repair [3, 135–137]. Notably, chromatin architecture influences how different nuclear regions interact with ionizing radiation (IR) and its secondary products [2, 126], particularly reactive oxygen species (ROS), thereby introducing an additional layer of complexity to the induction of DNA double-strand breaks (DSBs) by different IR types (**Figure 2(2)**).

2.2 Differential interaction of chromatin with low-LET and high-LET radiation

Photonic IR (e.g., X-rays and gamma rays), which is indirectly ionizing, primarily damages biomolecules through the indirect effect, wherein radiation induces the radiolysis of water, generating ROS that subsequently damage DNA [27]. Since a single ROS molecule is required to induce a single-strand break, the generation of a DSB requires two ROS molecules acting in close proximity to the DNA backbone [27]. In contrast, high-linear energy transfer (high-LET) charged particles, such as heavy ions, induce DNA damage predominantly *via* the direct effect, where energy is transferred directly to the DNA molecule upon particle impact (**Figure 2(1)**) [27].

Another fundamental distinction between low-LET and high-LET IR types lies in their dose deposition patterns: while a single high-LET particle deposits its energy continuously along its track, culminating in the characteristic Bragg peak, an equivalent radiation dose delivered by (a high number of) "low-LET" photons is distributed over numerous stochastic interactions, each transferring the photon's entire energy into the surrounding medium (**Figure 2(1)**) [5].

Based on described physical processes, photonic IR generates usually simple DSBs that are randomly distributed throughout the nuclear volume [138], whereas high-LET heavy ions induce complex DSB clusters confined to narrow regions along the particle track (**Figure 2(1)**) [13, 139, 140].

2.3 Differential interaction of IR with euchromatin and heterochromatin

Heterochromatin (Hc), which is predominantly transcriptionally inactive, is tightly packed and enriched with structural heterochromatin-binding proteins such as HP1. It is also less hydrated than euchromatin (Ec). These structural properties influence the way different IR types interact with chromatin and induce DSBs (**Figure 2(2)**) [2].

ROS generated by photonic IR are short-lived [141, 142] and thus predominantly damage DNA located in their immediate vicinity [143]. In this context, the accessibility of DNA plays a crucial role: Exposed DNA loops in Ec are more vulnerable to ROS-mediated damage than the DNA in Hc, which is shielded by structural proteins such as HP1 [2]. Additionally, Ec has a higher water content than Hc, leading to an increased local production of ROS [142]. As a result, Ec is generally more susceptible to DSB formation by photonic IR than Hc (**Figure 2(2)**).

By contrast, high-LET heavy particles inflict DNA damage mostly directly, either through collisions with the radiation particle itself or *via* secondary electrons generated along the particle track [27]. Because chromatin architecture provides no inherent protection against direct particle impact, the primary determinant of DNA damage severity in this case is chromatin density. Given its compact structure and higher DNA density per unit volume, Hc represents a more sensitive target for high-LET heavy ions than Ec (**Figure 2(2)**).

These mechanistic differences result in distinct patterns of DSB formation (**Figure 2**):

- Low-LET photonic IR induces simple, spatially dispersed DSBs with comparable multiplicity in both Ec and Hc, though with lower efficiency in Hc [2, 126].

- High-LET heavy ions induce DSBs of lower multiplicity in Ec and significantly more complex clustered DSBs in Hc, but with similar efficiency across compartments [11, 12].

The nature of these DSBs influences the formation of chromosomal translocations (CHTs). Simple DSBs induced by photonic IR predominantly give rise to reciprocal CHTs, whereas the clustered DSBs generated by high-LET heavy ions lead to extensive chromatin fragmentation, facilitating the formation of complex translocations and chromosomal aberrations (CHAs) (**Figure 2(4)**) [13, 140].

2.4 Distinct microdosimetric DSB damage patterns induced by different accelerated ions with similar LET and low energy

In the preceding discussion, we outlined the differences in DNA damage induced by sparsely and densely ionizing radiation within two fundamental structural and functional chromatin compartments, euchromatin and heterochromatin. Densely ionizing particles were initially considered as a single homogeneous category, assumed to produce similar DNA damage patterns. While this assumption is broadly valid, our previous research has revealed a striking and, in some respects, groundbreaking phenomenon—different ion species can generate markedly distinct microdosimetric DSB patterns despite having comparable LET values and similar low energy (**Figure 3**) [13].

Specifically, our study compared boron-11 (^{11}B) and neon-20 (^{20}Ne) ions, which exhibited LET values of 135 keV-μm^{-1} and 132 keV·μm^{-1}, respectively, and energies of 8.3 and 46.8 MeV·n^{-1} [13]. The results demonstrated that DSB clusters induced by ^{20}Ne ions had significantly higher multiplicity compared to those generated by ^{11}B ions. This increased multiplicity correlated with substantially slower DSB repair kinetics in cells exposed to ^{20}Ne, in contrast to those irradiated with ^{11}B. Notably, the repair kinetics of ^{11}B-induced DSBs, although significantly slower than those observed following γ-ray exposure, were still more comparable to γ-ray-induced repair dynamics than to those triggered by ^{20}Ne ions.

Even 96 hours post-irradiation, a substantial fraction of unrepaired DSBs persisted in cells exposed to ^{20}Ne ions, whereas the majority of DSBs induced by γ-rays or ^{11}B ions were efficiently repaired within 24 hours. Given that the variations in DSB repair kinetics correlated with increased apoptotic cell death following irradiation, it is evident that microdosimetric differences in DSB patterns generated by different ions with similar LET and energy (**Figure 3**) have functional consequences for post-irradiation cell survival.

These findings underscore the critical importance of considering microdosimetric heterogeneity when evaluating the biological effects of different ion species [13, 144, 145]. In the case of ^{11}B and ^{20}Ne ions, as studied by Jezkova et al. [13], simulations using the RITRACK software revealed that energy deposition, ionization events, and

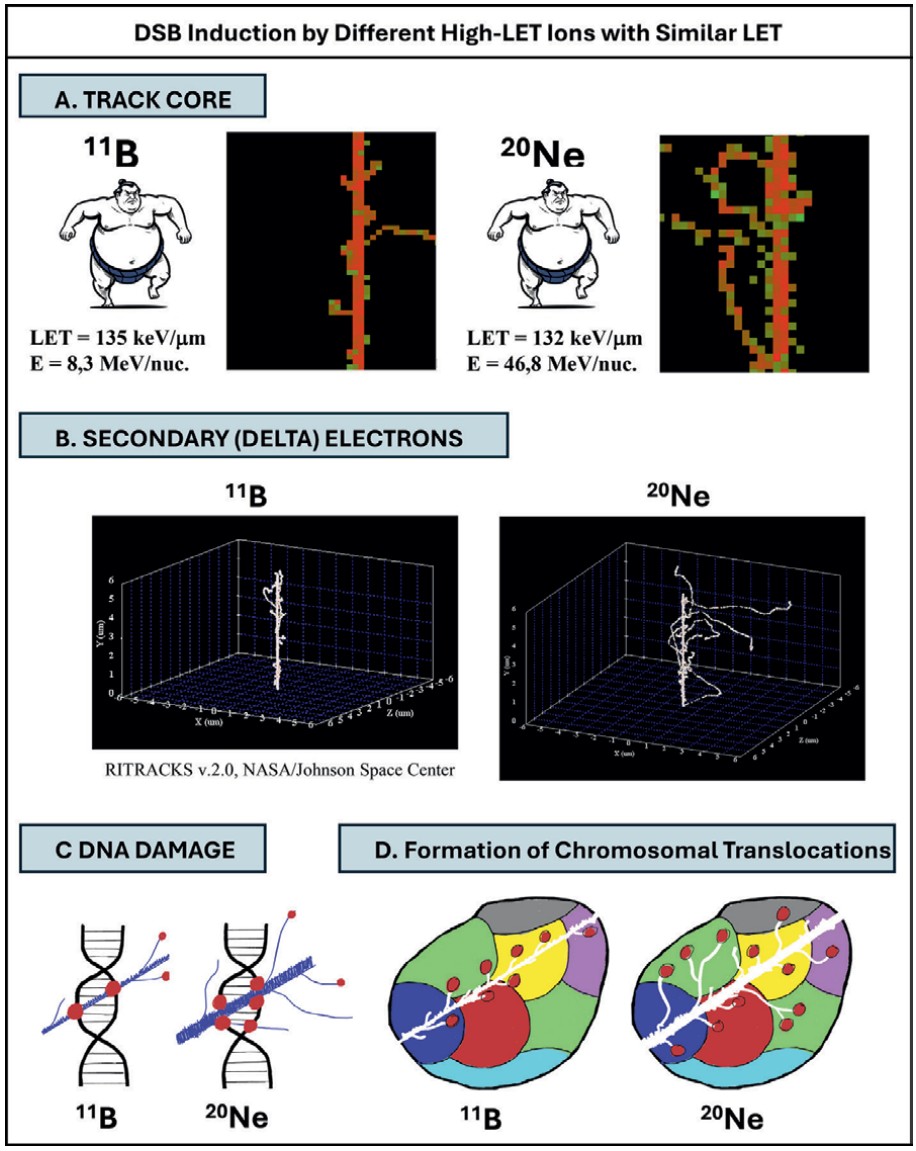

Figure 3.
DSB induction by different high-LET ions with similar LET. Despite their similar linear energy transfer (LET) values and low energy levels, ^{11}B ions (LET = 135 keV.µm^{-1} and energy of 8.3 MeV/nucleon) and ^{20}Ne ions (LET = 132 keV.µm^{-1} and energy of 46.8 MeV/nucleon) produce ionization tracks and DNA damage with distinct characteristics. A) Differences in the core of the ionization tracks as simulated by the RITRACK software. B) Variations in the delta electron tracks as simulated by the RITRACK software. C) An explanation of the relationship between the characteristics of the ionization particle track core and the multiplicity of double-strand breaks (DSBs) induced by ^{11}B and ^{20}Ne ions. D) The implications of delta electron distribution and range for the complexity of generated chromosomal translocations (illustrated schematically).

DNA damage are concentrated along a thin particle track core, with ^{20}Ne exhibiting a wider track core compared to ^{11}B (**Figure 3A**).

Given the dimensions of the DNA molecule, the broader track core of ^{20}Ne enables the efficient induction of multiple DSBs in close proximity, leading to highly complex damage clusters. In contrast, the narrower track core of ^{11}B results in a phenomenon reminiscent of the "overkill" effect—where the same total energy is confined to an excessively small volume. This leads to highly localized and extreme DNA damage at sites intersected by the track core, while leaving adjacent regions with significantly fewer DSBs compared to ^{20}Ne. Consequently, ^{11}B generates DSB clusters of lower multiplicity than ^{20}Ne, despite the similar ionization density of these ions (**Figure 3C**).

Another critical phenomenon regarding biological effects is the complexity of chromosomal translocations and aberrations in general. A key factor influencing this complexity is the distribution and range of secondary electrons emitted by the traversing ion (if we consider the scenario in which only one or a few particles traverse the nucleus). In the case of ^{20}Ne ions, the range of these secondary electrons is significantly greater than that of ^{11}B ions (**Figure 3B**). As a result, ^{20}Ne ions can induce DSBs across multiple chromosomal territories within the nucleus (**Figure 3D**).

The microdosimetric pattern of DNA damage induced by ^{20}Ne ions results in more complex and challenging-to-repair DSB clusters compared to ^{11}B ions (**Figure 3**); however, we can reasonably assume that it also contributes to the formation of chromosomal translocations of greater complexity (**Figure 3D**) [13], although this hypothesis could not be directly confirmed in the referenced study.

These insights highlight the need for further research to optimize ion species selection for cancer radiotherapy [146, 147] and to assess potential risks associated with astronaut exposure to different types of space radiation [80, 148–150].

2.5 Chromatin architecture, chromothripsis, and complex chromosomal aberrations occurring spontaneously

Chromothripsis is a relatively recent and paradigm-shifting phenomenon in cancer biology, characterized by the shattering of one or more chromosome segments into numerous fragments, which are subsequently reassembled in a highly disordered manner [151–154]. This chaotic process results in extensive genomic rearrangements, including complex karyotypes, even in the absence of ionizing radiation (IR) or other external mutagenic factors. Traditionally, cancer has been viewed as a disease driven by the accumulation of DNA damage over time. However, chromothripsis challenges this notion by suggesting that, in some cases, a single catastrophic event may suffice to drive tumorigenesis [154].

Despite significant advances, the underlying mechanisms of chromothripsis remain largely enigmatic. Several models have been proposed [151, 154–156], yet the precise triggers and sequence of molecular events remain under investigation. Interestingly, chromothripsis is frequently observed in preleukemic disorders, such as myelodysplastic syndromes (MDS) [157, 158], and often arises as a secondary event following radiotherapy or chemotherapy [155]. Chromosomal rearrangement characteristic for chromothripsis can be observed even in cells irradiated with a proton microbeam irradiation system [159]. This suggests that while chromothripsis can occur spontaneously, it may also be exacerbated by prior "chromatin stress." The resulting genomic alterations include complex deletions, translocations, and other structural rearrangements [151, 157].

Intriguingly, DSB breakpoints in chromothripsis are not randomly distributed; instead, they appear to occur with higher frequency in specific chromosomal regions [157]. However, unlike classical leukemia-associated breakpoints (e.g., in CML, AML, and APL) [160, 161], these regions are not sharply defined and do not correspond to known fragile sites [157]. This raises the possibility that chromatin architecture plays a fundamental role in both the formation of recurrent breakpoint regions and the subsequent rearrangement of fragmented chromatin, albeit in a stochastic manner [162].

A critical and still unresolved question is whether specific chromatin configurations predispose certain genomic loci to spontaneous DSB formation or whether these architectural features arise secondarily as a consequence of pre-existing pathological processes [162]. In any case, the example of MDS suggests that severe, high-multiplicity DSB clusters can arise spontaneously—without the involvement of external radiation or other mutagens. Further research is essential to elucidate how chromatin organization influences the susceptibility of specific genomic regions to such catastrophic events and whether individual variations in chromatin architecture correlate with a predisposition to MDS and cancers associated with chromothripsis.

3. DSB repair and formation of chromosomal translocations

3.1 DSB repair strategies in mammalian cells to prevent chromosomal aberrations

The repair of DNA double-strand breaks (DSBs) represents additional critical step following DSB induction in the process of chromosomal aberration (CHA) formation (**Figures 1** and **4**). The first parameter that comes to mind in the context of DSB repair is its fidelity, as accurate repair is essential to prevent genome erosion resulting from the accumulation of gene mutations. However, when examining DSB repair from the perspective of cellular priorities, it becomes evident that its primary function is to prevent the formation of CHAs [163]. These aberrations can lead to mitotic cell death [164]—one of the most common outcomes in irradiated cells—or, if transmitted to daughter cells, can result in severe genetic defects [163].

Even when CHAs do not directly interfere with mitotic progression or cause extensive genetic losses, they remain highly detrimental. For example, balanced chromosomal translocations, which are stably inherited by daughter cells, frequently exhibit strong oncogenic potential [33]. Such rearrangements are well-documented in the etiology of various leukemias, including chronic myeloid leukemia (CML), acute myeloid leukemia (AML), and acute promyelocytic leukemia (APL) [33].

Therefore, in the context of DSB repair fidelity, the most critical factor is the proper rejoining of DNA ends, which largely depends on the rapid stabilization of free DNA termini. On the other hand, certain genes are of exceptional importance, where even minor mutations can have severe consequences for cellular function and human health. Fortunately, the risk of functionally significant mutations remains relatively low, given that only approximately 1–2% of the human genome comprises protein-coding sequences [165], with an even smaller fraction directly involved in cellular proliferation and survival (and potentially cancer development) [166].

The optimal repair strategy also depends on the extent of DNA damage. When a high number of DSBs occur, such as following exposure to high radiation doses, the

rapid stabilization of free DNA ends becomes critical. Conversely, when only a few DSBs are present, repair fidelity may be prioritized over speed. Additionally, genome size plays a role in determining repair pathway utilization, as organisms with larger genomes, which sustain greater damage, tend to rely more on efficient repair kinetics.

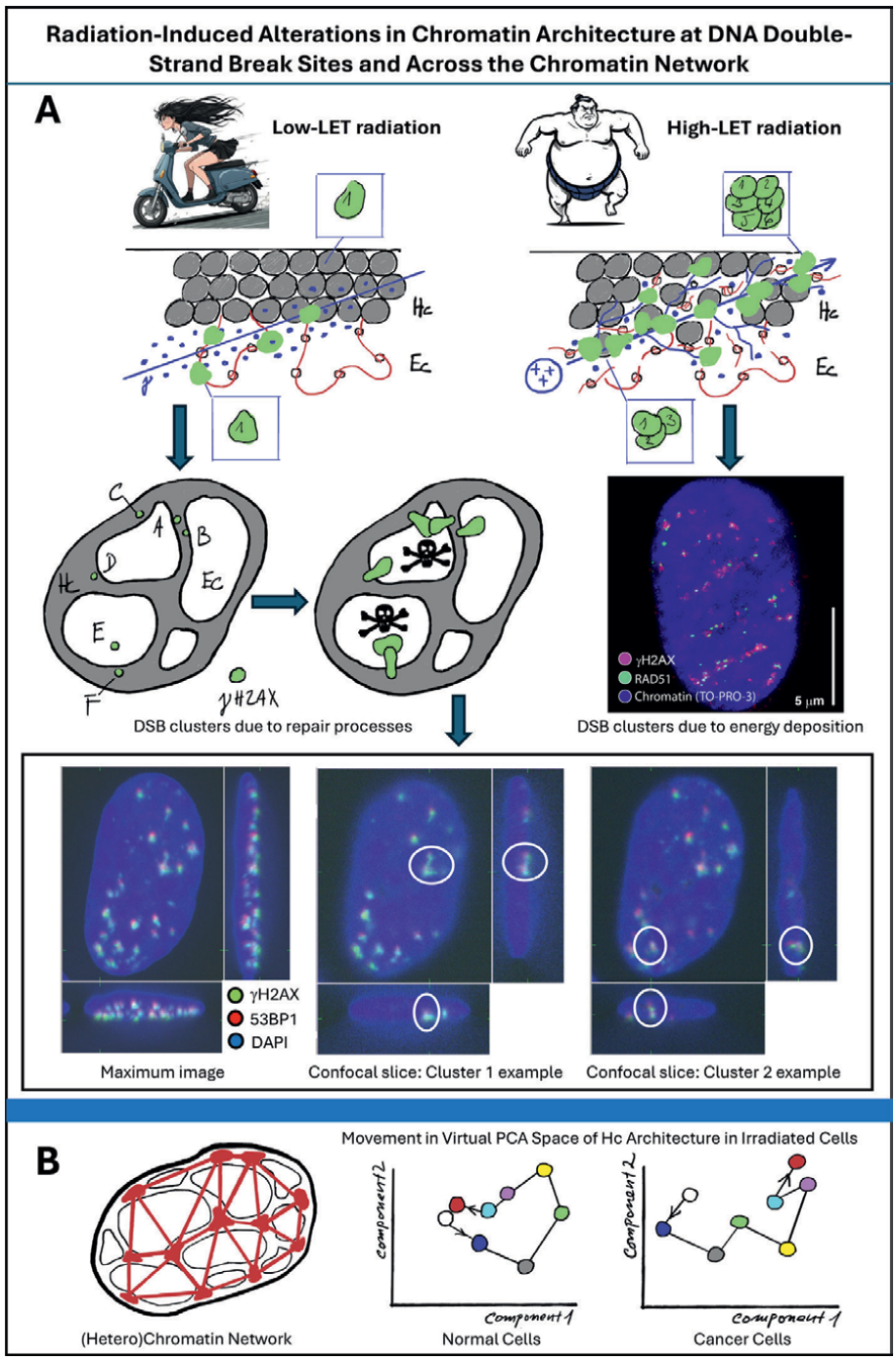

Figure 4.
Radiation-induced alterations in chromatin architecture at DNA double-strand break sites and across the chromatin network. Ionizing radiation (IR) impacts chromatin architecture at the sites of individual double-strand breaks (DSBs) and influences the entire chromatin network as a system. As previously elucidated in **Figure 2***, chromatin damage depends on the interplay between the type of radiation and the chromatin domain involved. In summary, indirectly ionizing photon radiation predominantly damages DNA through reactive oxygen species (ROS) generated by the radiolysis of water. These ROS can be partially attenuated by the abundant proteins that specifically bind to heterochromatin. Consequently, and because euchromatin is more hydrated, euchromatin is more sensitive to DSB formation induced by photon radiation compared to heterochromatin. Both euchromatin and heterochromatin predominantly exhibit simple DSBs. Given the energy deposition characteristics of photon radiation, DSBs are dispersed throughout the nucleus and are mostly distantly isolated, even for relatively high radiation doses on the order of Gy. Therefore, chromosomal translocations are largely a result of the mobilization of DSBs located in heterochromatin following the decondensation of damaged heterochromatin domains, which is necessary to facilitate ongoing repair processes. Heterochromatic DSBs may protrude into the nearest nuclear subcompartment with low chromatin density. Within these subcompartments, protruding DSBs can interact with existing DSBs or additional heterochromatic DSBs protruding into the same nuclear compartment. This leads to the secondary formation of DSB clusters as a consequence of repair processes. Although it may seem that this movement of DSBs toward repair factories is targeted, it is actually an incidental artifact inherently linked to the repair mechanism in heterochromatin. The architecture of chromatin thus statistically governs not only the spatial proximity of individual genetic loci but also determines which nuclear subcompartment a specific DSB will protrude into. Consequently, the probability of translocation events between specific genetic loci depends not only on their spatial proximity prior to DSB formation after irradiation but primarily on the chromatin architecture between these specific loci (DSBs). For example, if the probability of translocation formation was solely dependent on the distance between loci prior to irradiation (the Position-First Hypothesis), the likelihood would be the highest for loci A and B. However, the chromatin barrier between them causes DSBs A and B to protrude into different nuclear subcompartments, thus minimizing the probability of their interaction despite their close proximity. The highest probability of interaction and potential chromosomal translocation formation would, therefore, occur between DSBs A and C, which are further apart than A and B but protrude into the same nuclear subcompartment, significantly increasing their likelihood of interaction. DSB D also protrudes into the same subcompartment as A and C but is considerably farther away than A is from C. DSBs E and F are so far apart that they protrude into a different subcompartment than A, C, and D. The probability of interactions between E and F with A, C, and D is therefore very low; however, the interaction between E and F is highly probable, as E resides within the same nuclear subcompartment characterized by low chromatin density, into which F protrudes. It is important to note that although the probability of long-range interactions is quite low, there can occasionally be interactions between loci at opposite ends of the nucleus. The sites of DSB interactions are indicated by skull symbols in the illustration and by white circles in actual nuclear images obtained through immunofluorescence confocal microscopy, shown across all three planes. Nuclear (chromatin) structures are stained blue (DAPI), γH2AX foci are stained green, and 53BP1 foci are stained red. The left panel represents the maximum image obtained from the superimposition of all confocal slices through a human skin fibroblast nucleus irradiated with 1 Gy of gamma radiation (^{60}Co, 1 Gy/min) and visualized using immunofluorescence confocal microscopy 1 h after irradiation. Approximately 21 γH2AX foci are visible, mostly colocalizing with 53BP1 foci while remaining spatially separated. The middle and right panels show the confocal sections intersecting the locations where repairing foci are interacting, indicating potential sites for chromosomal translocation formation. In the case of densely ionizing charged particles, the situation differs significantly. As previously described (***Figure 2***), high-LET particles, by virtue of their energy deposition characteristics, generate complex and multiple DSB clusters by disrupting the architecture of chromatin along the particle's trajectory. Heterochromatin offers a greater number of DNA targets per unit volume; thus, it generates more clustered DSBs compared to euchromatin. In both euchromatin and heterochromatin, significant chromatin fragmentation (akin to a form of chromothripsis) can occur, facilitating interactions among these fragments. Consequently, DSB clusters arise directly as a result of the energy deposition from radiation. The clusters of DSBs concentrated along the six particle tracks in a human skin fibroblast nucleus irradiated with 4 Gy of ^{15}N ions (LET 180 keV. μm^{-1}, energy 13.1 MeV/n) are visualized in a confocal image (xy-plane) obtained from a Leica SP5 microscope after deconvolution using Lightning software. Chromatin is stained blue (DAPI), γH2AX foci are stained red, and RAD51 foci are stained green. Panel B illustrates the response to irradiation of the entire chromatin network as a system, highlighting the responses in normal and tumor cells. As discussed in part 4 of this chapter, the topology of heterochromatin domains, marked by antibodies against H3K9me3, was studied over time following irradiation using single-molecule localization microscopy (SMLM). Changes in the nanoarchitecture (topology) of heterochromatin domains observed between 0 and 24 h post-irradiation were analyzed and transformed using principal component analysis (PCA) to create a virtual two-dimensional topological space [25]. In normal cells, changes in the topology of heterochromatin domains create a closed cycle, indicating that the architecture of the (hetero)chromatin network returns to its pre-irradiation state after 24 hours. In contrast, the changes in tumor cells are more irregular, and closure of the cycle does not occur, which may contribute to, if not primarily cause, the genomic instability observed in tumor cells post-irradiation. For instance, the failure to restore the architecture of heterochromatin domains may lead to the mobilization of transposons. The nucleus in Panel A is reproduced from Toufar J, EBJ (Springer Nature) 2025, manuscript under review.*

3.2 Core DSB repair pathways in mammalian cells

Consistent with the repair strategies outlined above, mammalian cells have evolved two primary pathways to mitigate the harmful effects of DSBs—non-homologous end-joining (NHEJ) [46, 167, 168] and homologous recombination (HR) [167, 168]—along with several backup mechanisms of uncertain classification, including alternative NHEJ (alt-NHEJ) and microhomology-mediated end-joining (MMEJ) [50, 51, 169]. These pathways differ fundamentally in terms of repair accuracy, speed, and their consequences for genomic integrity.

NHEJ is a fast yet error-prone mechanism that involves the direct ligation of DNA ends without the need for extensive sequence homology. It can efficiently repair a large number of DSBs throughout the cell cycle [170]. However, before rejoining, the processing of free DNA ends is often required [46], leading to small insertions or deletions (indels) at break sites. Despite its error-prone nature, NHEJ serves as a robust frontline defense against CHAs.

In contrast, HR is a highly precise but slower repair mechanism, primarily restricted to the late S and G2 phases of the cell cycle, where a homologous sister chromatid is available as a repair template [171]. Due to this requirement for an intact homologous sequence, HR is limited in its capacity to repair all DSBs throughout the cell cycle.

Backup repair pathways, regardless of their specific mechanisms, tend to be significantly mutagenic [46, 50, 172]. For instance, MMEJ [173] utilizes short homologous sequences (microhomologies) flanking the break site to facilitate repair, often resulting in deletions of the intervening DNA sequence. Additionally, the use of repetitive sequences from different chromosomes can lead to misrepair, potentially generating CHAs [174, 175].

3.3 Pathway choice and chromatin architecture as a regulatory factor

The selection of a specific repair pathway at each DSB site is thus a critical determinant of CHA formation [5, 55, 176]. Although substantial progress has been made in understanding the molecular mechanisms underlying pathway choice, many aspects remain unresolved [5, 55, 176]. Numerous factors have been proposed to influence pathway selection at both nuclear and local DSB levels [5, 55, 176], including cell cycle phase, the chemical properties of DSB ends, the nature and distribution of DSBs within the nucleus, chromatin architecture and epigenetic modifications at the damage site, and overall DSB burden. However, the sheer number and diversity of these factors raise the question of how cells can process such a vast array of signals rapidly enough to ensure timely and efficient DSB repair [5, 177].

It is therefore plausible that an integrated signaling mechanism exists to consolidate these diverse nuclear and local signals into a single, easily interpretable signal that dictates the initiation of either NHEJ or HR [5]. One potential candidate for such a regulatory mechanism is chromatin architecture, which could modulate DSB accessibility to repair proteins, ensuring the timely assembly of particular repair complexes [5, 177, 178]. This regulation is reflected in the formation of ionizing radiation-induced foci (IRIF) [179] that serve as recruitment hubs for DSB repair factors and may provide a structural framework for repair pathway selection [5].

There are multiple perspectives on how the DNA double-strand break (DSB) repair network is regulated within chromatin architecture, particularly in the context

of euchromatin and heterochromatin [5, 177, 178]. Importantly, each of these perspectives presents fundamental challenges and unresolved questions.

Euchromatin (Ec) is the transcriptionally active compartment of the genome, enriched in functionally significant and highly expressed genes, including tumor suppressors and protooncogenes [132, 133]. Given its crucial role in genome functioning, it could be reasonably assumed that Ec preferentially undergoes precise repair *via* homologous recombination (HR), at least its highly expressed parts [180]. In contrast, heterochromatin (Hc) is highly compacted and largely transcriptionally inactive [132, 133], which creates a structural barrier to the repair machinery [1, 127, 181]. HR is thus generally impeded in Hc unless the damaged domain undergoes decondensation [1, 182, 183]. This structural constraint and genetic activity of Ec suggest a model in which Ec is a prime candidate for HR-mediated repair, while the more error-prone non-homologous end joining (NHEJ) might be sufficient for DSB repair in heterochromatin. However, some studies suggest that the intricate architecture of heterochromatin may facilitate HR [184–186], or at least promote resection-based repair mechanisms [187]. This offers an alternative regulatory framework for DSB repair pathway networks that does not solely rely on the significance of transcribed gene sequences (transcriptional activity), but rather on the compatibility of chromatin architecture with specific repair mechanisms.

Moreover, a significant challenge to the model proposing that HR is the primary mechanism for actively transcribed genes in order to ensure precise repair is the considerably slower repair kinetics of HR compared to NHEJ. HR is a slow and intricate process that often requires several hours to complete, making it difficult to reconcile with the need for timely reactivation of critical genes following DNA damage. This raises an important question: How do cells balance the necessity for precise repair with the urgency of restoring transcriptional activity?

Given the uncertainties regarding the factors that activate HR mentioned above, an alternative perspective on the regulation of repair pathway network also holds merit [188, 189]. A plausible model proposes that NHEJ is initially activated throughout the nucleus to rapidly repair as many DSBs as possible [188, 189]. Subsequently, repair at certain DSB sites that are resistant to NHEJ or specifically marked for HR—such as highly transcribed genes or centromeres—may be redirected toward HR, potentially guided by specific epigenetic modifications or stalling repair.

Several factors could contribute to the failure of NHEJ at certain DSB sites, prompting a shift to HR: (i) high complexity or multiplicity of DSBs, as seen after exposure to high-LET IR, where Ku proteins fail to bind short DNA fragments [190]; (ii) restrictive chromatin architecture, such as the highly compacted structure of heterochromatin [191]; or (iii) the presence of single-ended DSBs, which are inherently unsuitable for NHEJ [45].

Regarding epigenetic marks, specific histone modifications have been proposed to facilitate the recruitment of HR machinery to distinct genomic regions. For instance, euchromatic histone modifications, such as trimethylation of lysine 36 on histone 3 (H3K36me3) and dimethylation of lysine 4 on histone 3 (H3K4me2), have been linked to HR recruitment at highly transcribed genes [181, 192–194]. Notably, both of these modifications are also observed in centromeric regions—areas characterized by their heterochromatic nature that nonetheless exhibit active transcription—where they have been shown to initiate precise HR repair [109, 184, 195, 196]. Similar findings in nucleolar repeats, but not at pericentromeric repeats, suggest a broader role for these modifications in regulating HR [184]. In contrast, trimethylation of lysine 9 on

histone 3 (H3K9me3), a marker associated with constitutive heterochromatin, has also been implicated in directing HR, particularly within heterochromatic regions [197]. Interestingly, while the aforementioned studies indicated that H3K36me3 can initiate homologous recombination (HR), other research has shown that it may also reduce chromatin accessibility for repair factors and promote NHEJ [181, 198]. This suggests that our understanding of the epigenetic codes associated with DSB repair remains incomplete or, more likely, multiple factors must cooperate to guide repair pathway selection within specific chromatin subcompartments, thereby regulating the cellular response to DSBs.

An intriguing insight derived from the discussion above is that HR, despite being a highly precise repair mechanism, is not exclusively reserved for highly expressed (functionally important) genes. Instead, it is also utilized in heterochromatic domains, including centromeres. This observation suggests that heterochromatic DNA sequences may be just as critical for cellular function as euchromatic sequences [106, 107, 199, 200]. In the case of centromeres, for instance, the DNA sequence may play a crucial role in sequence-specific attractive forces that facilitate interactions between homologous regions of double-stranded DNA molecules, thereby contributing to homologous chromosome pairing. The base sequence influences the modulation of DNA backbone structure and the surface charge pattern, which, in turn, enables the electrostatic recognition of sequence homology at distances extending up to several layers of water molecules [201, 202]. Moreover, errors in the repair of DSBs within centromeric regions are responsible for gross chromosomal rearrangements, such as translocations and isochromosome formations [203]. The functional significance of the heterochromatin network and its response to radiation-induced damage will be discussed later.

The present findings highlight the need for a deeper understanding of how chromatin organization affects the selection of repair pathways, with implications for repair fidelity, and how cells mitigate the risks associated with imprecise repair in different chromatin contexts.

3.4 Ionizing radiation-induced foci (IRIF) and DSB repair

Ionizing radiation-induced foci (IRIF), studied at the microscopic level using confocal immunofluorescence microscopy or at the nanoscale through super-resolution microscopy techniques (e.g., single-molecule localization microscopy, SMLM) [18, 25], have revealed numerous groundbreaking insights into the mechanisms of double-strand break (DSB) repair and the potential formation of chromosomal aberrations (CHA) in both normal and various cancer cell types exposed to different IR types [1–19, 23, 104–106, 108, 139, 140, 204–211]. Although much of the data is still under analysis, several conclusions can be drawn at this point (see, e.g., [15, 16, 209]):

i. Analysis of γH2AX foci, reflecting chromatin architecture at DSB sites, and repair foci formed by various repair proteins (e.g., 53BP1, RAD51, etc.), indicates that these repair foci (IRIF) exhibit specific sizes, geometries, and topologies, suggesting the functional significance of these architectural parameters for DSB repair.

ii. The micro- and nanoscale characteristics of specific IRIF types (e.g., γH2AX, 53BP1, RAD51) are generally surprisingly similar across different cell types,

but can vary, particularly in cancer cells exhibiting defects in certain repair mechanisms.

iii. The nano-topology of individual γH2AX foci, as well as foci formed by various repair proteins, exhibits remarkable similarity, further underscoring the functional significance of chromatin architecture and spatial organization at DSB sites.

iv. The similarity between different types of IRIF changes over time after irradiation, with both the extent of this similarity and the dynamics of its evolution depending on the IRIF and cell type.

v. The sizes of γH2AX and 53BP1 foci are closely correlated; however, this correlation may be disrupted in cancer cells.

vi. The similarity in the topology of IRIF is greater for DSBs localized in Hc (marked by H3K9me3) compared to those in Ec [209].

Considering these findings, along with the observed correlation between alterations in IRIF topology in U87 cancer cells and the ability of these cells to resolve complex DSBs [210], it is evident that IRIF analysis provides valuable insight into DSB repair processes at the single-molecule level. This underscores the crucial role of chromatin architecture at individual DSB sites and the organization of repair complexes in the DSB repair mechanism, ultimately influencing the generation of chromosomal aberrations (CHA) [5]. However, establishing a precise relationship between IRIF parameters and the specific outcomes in terms of CHA formation remains a significant challenge.

3.5 DSB repair within the chromatin environment

In the previous chapter, we discussed how chromatin architecture at individual DSB sites can influence the crucial decision-making process of selecting the optimal repair pathway for each DSB. In this chapter, we will specifically examine the impact of chromatin architecture and its dynamic modifications during DSB repair on the formation of chromosomal translocations (CHT), without addressing whether the repair occurs *via* NHEJ, HR, or alternative pathways (**Figure 4**).

A fundamental question regarding the mechanism of CHT (and CHA) formation concerns the mobility of free DSB ends [1, 6, 212–216]. Based on various observations of this mobility, two primary hypotheses have been proposed to explain the formation of CHT:

i. the Position-First Hypothesis, and

ii. the Breakage-First Hypothesis.

The Position-First Hypothesis (**Figure 1**, bottom panel, *a* and *b*) (reviewed in [3]) posits that chromosomal territories and chromatin domains within the nucleus are spatially organized according to non-random, albeit stochastic, principles. Given that DSBs exhibit mobility similar to that of unaltered chromatin (reviewed in [3]), this three-dimensional nuclear architecture plays a crucial role in determining the

likelihood of chromosomal translocation formation following IR exposure. According to this hypothesis, the probability of translocation formation between two specific loci is directly proportional to their spatial proximity. This type of result has been observed in experiments where cells were exposed to sparsely ionizing photon radiation, which generates simple and spatially isolated DSBs.

Contrary to this, experiments involving exposure to alpha particles or accelerated heavy ions, which represent densely ionizing radiation, led to dramatically different conclusions. In these experiments, the passage of particles through the nucleus caused significant chromatin fragmentation, accompanied by a marked increase in the mobility of damaged chromatin compared to undamaged chromatin (reviewed in Ref. [3]). These observations gave rise to the Breakage-First Hypothesis (**Figure 1**, bottom panel, *a* and *c* plus *d*) (reviewed in Ref. [3]), which suggests that the risk of chromosomal translocation formation is not only influenced by the non-random architecture of chromatin in the nucleus but also by the increased mobility of DSBs [6, 112, 215]. An extension of this hypothesis suggests that DSBs are not repaired at their site of origin, as proposed by the Position-First Hypothesis. Instead, they migrate to specific nuclear subcompartments, referred to as "repair factories," where multiple DSBs are repaired simultaneously [6].

The Position-First Hypothesis provides a compelling explanation for why certain translocations, particularly commonly observed oncogenic translocations, occur more frequently than others. Additionally, the unique chromatin architecture in different individuals may result in the closer proximity of specific loci, such as ABL and BCR, in some of them compared to others [96–99], potentially predisposing them to develop, in this case, chronic myelogenous (CML) leukemia or other type of leukemia. In contrast, the Breakage-First Hypothesis accounts for the occurrence of complex CHT, which can occasionally be observed even in cells exposed to photon radiation—a phenomenon that is difficult to explain through the Position-First Hypothesis alone.

In our previous studies [1, 3], we investigated the mobility of DSBs in cells exposed to gamma radiation (^{60}Co) and unexpectedly observed IRIF behavior consistent with both the Position-First and Breakage-First Hypotheses. Using time-lapse microscopy to track the mean squared displacement (MSD) of 53BP1 foci labeled with RFP in live cells, we found that while the majority of DSBs were repaired at their sites of origin, a distinct subset of 53BP1 foci exhibited significantly increased mobility. Notably, this highly mobile fraction corresponded to DSBs localized within heterochromatin [1].

Further analysis revealed that these mobile heterochromatic DSBs colocalized with the epigenetic marker H4K5ac within the first 20-min post-irradiation, suggesting that their increased mobility was linked to damaged Hc domain decondensation (**Figure 4A**) [1]. This decondensation is crucial for the repair process for several reasons:

1. *Accessibility of repair proteins*—Heterochromatin is inherently less accessible to certain repair factors. Only upon decondensation do heterochromatic DSBs become fully available to the repair machinery. For example, while the small DSB sensor protein NBS1 can rapidly access Hc domains marked by HP1β and containing DSBs induced by laser micro-irradiation, the much larger scaffold protein 53BP1 can only do so after Hc decondensation [127, 217]. Similarly, repair proteins such as RPA, which stabilize single-stranded DNA overhangs generated during homologous recombination (HR), can be efficiently replaced by

RAD51—facilitating the recombination step of HR—only after the decondensation of the damaged Hc domain [183].

2. *Suppression of illegitimate recombination* – Heterochromatin is enriched in repetitive sequences, which poses a risk of aberrant recombination. Decondensation helps mitigate this risk by regulating the accessibility of these sequences [183].

Thus, chromatin architecture serves as a fundamental regulatory factor in DSB repair, as outlined earlier (**Figure 1**). However, Hc decondensation also has a potentially detrimental consequence. As damaged heterochromatin unfolds, DSBs protrude from their original heterochromatic compartments into adjacent nuclear subcompartments with lower chromatin density. Within these subcompartments, mobilized heterochromatic DSBs can interact with other DSBs, either pre-existing or those that have similarly protruded from different heterochromatin domains (**Figure 4A**) [1, 3].

It is important to emphasize that these interactions do not reflect an active, targeted migration of DSBs into dedicated repair factories. Rather, they represent an incidental byproduct of the repair process—an inherent consequence of chromatin dynamics that creates a potential substrate for chromosomal translocations (CHTs) (**Figure 4A**) [1, 3].

A groundbreaking finding here is that the local chromatin architecture between DSB sites significantly influences the likelihood of their interaction, potentially leading to chromosomal translocations. Our results, therefore, bridge aspects of both the Position-First and Breakage-First Hypotheses (**Figure 4A**) [1, 3]:

1. Most DSBs are relatively immobile and are repaired at their sites of origin, consistent with the Position-First Hypothesis. However, heterochromatic DSBs become mobilized, leading to the sporadic formation of DSB clusters as a secondary consequence of repair processes within heterochromatin, rather than through the directed migration of multiple DSBs into repair factories. This phenomenon is partially consistent with the Breakage-First Hypothesis.

2. The probability of chromosomal translocation between two loci is influenced not only by their spatial proximity within the nucleus but is primarily dictated by the chromatin architecture between them. This suggests that the process integrates aspects of both the Position-First and Breakage-First Hypotheses.

However, the concept of repair factories may still hold relevance at the nanoscale [218]. Recent studies have demonstrated that γH2AX foci, when analyzed at super-resolution, consist of multiple γH2AX subclusters [15, 219, 220]. It remains unclear whether these subclusters reflect:

i. multiple closely spaced DSBs generated even by photon radiation,

ii. DSBs actively migrating into a repair nanofactory for processing, or

iii. internal heterogeneity within γH2AX foci, where molecular-level resolution (e.g., SMLM imaging) reveals competition in antibody binding—especially when co-staining additional repair proteins alongside γH2AX.

Overall, our findings emphasize that chromatin architecture at and between specific DSB sites plays a crucial role in DSB misrejoining and the formation of CHTs [1, 3]. The dynamic reorganization of chromatin during DSB repair, particularly in heterochromatin, is not merely a passive consequence but an active and integral component of the mechanisms governing DSB repair and chromosomal aberration formation [1, 3].

3.6 Chromatin topology, DSB patterns induced by different types of ionizing radiation, and the mechanism of chromosomal translocation formation

If we set aside the mobility of heterochromatic DSBs driven by the decondensation of damaged heterochromatic domains, as discussed earlier (**Figure 4A**), it becomes evident that the non-random spatial organization of chromosome territories and loci within the nuclear landscape (chromatin texture) creates a probability field for interactions between these territories and specific loci. If DSBs occur within these regions, such interactions may facilitate the formation of CHT. In addition to the influence of chromatin architecture, as discussed in the previous chapter, the probability of specific translocations also depends on the spatial distribution of DSBs within the nucleus, which in turn is dictated by the type of incident-ionizing radiation (**Figures 2** and **4**).

DSBs induced by photonic-ionizing radiation (IR) tend to be more widely spaced compared to those generated by high-linear energy transfer (high-LET) heavy ions. As a result, photonic IR predominantly leads to interchromosomal translocations, where breaks on distinct chromosomes are misrejoined. These translocations typically involve only two chromosomes and are often reciprocal (**Figure 2**).

In contrast, high-LET IR, due to its capacity to induce extensive chromosome fragmentation along the particle track, generates most DNA breaks within individual chromosome territories or at interchromosomal boundaries between neighboring chromosomes. This pattern of DSB distribution favors the formation of complex chromosomal translocations (CHTs), frequently of the intrachromosomal type (**Figure 2**). Such phenomena have been observed in individuals exposed to plutonium contamination, including workers at the Mayak Production Association in the former Soviet Union following nuclear incidents.

Finally, it is important to note that in the case of densely ionizing particles, the microdosimetric pattern of radiation energy deposition—and consequently the spatial distribution of DSBs—depends on both the LET and the energy of the radiation. As discussed in Section 2.4, different ions can generate DSB clusters of varying multiplicity, even when they have nearly identical LET values and similar energy levels. Evidently, additional ion-specific parameters influence the width of the ionization track core and associated DSB distribution. However, simulations using the RITRACK software have demonstrated that substantial differences can also be expected in the distribution and range of secondary (delta) electrons (**Figure 3B**).

For instance, when revisiting the comparison between ^{11}B and ^{20}Ne ions, as reported by Jezkova et al. [13], it was observed that electrons emitted by ^{20}Ne ions exhibit a significantly greater range than those emitted by ^{11}B ions (**Figure 3B**). Consequently, in cells irradiated with ^{20}Ne, DSBs can occur not only within the core ionization track but also across multiple chromosomes (**Figure 3C**), leading to a broader spectrum of affected chromosomes compared to cells exposed to ^{11}B ions (**Figure 3D**). Although direct experimental evidence was not provided in the study by Jezkova et al. [13], these findings suggest that ^{20}Ne ions may induce more complex

chromosomal translocations and karyotypic rearrangements than ^{11}B ions, despite their similar LET and energy (**Figure 3D**).

4. Application of single-molecule localization microscopy: Analyzing heterochromatin organization and topology following irradiation—Systemic response of the heterochromatin network

The results presented in this chapter demonstrate that, following radiation treatment, heterochromatin undergoes complex reorganization that significantly impacts double-strand break (DSB) repair [176]. To investigate this reorganization in greater detail, we employed single-molecule localization microscopy (SMLM) [221, 222], an advanced super-resolution imaging technique [223], which enables precise localization of individual blinking fluorescently labeled molecules with nanometer-scale precision (~10 nm). Specifically, we used SMLM to analyze H3K9me3 methylation sites. Blinking events of the dye molecules were detected through a time series of image frames captured by a highly sensitive CCD camera [224]. These blinking events were registered in a coordinate matrix, known as the "ortho-matrix" [14]. From these data, artificial images were generated, and geometric as well as topological analyses were performed (**Figure 5**).

For topological analysis, persistent homology analysis [225–227], persistent imaging [228], and principal component analysis (PCA) [229] were applied, as described in detail in Ref. [25]. Significant structures within the point pattern were identified using the following approach: Each point (i.e., component, corresponding to dimension 0 in persistent homology) recorded in the SMLM coordinate ("orte") matrix was enclosed by an expanding circle. Each component was represented by a bar, which terminated at the radius value when the expanding circles of two components merged. At this point, two separate components (two bars) combined into a single component (one remaining bar).

During this process, some enlarged components formed closed loops (in the simplest case, a triangle) that left a free space inside, not covered by the expanding circles. These free spaces were considered as topological "holes," and their appearance was marked by the initiation of a new bar (dimension 1 in persistent homology). Once the hole was entirely covered by the growing circles, the corresponding bar ended. As components merge and their number decreases, the number of holes first increases and then decreases as more and more holes are closed.

For each cell, the barcode results were converted into a "bar lifetime vs. bar birth" diagram, which was then transformed into a pixel scan (persistent image), where pixel intensity corresponded to the number of points in the diagram. Persistent images were generated for each cell in the experiment and subsequently analyzed using PCA. In PCA, each pixel's value—ranging from the 1st to the nth pixel—was treated as an n-dimensional vector space. The dimension with the largest variance became PCA component 0 in the final diagram. PCA component 1, always perpendicular to component 0, captured the second largest variance.

This approach allowed the complexity of the point pattern to be reduced to a two-dimensional (2D) latent space, where only two principal components described the primary features and their variations. Consequently, small fluctuations, often considered "biological noise," were effectively disregarded.

These topological processes have been utilized to distinguish different cell types [106], to analyze chromatin organization changes during DNA repair following

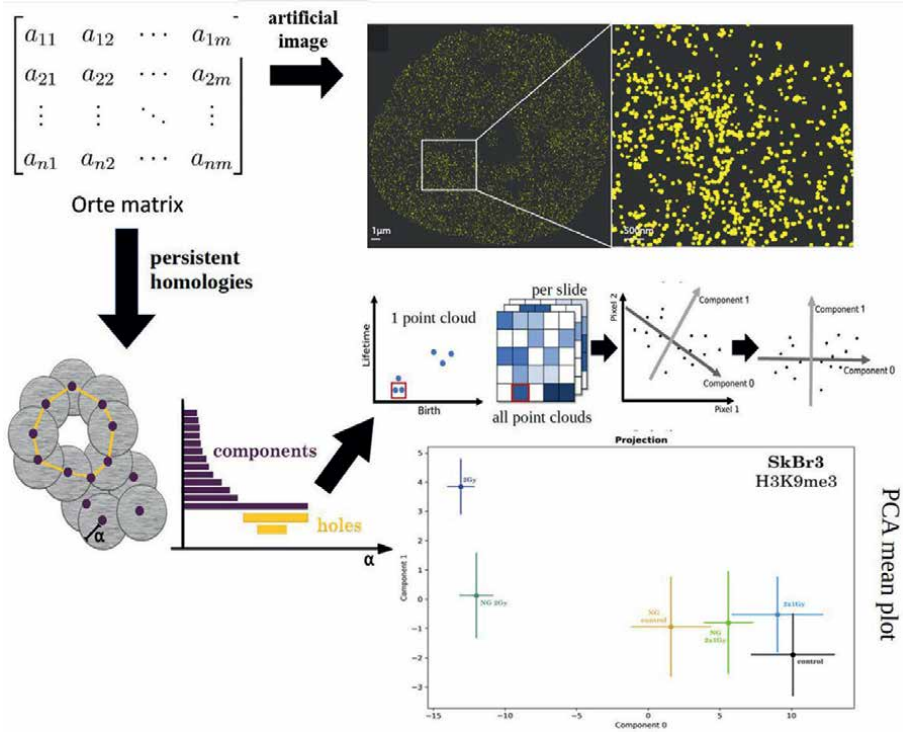

Figure 5.
Schematic representation of the different evaluation steps of SMLM datasets. After acquisition of a time series of image frames, the coordinates and other values of all the blinking events of a cell nucleus were integrated into a matrix, the so-called orte-matrix (top-left). The processes of persistent homology, persistent imaging, and principal component analysis are shown. The point pattern is transferred into a bar code description of components and holes. The lengths of the bars (difference of the α values of the end and the beginning of a given bar) give the lifetime and the beginning the birth in the one point cloud. The one point clouds for all cell nuclei that are considered for an evaluation are transferred in pixel images (persistent imaging). Each pixel is compared for all images. The results of these comparisons span an n-dimensional orthogonal vector space. The variations of the pixel values determine the components of the principal component analysis. Finally, the outcome for the components (orthogonal vector values) with the largest variation and the second largest variation (PCA) determine the latent space (graph bottom right) (for further details see text). Modified from Bartosova M. et al., Nanoscale 2025 (under review) (published under a BY-CC license).

radiation-induced damage [25, 230], and to investigate the effects of gold nanoparticles on chromatin structure, both with and without irradiation [230].

In **Figure 6**, the density distribution shown in **Figure 6A** represents the fluorescence intensity pattern as it would appear in a standard fluorescence microscopy image of a cell nucleus. However, this staining pattern results from the diffraction of all individual dye molecules (here, 53,000), as depicted in **Figure 6B**.

For the analyses presented here, SkBr3 cells [231], HeLa cells [15, 232], and Jurkat cells [233] were used. Representative nuclei for SkBr3 cells and HeLa cells are shown in **Figure** 7.

SkBr3 cells were exposed to photon-ionizing radiation at varying doses (0, 0.1, 0.5, 1.0, 2.0, 4.0, and 8 Gy), followed by DSB repair. Thirty minutes post-irradiation, the cells were fixed and immunostained with fluorescent antibodies targeting heterochromatin (H3K9me3). At first glance, the spatial distribution of labeled heterochromatin appeared similar between irradiated and non-irradiated samples.

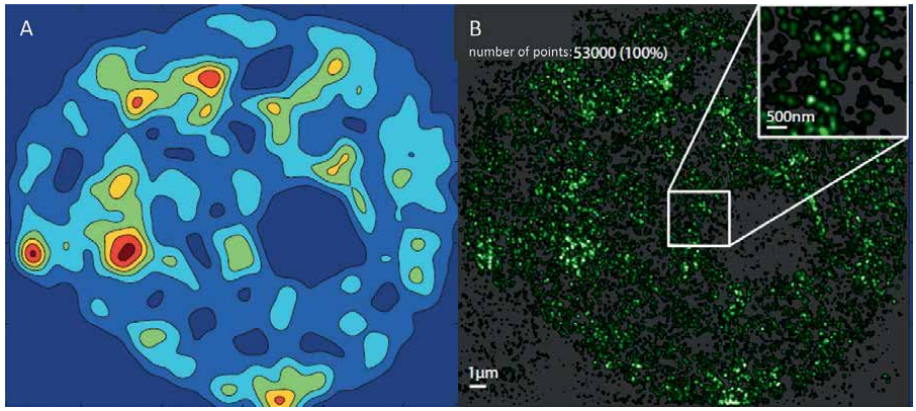

Figure 6.
Density distribution of staining H3K9me3 in a cell nucleus as being obtained by standard fluorescence microscopy (A), super-resolution imaging of single-labeling molecules as obtained by SMLM (B).

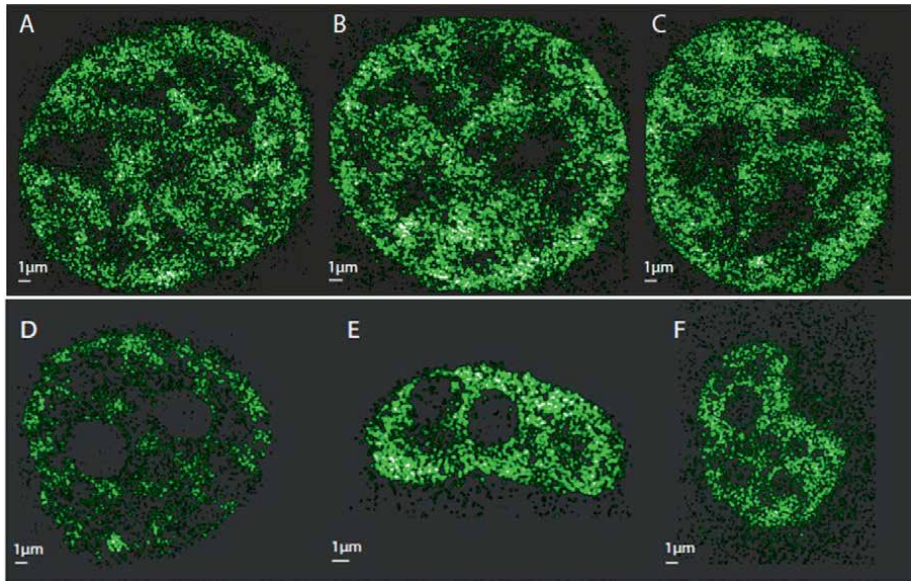

Figure 7.
Typical examples of SMLM images of SkBr3 (A-C) and HeLa (D-F) cell nuclei after H3K9me3 labeling.

To assess potential differences, pairwise distances between all labeled points were measured in approximately 25 nuclei, and the nearest-neighbor distances were calculated and averaged for all points (**Figure 8**). Interestingly, the nearest-neighbor distance distribution in cells exposed to 8 Gy was nearly identical to that of the control group. However, cells irradiated with 4 Gy exhibited a significant shift toward smaller distances, whereas cells exposed to doses below 4 Gy displayed a broader distribution with increased distances, suggesting a reduction in heterochromatin compaction within the cell nuclei.

Although the nearest-neighbor distances in the 8 Gy-exposed samples were highly similar to those in the control group, the topological distribution of the point pattern,

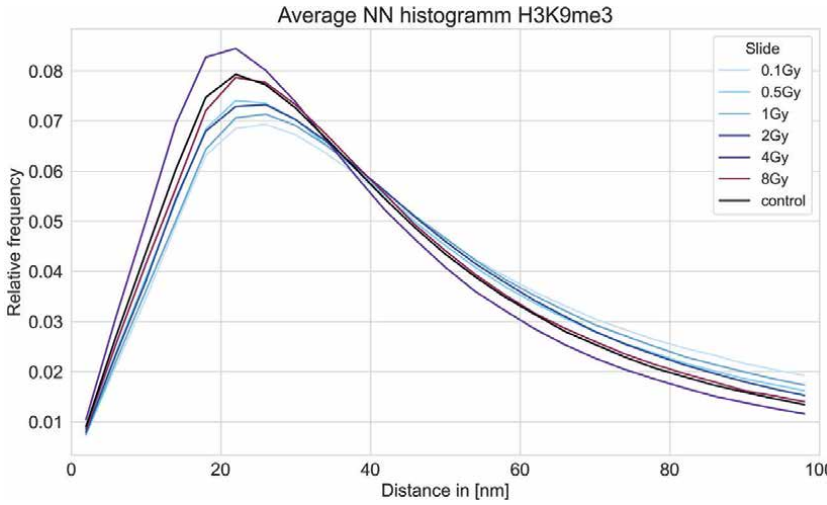

Figure 8.
Relative frequency of next neighbor distances of H3K9me3 labeling points in SkBr3 cell nuclei after exposure to different doses of photonic radiation between 0 and 8 Gy.

as analyzed using persistent homology, persistent imaging, and PCA, showed a striking divergence. This difference is evident in the latent space representation in **Figure 9**. The key structural features of heterochromatin were rearranged following irradiation, differing significantly from the non-irradiated control.

To assess whether the observed changes in heterochromatin topology were reversible, we irradiated HeLa cells with 1 Gy of X-rays and monitored the repair process over 24 hours. Thirty minutes post-irradiation, heterochromatin organization was

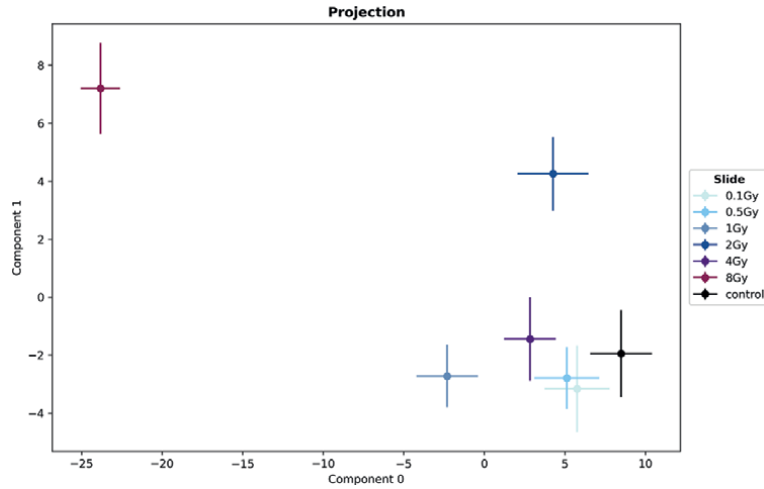

Figure 9.
Two-dimensional latent space after PCA of the persistent imaging data of the H3K9me3 loci in nuclei of SkBr3 cells 30 min after exposure to different doses of photonic ionizing radiation. Mean values of component 1 vs. component 0 are shown. In this latent space, "component 0" is the vector in the n-dimensional orthogonal vector space of persistent imaging with the largest variability (variance). Component 1 is a vector orthogonal to component 0 with the second largest variance. The error bars of the components represent the standard deviation. The application of ionizing radiation leads to a significant shift in both components (0 and 1).

markedly altered, suggesting chromatin relaxation to facilitate repair. After 24 hours, once repair was expected to be complete, heterochromatin architecture had nearly returned to the control state (**Figure 10**).

For comparison, cells were subjected to heat treatment at 43°C prior to irradiation with 1 Gy of X-rays. Similar to the non-heated samples, heterochromatin reorganization was observed 30-minute post-irradiation, indicating a rapid chromatin response to facilitate repair. However, after 24 hours, the chromatin structure did not revert to the control state and remained significantly altered due to the heat treatment (**Figure 10**). Given that heat exposure is known to increase cellular radiosensitivity, these results are consistent with previous findings.

To investigate irreversible heterochromatin reorganization following radiation exposure, we studied the highly radiosensitive Jurkat cells. A representative Jurkat cell nucleus is shown in **Figure 11**, where H3K9me3 labeling reveals an inhomogeneous distribution (**Figure 11**, right). Cells were irradiated on ice with either 3 or 10 Gy and subsequently incubated at 37°C to allow for DNA repair. Two-dimensional latent space analysis demonstrated that 1 h post-irradiation, heterochromatin organization had significantly diverged from that of non-irradiated control cells. After 24 hours, the chromatin structure had undergone further changes but had not returned to its pre-irradiation state. Furthermore, the extent and nature of these changes were strongly dose-dependent, with 3 and 10 Gy exposures leading to nearly opposite outcomes.

In addition to irradiation, we examined the effects of low temperature (on ice specimens) on chromatin organization. This so called "freezing" induced a downregulation of most cellular functions, accompanied by heterochromatin relaxation. Upon rewarming to 37°C, the cells recovered, and heterochromatin regained an organized structure, though it differed from both the control and irradiated samples (**Figure 11**).

The findings presented in this chapter demonstrate that chromatin responds to irradiation not only locally at DSB sites but also as a cohesive network, functioning

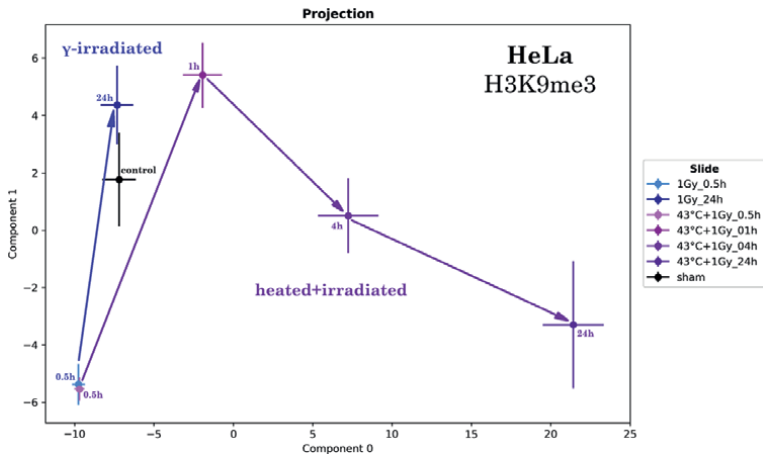

Figure 10.
Two-dimensional latent space after PCA of the persistent imaging data of the H3K9me3 loci in nuclei of HeLa cells. Thirty minutes after exposure to a dose of 1 Gy of photonic radiation both specimens (with and without heat treatment) showed the same changes in topology of heterochromatin. While the non-heated specimen nearly reached the control situation after 24 hours, the heat exposed specimen differed significantly in both components of the latent space. Mean values of component 1 vs. component 0 are shown. In this latent space "component 0" is the vector in the n-dimensional orthogonal vector space of persistent imaging with the largest variability (variance). Component 1 is a vector orthogonal to component 0 with the second largest variance. The error bars of the components represent the standard deviation.

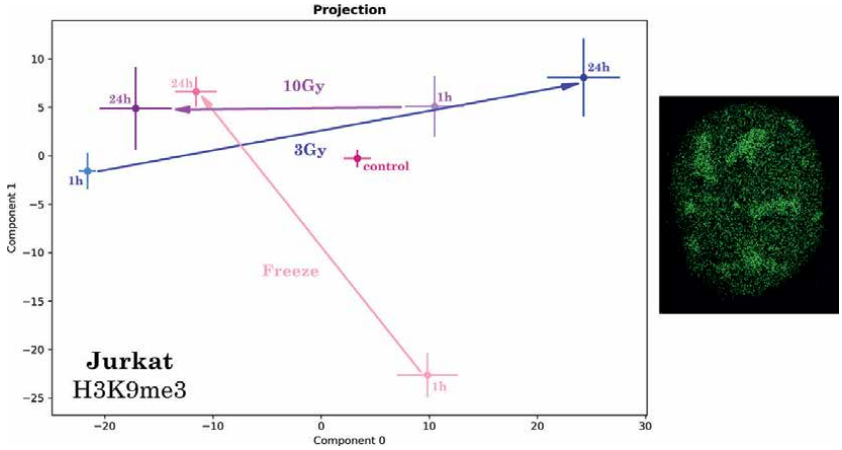

Figure 11.
Left: Two-dimensional latent space after PCA of the persistent imaging data of the H3K9me3 loci in nuclei of Jurkat cells. One hour and 24 hours after exposure to a dose of 3 and 10 Gy of X-rays on ice, both specimens showed opposite changes in topology of heterochromatin strongly different from the non-irradiated, not-frozen (i.e. not put on ice) control. Also, the on-ice (= freeze) control showed a different heterochromatin re-organization during recovery. Mean values of component 1 vs. component 0 are shown. In this latent space "component 0" is the vector in the n-dimensional orthogonal vector space of persistent imaging with the largest variability (variance). Component 1 is a vector orthogonal to component 0 with the second largest variance. The error bars of the components represent the standard deviation. Right: SMLM image of a Jurkat cell nucleus after labeling with antibodies against H3K9me3 (heterochromatin).

as an integrated system. Notably, substantial reorganization was observed in heterochromatin domains, with this restructuring appearing to be dependent on the nature of the damaging agent (e.g., type of ionizing radiation, high or low temperature), radiation dose, and cell type.

While in normal irradiated cells, the architecture of the (hetero)chromatin network typically returns to its pre-irradiation state within approximately 24 hours, this restoration does not occur in cancer cells, which often harbor defects in DSB repair. Whether this alteration in (hetero)chromatin network architecture persists beyond 24 h or becomes permanent remains to be investigated. However, it is highly likely that the inability of cancer cells to restore the irradiated (hetero)chromatin architecture to its original state contributes to post-irradiation genomic instability. This instability may, in turn, be linked to processes such as transposon mobilization.

5. Conclusions and future perspectives

The mechanism of chromosomal aberration formation here specifically focused on radiation-induced chromosomal translocations, which involves several critical processes, beginning with the deposition of radiation energy in the chromatin environment and concluding with the repair of double-strand breaks (DSBs), again within the chromatin context. The results presented in this chapter clearly demonstrate that the physical identity of the incident ionizing radiation, chromatin architecture, and the cell's ability to repair DSBs in the chromatin environment play crucial roles in the induction of chromosomal translocations. Moreover, it appears that chromosomal translocation formation does not depend solely on these factors individually but rather on the interaction between them.

While in cells irradiated with densely ionizing radiation, chromosomal translocations arise as a direct consequence of radiation energy deposition and local chromatin fragmentation, in cells exposed to sparsely ionizing radiation, chromosomal translocations are primarily a secondary result of DSB repair processes within heterochromatin. Heterochromatin plays multiple roles in the process of chromosomal translocation (and aberration formation more generally). On one hand, heterochromatin serves as a protective barrier, shielding DNA to some extent from the effects of reactive oxygen species (ROS) and thus from damage caused by sparsely ionizing radiation. On the other hand, the architecture of heterochromatin complicates the repair of heterochromatic DSBs, increasing the risk of chromosomal translocations due to the mobilization of heterochromatic DSBs.

Furthermore, the (hetero)chromatin network responds to irradiation not just locally but as a cohesive system, with regulation of this system potentially disrupted in cancer cells. This disruption is likely associated with post-irradiation genomic instability, which may be linked to phenomena such as transposon mobilization.

Although significant progress has been made in understanding the mechanism of chromosomal translocation formation, largely through the application of advanced microscopic techniques, many fundamental questions remain unresolved. For example, how do cells select the most appropriate repair mechanism at individual DSB sites? The application of super-resolution microscopy techniques, such as the extensively discussed single molecule localization microscopy (SMLM), alongside DNA damage and repair analysis in individual live cells, offers great promise for further elucidating the mechanisms underlying chromosomal translocation (and aberration formation more broadly).

Further research on the relationship between the physical parameters of ionizing radiation, chromatin architecture, DNA damage, DNA repair, and the formation of chromosomal aberrations is particularly important with respect to the development of hadron therapy for cancer treatment and astronaut protection during planned manned space flights to Mars.

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Chapter 4

Nanomaterial-Induced Genotoxicity: Insights into Chromosomal Aberrations and Cellular Integrity

Jyotishman Tamuli, Sarbani Giri and Rahul Saikia

Abstract

Nanomaterials have various uses in modern life due to their adjustable physiochemical properties and controlling shape and size. Recently, the biomedical and pharmaceutical industry has started extensively using nanomaterials in different fields, including enzyme immobilization, drug delivery, biosensing, and imaging. Despite having a wide range of applications, nanomaterials have significant hazardous effects on the environment and health. It causes toxicity due to unwanted interactions with biological systems and has the potential effect of DNA damage, which may lead to cancer. Nanomaterials can affect the cell by causing intranuclear protein aggregation, which interferes with the cell proliferation and division process. Some nanomaterials possess genetic toxicity by DNA strand break, producing reactive oxygen species and targeting the histone protein. Exposure to nanomaterials induces carcinogenicity in different species by increasing micronuclei content and causing inflammation in various manners. Understanding the mechanisms underlying nanoparticle-induced genotoxicity is crucial for developing safe nanomaterials. Toxicity assessment of nanomaterials is crucial to ensuring human and environmental safety. This chapter explores how nanomaterials can induce DNA damage, including oxidative stress, inflammation, and mitochondrial dysfunction. The consequences of such damage, such as disruption of cellular integrity, genotoxicity, and chromosomal aberrations, are also explained.

Keywords: nanomaterials, oxidative stress, DNA damage, genotoxicity, chromosomal aberrations

1. Introduction

Nanomaterial (NM) is an umbrella term for a group of substances whose size ranges between 1 and 100 nm in one dimension. Nanomaterials are microscopic-sized particles with exceptional properties, including significantly higher surface/volume ratio, immense absorption capacity, and site-specific delivery [1]. Due to their adjustable physiochemical properties and controlling shape and size, nanomaterials possess unique and beneficial properties (**Table 1**). Nanomaterials have

Sl no.	Types	Properties	Uses	Toxicity	References
1	Gold nanoparticle	Surface plasmon resonance, high light-reflecting ability, large surface volume ratio, excellent biocompatibility	Drug delivery, photothermal therapy, cancer therapy, biosensor, imaging agent	Genotoxicity, cytotoxicity, immunotoxicity	[2]
2	Silver nanoparticle	Efficient light-absorbing and scattering, large surface area for coordination	Medical devices, antibiotic agents in wound dressing, and textile industries. Antibacterial, antiviral, antimicrobial	Genotoxicity, cytotoxicity	[2–4]
3	Carbon nanotubes	High sensitivity, selectivity, high thermal stability, and electric conductance	Gas sensors, small molecular sensors, electrochemical sensing	Developmental toxicity, hepatotoxicity, pulmonary toxicity	[1, 5]
4	Titanium oxide	Chemical stability and chemical innerness, high photoactivity, quantum efficacy	Water and wastewater purification, modulate the toxicity of synthetic dyes and medicines.	Genotoxicity, cytotoxicity, skin and eye irritation	[2, 5]
5	Graphene oxide	Superior electrochemical activity, high carrier mobility	Biosensor, optical and optoelectronic uses, drug delivery	Neurotoxicity, genotoxicity, inflammation, oxidative stress	[6]
6	Zinc oxide	UV-filtering, high catalytic activity, photochemical properties	Bioimaging, antibacterial, antifungal, sunscreen, drug delivery	Hepatotoxicity, increased oxidative stress, reduced cell viability	[7, 8]
7	Copper oxide	Longer shelf life, chemically inert, thermal stability	Packaging, environmental remediation, catalyst in industrial process	Genotoxicity, cytotoxicity, skin allergy and irritation	[9, 10]
8	Platinum nanoparticle	Rich electronic structure, surface plasmon resonance, high catalytic activity	Environmental catalysis, energy-related catalysis, glucose sensors, fuel cell	Hepatotoxicity and nephrotoxicity	[11]
9	Silica nanoparticle	High thermal stability, biocompatibility, porosity	Biosensor, drug delivery, and improving the hydration potential and strength of cement	Genotoxicity, immunotoxicity, reproductive toxicity	[2, 12]
10	Quantum dots	Discrete energy levels, great light stability broad absorption spectra, restricted emission spectra	Energy storage, bioimaging, gene and drug delivery, sensors, catalysis	Cytotoxicity	[13]

Table 1.
Types of nanomaterials, their properties, uses, and toxicity.

nanostructure-dependently different properties from their bulk counterparts, and they may not be predicted by the components [14]. In recent times, the production and development of nanomaterials in different industrial, groundwater remediation, biomedical, pharmaceutical, and agricultural fields have been accelerated. The biomedical and pharmaceutical industries have started extensively using nanomaterials in various fields, including enzyme immobilization, drug delivery, wound dressing, biosensing, and imaging [9, 15]. Attributable to the evolution of nanotechnology, the substantial demand for engineered nanoparticles (NP) and their non-restricted discharge into the environment has contributed to the significant impact on both the environment and human health [16, 17]. Nanomaterials may react with different biomacromolecules in the environment or biological system, which may change the transport, fate, and toxicity of the nanomaterials. Another kind of transformation in nanomaterials may also occur due to redox reactions, aggregation, and dissolution [18].

On the basis of the origin of nanomaterials, they can be categorized into three types: natural, incidental, and engineered nanomaterials. Natural nanomaterials are formed through mechanical or biogeochemical reactions without human interference. Incidental nanomaterials are unintentionally produced by industrial processes or human activities. Engineered nanomaterials are designed and manufactured with specified physical and chemical characteristics. They are made in industries and laboratories with desired features for different uses [19].

In general, based on composition, nanomaterials are classified as metal-based, carbon-based, dendrimers, and composites. Metal-based NMs are composed of metals or metal oxides. They have specific properties like surface plasmon resonance, high electromagnetic reactivity, and high light-reflecting and absorbing ability. Metal-based nanomaterials are used in different biomedical applications, including drug delivery, biosensing, cancer radiotherapy, and chemotherapy enhancement [2]. Carbon-based NMs consist of carbon and possess unique optical features, immense thermal stability, electrical conductivity, and exceptional mechanical properties. Carbon-based nanomaterials have been used in multidisciplinary fields, including energy storage, wastewater treatment, biomedical devices, and sensors [20]. Dendrimers are branched polymeric molecules with a central core. They have significant compatibility with the biological system and are used for cancer diagnosis, gene therapy, and targeted drug delivery. Composites are constituted by two or more nanoparticles with special characteristics. The components of composite nanomaterials have common contact interfaces.

2. Route of exposure and toxicity of nanomaterials

The increasing manufacture and utilization of nanomaterials (**Table 1**) in various fields of the present-day world are accelerating their exposure to the environment and humans. The NMs can pollute water and soil, infiltrate biological systems *via* a variety of routes, and result in hazardous reactions [21]. There are numerous ways by which humans come into contact with nanomaterials, including through food and water consumption, direct skin contact from cosmetics, and inhaling airborne nanomaterials, as shown in **Figure 1**. Shape, size, surface charge, and coating all affect a nanomaterial's stability and durability, which in turn determine its potential for toxicity. Furthermore, the toxicity of nanomaterials varies according to the body's absorption pathways, which include the skin, eye, digestive tract, or nasal cavity. The physical and chemical characteristics also influence the absorption mechanism.

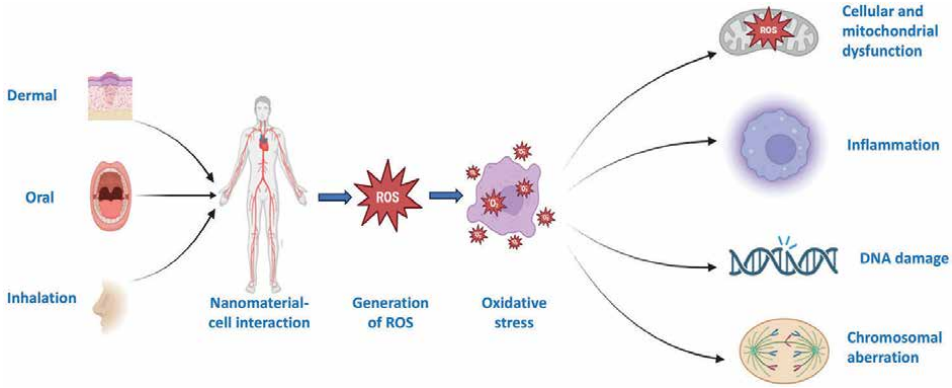

Figure 1.
Schematic illustration of the route of exposure and toxicity of nanomaterials.

The nanomaterials may be selectively absorbed and distributed throughout the body due to their surface properties [21].

Many factors contribute to nanomaterial toxicity, including:

- Their incredibly small size and significantly higher surface/volume ratio.
- Their non-specific binding with other molecules.
- Their accumulation in essential organs inside the body.
- Their natural resistance to the body's excretory system.
- Their capability to emit harmful free radicals continuously.

Since living organisms were rarely in contact with nanomaterials during their evolutionary history, they have not developed any defense mechanism against nanomaterial toxicity. Predicting the transport, reactivity, toxicity, and destiny of nanomaterials in the environment is made more difficult by the absence of a natural analog [16, 22].

2.1 Mechanism of nanotoxicity

One of the most important variables affecting the complex process of transporting NMs into cells and the nucleus is their size. Nanomaterials that are smaller than 80 nm can only enter nonphagocytic mammalian cells *via* the commonly employed uptake pathways of clathrin- or caveolin-mediated endocytosis. Passive diffusion through the nuclear pore complex is limited to molecules with a diameter of less than 10 nm, so only the extremely small nanomaterials can passively diffuse into the nucleus and react with the nuclear DNA. However, a signal-dependent mechanism enables the nuclear pore complex to transport bigger nanomaterials up to 39 nm inside the nucleus. Furthermore, nanomaterials can access DNA through the breakdown of the nuclear membrane during the cell cycle's mitosis phase [23]. Once inside the cell, the NM can cause toxicity through a variety of mechanisms. Nanomaterials can generate free radicals through several mechanisms, including surface-bound radicals, electron transfer, phagocytic cell response to foreign material, metal ion release,

and Fenton reaction. The production of excessive free radicals could overwhelm the body's antioxidant capacity, leading to oxidative stress—the primary molecular mechanism of nanotoxicity. Excessive levels of free radicals damage different cellular components *via* oxidation of DNA, proteins, and fats. Oxidative stress can contribute to the development or exacerbation of inflammation by upregulating activator protein-1, redox-sensitive transcription factors (such as NF-κB), and kinases implicated in inflammation as shown in **Figure 2** [24]. The liver and spleen are major targets of oxidative stress because of the sluggish clearance and tissue buildup of potentially free radicle-producing nanomaterials and the abundance of phagocytic cells in the reticuloendothelial system organs. Furthermore, high-blood-flow organs like the kidney and lungs that are exposed to nanomaterials may also be impacted [25].

Nanomaterials can affect or interfere with cellular activity, interact with biological components, or generate reactive oxygen species. Interactions between NMs and the cell nucleus and mitochondria are considered the primary causes of toxicity (**Figure 1**). Fullerenes, block copolymer micelles, carbon nanotubes, and silver-coated gold NPs are some nanomaterials that may localize to mitochondria and cause apoptosis and reactive oxygen species (ROS) formation. Nanomaterial-induced cell cycle arrest, mutagenesis, DNA damage, and apoptosis can also serve as a potential source of toxicity. Some studies claim that nanomaterials may contribute to the overexpression of xanthine oxidase and NADPH oxidase, which are sources of free radicals in neutrophils and macrophages [24]. Since nanomaterials interact with their surroundings instantly, other toxicity pathways should also be considered. Thrombosis and hemolysis can occur from interactions of nanomaterials with blood components when they get absorbed into the systemic circulation. According to Aillon et al., interactions between nanomaterials and the immune system accelerate immunotoxicity. Moreover,

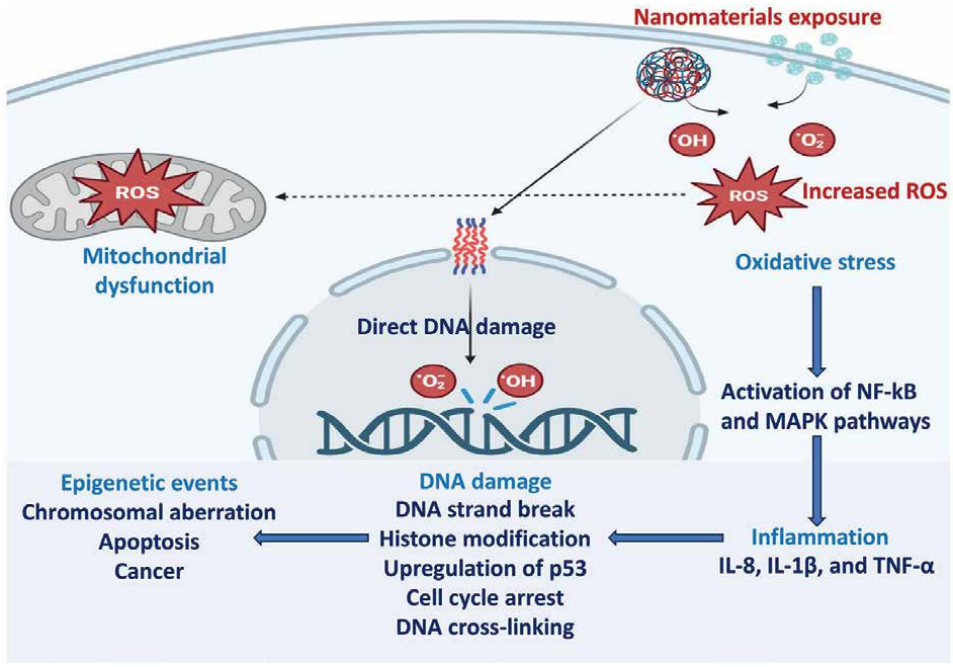

Figure 2.
Schematic representation of the mechanism of NM-induced genotoxicity.

in the liver, further metabolic transformation of nanomaterials can be eventuated by cytochrome P450, which may result in hepatotoxicity [25].

The toxicity of nanomaterials increases in a dose-dependent manner. The existence of diverse functional groups might alter the micronutrients, proteins, and biomolecules inside the body. When nanomaterials interact with proteins, they may disrupt protein unfolding, fibrillation, and thiol cross-linking, and reduce enzymatic activity, all of which could result in adverse health consequences [26]. Because of impurity residues and poor finishing, the synthesis techniques are additionally anticipated to impact the nanomaterial's toxicity [1].

2.2 Nanomaterials induced genotoxicity and chromosomal aberrations

There are several mechanisms by which nanomaterials cause genotoxicity

- Primary direct mechanism: direct interaction of nanomaterials with DNA
- Primary indirect mechanism: nanomaterials interact with molecules that are involved in cell cycle, cell division, and repair mechanism
- Secondary mechanism: nanomaterial-dependent inflammation produces ROS, which causes DNA damage [26].

If the NMs settle inside the nucleus, direct contact between them and the DNA molecule or proteins linked to DNA could cause physical harm to the genetic material. Studies have demonstrated that silica and titanium dioxide nanomaterials can penetrate the nucleus and form intranuclear protein aggregates, which leads to the inhibition of cell cycle replication, transcription, and cell proliferation. Quantum dots can enter the nucleus *via* the nuclear pore complex, interact with histone proteins, and damage the DNA (**Table 2**) [25].

Additionally, it has been shown that NMs interact with DNA repair genes and change their activity, leading to defective DNA repair mechanisms. Errors in DNA repair were caused by the altered expression of genes associated with DNA damage/repair, including Gadd45, RPA1, XRCC1 and 3, RAD51C, and FEN1, in human lung and brain cell lines that were exposed to silver nanoparticles [28].

The majority of genotoxicity caused by NMs arises from secondary mechanisms, such as injury to mitochondria and lysosomes, which cause the generation of reactive free radicals and inflammation-induced oxidative stress. All these together eventually result in DNA damage. These secondary mechanisms need to reach certain threshold dosages to cause the initial damage, such as inflammation. Nevertheless, in certain instances, evidence of primary genotoxicity induced by specific nanomaterials, including remarkably inflammation-independent DNA damage, was found [23].

Srivastav et al. [8] demonstrated the genotoxic potential of zinc oxide NP in mice test systems by assessing different forms of chromosomal aberrations due to the generation of ROS and associated cytotoxicity [8]. A recent study by Durairaj et al. [15] reported that silver nanorods-treated *A. cepa* root tips showed concentration-dependent genotoxic effects and chromosomal aberrations [15]. Catalán et al. [5] pointed out that the treatment of carbon nanotubes and titanium oxide-induced chromosomal aberrations in cultures of isolated human lymphocytes [5]. Along with these experiments, some other important nanomaterial-induced genotoxic effects and chromosomal aberrations have been given in **Table 2**.

Sl no.	Types of nanomaterial	Test/assay	Model	Dose and time	Finding	References
1	Aluminum oxide	MN assay CA assay	Rat	500–2000 mg/kg bw; 18–48 h	MN induction increases at 1000 and 2000 mg/kg bw treated groups as compared to control. 24 hours of oral treatment increase the abnormal metaphases and total chromosomal aberrations in bone marrow cells. Different structural changes, such as chromatid and isochromatid breaks, acentric fragments, and translocation, have been observed in the treated groups.	[14]
2	Carbon nanotubes	CA assay	Human lymphocytes cell line	6.25–300 μg/ml; 24–72 h	Short single-walled carbon nanotube treatment of 300 μg/ml for 48 h produced a significant rise in chromatid and chromosome-type abnormalities with or without gaps. Short multiwall carbon nanotube exposure for 48 h at a conc. of 50–300 μg/ml induced chromosome type and total chromosomal aberrations in a dose-dependent manner.	[5]
3	Titanium dioxide	CA assay	Human lymphocytes cell line	6.25–300 μg/ml; 24–72 h	300 μg/ml treatment of titanium dioxide for 48 h exhibited a substantial upregulation of cells with chromatid-type chromosomal aberrations with or without gaps as compared to the control group.	[5]
4	Graphene oxide	MN assay Comet assay	Mice	10–40 mg/kg bw; 1–5 d	10, 20, and 40 mg/kg bw of graphene oxide nanoparticle administration increased the MNPCE frequency throughout all treated groups in contrast to the control. In treated groups, the PCE/NCE ratio decreased in a dose-dependent manner. The comet assay demonstrated significant increases in the DNA damage parameters like tail length, tail movement, and % DNA in the tail in all the treated experimental groups.	[6]

Sl no.	Types of nanomaterial	Test/assay	Model	Dose and time	Finding	References
5	Zinc oxide	MN assay CA assay	Mice	300–2000 mg/kg bw; 3 d	At 2000 mg/kg bw of zinc oxide treatment, the mean ratio of PCE/NCE was decreased. Zinc oxide treatment increased the total aberrant cell percentage and CA frequency in treated groups as compared to control. Different types of abnormalities including chromosomal rings, chromosomal breakage, and pulverized chromosomes, were induced in the treated groups.	[8]
6	Silver nanorods	Microscopic study Biochemical assay	*Allium cepa*	5–15 μM; 4 h	The mitotic index of silver nanorods-treated *A. cepa* root tips decreased significantly as compared to the control. 10 and 15 μM-treated root tips produced different chromosomal aberrations, including chromosome break and bridge formation. Disturbed metaphase and chromosome loss have been found in the treated groups.	[15]
7	Copper oxide	CA assay	*Allium cepa*	20–2000 μg/ml; 12 h	Treated groups showed different chromosomal aberrations, including chromosomal bridges, metaphase and anaphase stickiness, and broken and lag chromosomes. The frequency of chromosomal aberrations caused by copper oxide is not dose-dependent. The elevated percentage of MI indicates uncontrolled cell proliferation in the treated root tips.	[9]
8	Dental nanocomposite KelFil	Comet assay, MN assay, CA assay	Human lung fibroblast cell line (MRC-5)	0.08–8.00 mg/ml; 6–48 h	0.08, 0.79 and 8.00 mg/ml KelFil treatment on the MRC-5 cell line for 24 h resulted in no adverse effects on the cell line. No comet formation was observed during the test. The CA assay was done after 48 hours of exposure, and no significant cytotoxicity was found.	[27]

Sl no.	Types of nanomaterial	Test/assay	Model	Dose and time	Finding	References
9	Silica	Comet assay RT-PCR	Rats	5–50 mg/ml; 4–24 h	50 mg/kg bw of silica NP exposure for 4 h caused an alarming increase in OGG1-sensitive DNA damage in the liver cell. RT-PCR data demonstrated the activation of inflammatory and immune responses. IL-6 and Th17-derived cytokines increase at 1 h and again at 4–8 h after silica NP exposure.	[12]
10	Silver	Comet assay, MN assay	Tadpoles	10–80 mg/L; 24–96 h	Exposure to silver NP increases the frequency of MN in a dose-dependent manner. At 29°C, the frequency of MN is higher than 20°C. The comet assay demonstrated the DNA damage in the NP-treated groups. Tail extent moment and olive tail moment, decreased head DNA, and increased tail DNA in the treatment group relative to the control group is indicative of DNA damage.	[4]

Table 2.
List of different nanomaterials that induce genotoxicity and chromosomal aberrations.

2.3 Fundamental routes to genotoxicity induction

2.3.1 Oxidative stress

Certain transition metal ions are released by titanium, zinc, iron, and cadmium nanoparticles. These ions have the ability to transform cellular oxygen metabolic products (H_2O_2 and O_2–) into hydroxyl radicals ($^{\bullet}OH$), which can damage DNA. Additionally, Fe (II) can convert molecular O_2 into H_2O_2, which can then diffuse through the nuclear and cellular membranes and react with Fe that is associated with DNA, resulting in the generation of hydroxyl radicals ($^{\bullet}OH$). These reactive hydroxyl radicles can cause cross-links of thymine-tyrosine in the chromatin. Likewise, free Fe ions can result in hydroxyl radical-generated purine and pyrimidine alterations. Therefore, iron-containing nanomaterials could potentially cause an excess of iron to accumulate in the cells, which would subsequently feed the Fenton reaction, which generates extremely reactive hydroxyl radicals. Moreover, oxidative stress triggers particular signaling pathways, such as NF-κB and MAPK, which, coupled with the deterioration of antioxidant defenses, release pro-inflammatory cytokines. The ramification of this signaling cascade is the induction of inflammation (**Figure 2**). This defensive response prompts

additional ROS release from inflammatory cells (such as neutrophils), creating a vicious cycle of events that is also essential to the pathogenic effects of particle exposure [25].

2.3.2 Inflammation

Studies showed that, when A549 cell lines were treated with single-walled carbon nanotubes, the expression level of interleukin-8 (IL-8) was significantly increased. After Sprague-Dawley rats were treated with silver NMs, vascular endothelial cells in the brain expressed more cytokines, including prostaglandin E2, TNF-α, and IL-1β. Additionally, nanoparticles cause the blood-brain barrier to break down, resulting in neurodegeneration, neuronal abnormalities, and a neuroinflammatory reaction [21]. All these together can lead to DNA damage exhibited as point mutation, chromosomal disintegration, and DNA adduct formation, even though they are crucial defenses against infection and/or tissue damage. They can also prevent DNA repair and cause aberrant methylation patterns that change the normal patterns of gene expression. As a result, carcinogenesis has become closely linked to chronic inflammation [25].

2.3.3 DNA damage-responsive signaling

A recent study demonstrated that cadmium-telluride quantum dots significantly elevated p53 activity, leading to an upregulation of the downstream effector's Puma, Noxa, and Bax [29, 30]. Titanium oxide-induced DNA damage causes the accumulation of p53 in lymphocytes. Furthermore, it increased the phosphorylation of DNA damage checkpoint kinases (Chk1 and Chk2) and triggered a cellular reaction to DNA damage, including cell cycle arrest, DNA repair, or cell death. It has been reported that gold nanoparticles downregulate several DNA repair genes, including BRCA1, HUS1, AT-V1/V2, and ATLD/HNGS1, suggesting that nanoparticles may interact directly or indirectly with regulators of genome integrity. This in turn leads to more genomic instability [31]. The discharge of metal ions from transition metal nanoparticles may disturb the DNA damage-associated repair pathways. For instance, Co^{2+} can compete with Mg^{2+}, which might hinder repair enzymes' capacity to bind with damaged DNA, leading to ineffective genetic aberrations correction. Later on, this may result in chromosomal aberrations (**Figure 3**) [25].

2.4 Different tests to measure genotoxicity and chromosomal aberrations

2.4.1 Comet assay

The most commonly used genotoxicity test for NMs is the comet assay. Agarose gel electrophoresis is essentially used to separate intact DNA from the fragmented DNA. Under the electric charge, large intact DNA stays at the origin, whereas small fragmented DNA moves toward the anode to create a comet-like tail. Fluorescent dyes, such as ethidium bromide or propidium iodide, are typically used for visualization; however, nonfluorescent staining with silver nitrate has improved sensitivity and repeatability. The length and intensity of the tail can determine the degree of DNA damage caused by the toxicant. There is a linear relationship between DNA fragmentation and the relative tail intensity [23].

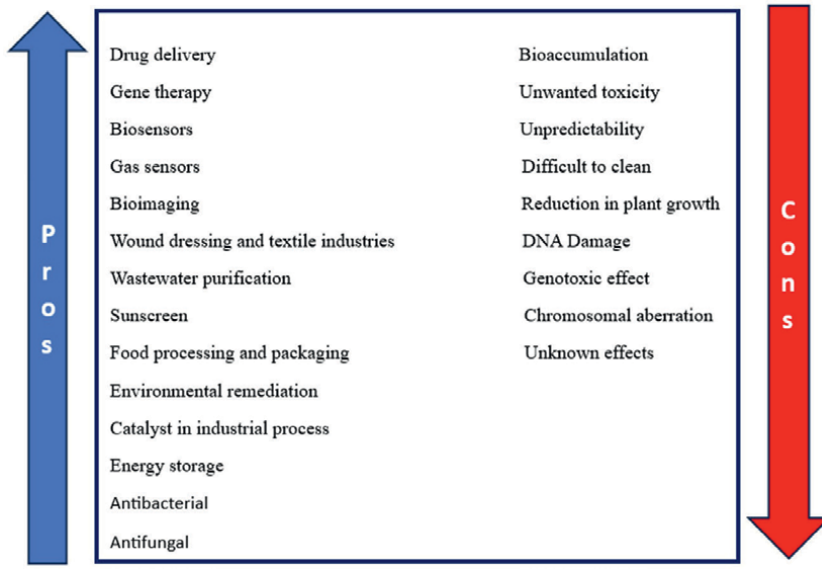

Figure 3.
Pros and cons of nanomaterials.

Advantages:

- Comet assay is highly sensitive to DNA damage.
- For comet assay, a minimum number of cells is required.
- It can be carried out in different eukaryotic cell types.

Limitations:

- Comet assay has low specificity and limited relevance to mutagenicity.
- Incomplete chromatin de-condensation in sperm makes it challenging to detect breaks in DNA strands [32].
- Variations in the results between different laboratories [6].

2.4.2 Micronucleus (MN) assay

Blood or bone marrow is extracted after a sufficient time to detect the treatment-induced induction of micro-nucleated immature erythrocytes. Following that, the cells are stained and evaluated for the detection of micronuclei using a light microscope, fluorescence microscope, or flow cytometry. Micronuclei are small nuclei-like structures generated when chromosomes or chromosome fragments cannot be incorporated into a primary nucleus during mitosis or meiosis. MN can occur when chromosomes fail to appropriately adhere to the spindle during the anaphase [26].

Advantages:

- For MN assay, regardless of its karyotype, any dividing cell population can be used.
- As micro-nucleated cells are stable, responses for a longer period can be made.
- It is comparatively easier, faster, **and** less subjective, and it ensures accuracy.

Limitations:

- MN assay demands meticulous cell preparation, spreading, fixation, staining, and scoring.
- Nanoparticles with fluorescence and adsorption features may alter the result.
- In certain cases, a pseudo-micronucleus can be identified [27].

2.4.3 Chromosomal aberration (CA) assay

This test identifies significant structural and numerical changes brought on by the test agent. It is frequently used to screen for suspected genotoxic carcinogens despite considering that the test involves a labor-intensive procedure. The *in vitro* CA assay involves treating cultured mammalian cells with the experimental sample and then exposing them to a reagent that stops the cell cycle at metaphase. Following that, the metaphase chromosome preparations are placed on slides. The cells are then examined under a microscope to determine the existence of chromosomal abnormalities based on their unique structures and numbers after being stained with Giemsa. In the *in vivo* form of the experiment, the test substance is administered to the test animal first, followed by the metaphase arresting reagent. Collected bone marrow cells are used for metaphase chromosomal preparations, which are subsequently stained and scored [25].

Advantages:

- In CA assay interactive scoring possible, semi-automated system.
- CA is Suitable to study nanomaterial-induced chromosomal aberrations.
- It is robust and cost-effective; potential mitotic index co-detection is possible.

Limitations:

- Low sensitivity in comparison to alternative techniques for low-dose exposure.
- Sometimes the abnormalities that arise from toxicity are not relevant to human risk.
- CA assay needs experience and expertise [27].

2.4.4 Ames test

The Ames test is a bioassay that employs bacteria to identify a chemical's potential to cause mutagenesis. It is also known as the bacterial reverse mutation test. It involves many strains of the *Salmonella typhimurium* bacteria, each with unique mutations in distinct genes that prevent them from synthesizing the amino acid histidine. Consequently, they need additional histidine as a growth supplement. In the presence of a test sample, microbial cultures are maintained on histidine-deficient agar plates. Only those bacteria that have undergone reverse mutations can restore the function of the histidine synthesis gene and will be able to survive. The number of colonies formed is proportional to the frequency of mutations caused by the dose of the test agents [25].

Advantages:

- It is simple and relatively inexpensive.
- Ames test is highly sensitive to genotoxic chemicals.
- It is a quick and convenient assay.

Limitations:

- *Salmonella typhimurium* is a prokaryote, so it is not a perfect model for human.
- Ames test is unable to sufficiently evaluate substances that are harmful to bacteria.
- In the Ames test, it is not possible to identify substances that are non-DNA reactive [6].

2.4.5 Cytokinesis-block micronucleus (CBMN) assay

The administration of cytochalasin B (actin polymerization inhibitor) inhibits the cell cycle during cytokinesis and produces binucleated cells. This process makes it simple to identify cells that have undergone cell division in the presence of a test chemical. The damaged genetic material lags behind during chromosome segregation and is not included in either of the daughter nuclei that arise when the test chemical causes chromosomal fragmentation or loss. Rather, they are contained within a micronucleus, and their abundance in binucleated cells indicates the genotoxicity brought on by the quantity of the test chemical. The CBMN assay is connected to kinetochore staining to investigate whether the micronuclei generated are the consequence of a clastogenic (chromosome fragmentation) or aneugenic (whole chromosome loss) mechanism of action [25].

Advantages:

- It is a rapid and sensitive test for quantification and classification of chromosomal damage.
- Micronuclei can be easily scored with an automated microscope-aided system.
- CBMN assay is well suited for biomonitoring of large populations.

Limitations:

- Typically examined by manual microscopy, so there are high chances of variability.
- For blood sample analysis, fresh blood samples must be processed within 48 hours.
- In CBMN assay the micronuclei manual detection is subjective and time-consuming.

3. Risk assessment and future perspective

Although researchers, companies, and governments are fully focused on nanomaterials, their recycling and waste management strategies have unfortunately received less attention. Research and publications on the fate of nanomaterials following the disposal phase of nanoproducts are scarce. Since nanomaterials have already shown hazards to the environment and human health, their toxicity can be reduced with appropriate waste management and recycling. A product made with nanomaterials could end up landfilled, burned, or recycled after its intended lifespan is completed. They can move through the soil, water, and air. If nanomaterials are not properly handled, sterilized, or recycled, they might transform into new contaminants [1].

Nanomaterial development and usefulness hold immense promise, but several key concerns must be addressed. Toxicity assessment of nanomaterials is crucial for ensuring human and environmental safety. Standardized manufacturing processes are needed for consistent and reliable nanoparticle production. Clear regulatory frameworks must be established to govern the use and disposal of nanomaterials. Public awareness and engagement are essential to foster informed decision-making. Finally, ethical considerations surrounding potential societal impacts require careful attention.

4. Conclusion

Nanomaterial interactions with cellular components result in oxidative stress, inflammation, and cell cycle arrest, impacting DNA integrity and stability. These may lead to genotoxicity, chromosomal aberrations, and cancer development. NM toxicity is a growing area of concern in nanomedicine and toxicology. Understanding the mechanisms underlying nanomaterial-induced toxicity is crucial for developing safe nanomaterials. In this book chapter, the mechanism of nanomaterial-induced DNA damage—such as oxidative stress, inflammation, and mitochondrial dysfunction—has been critically analyzed. Along with that, the impact of such damages has also been described, including genotoxicity, chromosomal abnormalities, and disruption of cellular integrity. This knowledge is essential for developing strategies to mitigate the potential risks associated with NM exposure. Further research is needed to fully elucidate the complex interplay between nanomaterials and the genome.

Over the last few years, different scientific data on NM toxicity have been sporadically publicized, but the genotoxicity part is inconclusive. Studies on nanomaterial-induced genotoxicity and chromosomal aberrations are inadequate; however, their effect on health research is undeniable.

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Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

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Appendixes and nomenclature

NM	nanomaterial
NP	nanoparticle
ROS	reactive oxygen species
IL	interleukin
MN	micronucleus
CBMN	cytokinesis-block micronucleus
CA	chromosomal aberrations
MI	mitotic index
PCE	polychromatic erythrocytes
NCE	normochromatic erythrocytes
MNPCE	micro-nucleated polychromatic erythrocytes
MNNCE	micro-nucleated normochromatic erythrocytes

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Chapter 5

Genome Instability in Chemotherapy-Induced Senescence

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Abstract

Cellular senescence, initially described as an irreversible growth arrest, has emerged as a complex and dynamic phenotype with significant implications in cancer biology and therapy. Chemotherapy-induced senescence represents a critical response to genotoxic stress, wherein cancer cells enter a state of permanent cell cycle arrest characterized by metabolic activity, morphological changes, and the secretion of pro-inflammatory factors known as the senescence-associated secretory phenotype (SASP). While chemotherapy-induced senescence serves as a barrier to tumor progression, it also contributes to genomic instability, polyploidy, and the creation of a pro-tumorigenic microenvironment, complicating its role in cancer treatment. This chapter explores the mechanisms by which chemotherapeutic agents, including doxorubicin, cisplatin, etoposide, busulfan, temozolomide, and others, induce senescence through DNA damage response (DDR) pathways involving p53, p21, and p16INK4a. These agents trigger genomic instability, leading to chromosomal aberrations and metabolic reprogramming, which can paradoxically promote tumorigenesis. The SASP, a hallmark of senescent cells, further influences the tumor microenvironment by modulating inflammation, tissue repair, and immune responses. Despite its anti-tumor effects, the persistence of senescent cells poses challenges, including therapy resistance and age-related diseases. Emerging strategies to target senescent cells, such as senolytic therapies and SASP modulation, offer promising avenues to enhance chemotherapy efficacy and mitigate adverse effects. This chapter highlights the dual role of chemotherapy-induced senescence in cancer therapy, emphasizing the need for a deeper understanding of its molecular mechanisms and the development of targeted interventions to harness its benefits while minimizing its detrimental consequences.

Keywords: senescence, chemotherapy-induced senescence, genomic instability, senescence-associated β-galactosidase, senescence-associated secretory phenotype

1. Introduction

Since the discovery of replicative senescence by Hayflick and Morehead in the 1960s, the concept of cellular senescence has undergone significant change. The widely held belief that cells grown in vitro can divide endlessly was decisively challenged by Hayflick. He proved by means of an array of rigorous investigations that human fibroblasts do not have an eternal state; rather, they undergo a senescent period that prohibits them from dividing further. According to Hayflick, senescent cells are

destined for a permanent growth arrest, making senescence an "eternal" fate. For several decades, our knowledge of senescence was based on this assumption. For a long time, "irreversibility" was thought to be a crucial feature that set senescence apart from other types of growth arrest, such as quiescence, which is a temporary form of growth arrest. But in more recent years, senescence's hallmarks have been discovered that, when taken together, describe a more complicated and distinct phenotype that goes beyond merely being another growth halt variation. The early perceptions of senescence are further complicated by the vast genetic, epigenetic, metabolic, and structural changes that characterize this phenotype. However, the growth arrest's steady nature has long been a fixed feature of the senescence concept [1, 2].

Steady phases of cell development arrest, or cellular senescence, occur when cells maintain their metabolic activity but do not react to external growth cues. Senescent cells (SenCells) frequently have a deformed and expanded appearance, a changed metabolism, and mitochondria that are not working properly. The initial marker used to identify them was the lysosomal enzyme senescence-associated β-galactosidase (SA-β-gal), which dyes positively toward a hydrogen potential of six. Perhaps the greatest prevalent molecular characteristic of SenCells is the overexpression of the tumor suppressor p16INK4a, which is also a commonly utilized biomarker both in culture and in vivo. Furthermore, the senescent phenotype is frequently characterized by downregulation of the nuclear lamina protein lamin B1 and stimulation of the p53/p21WAF1/Cip1 pathway. Immunostaining techniques can identify DNA damage foci called DNA-SCARS (DNA segments with chromatin alterations reinforcing senescence) and Senescence-Associated Heterochromatin Foci (SAHF) that are formed by senescence triggers that harm DNA. The Senescence-Associated Secretory Phenotype (SASP), which is another characteristic of senescent cells, is their capacity to release a wide range of cytokines, growth factors, and proteases, which can influence nearby cells. Since neither of those indicators is believed at present to be universal, senescence detection must be accomplished by combining multiple tests [3–5].

It is being demonstrated that the mechanisms of tissue transformation and repair throughout gestation and adolescence depend on the temporary buildup of SenCells. SenCells, on the other hand, proliferate and endure throughout organismal aging in a variety of organisms, contributing to the emergence of numerous age-related disorders. According to this theory, getting rid of them enhances lifespan and health by reversing a lot of medical disorders. They can accumulate due to extrinsic sources like anticancer compounds or ionizing and non-ionizing radiation or intrinsic factors like telomere shortening (also called replicative senescence) or oncogenic mutation (oncogene-induced senescence, or OIS). Cellular senescence brought on by receiving radiation or chemotherapeutic medications is known as therapy-induced senescence (TIS) in oncologic contexts and exhibits both pro- and anti-tumorigenic characteristics. These treatments try to cause senescence and cell death in the cancer cells they treat, but they also affect dividing stromal cells, which results in TIS stromal cells that can influence their surroundings through the SASP [6, 7].

Both cancerous cells and healthy, non-neoplastic cells that live in the microenvironment surrounding the tumor might experience TIS, a condition of cellular senescence brought on by anticancer therapies. TIS was initially noticed in 1999 when Chang and associates reported that several chemotherapeutic medications and ionizing radiation might cause phenotypic and biochemical alterations in human carcinoma cell lines that resembled the replicative senescence seen in healthy cells. Single-cell study has shown that high levels of p21 expression at early time points result in an ultimate fate of cellular senescence after chemotherapy, even though

p53 is not necessary for TIS, which can also be generated in p53-null and p53-mutant cancer cells. Interestingly, preliminary research revealed that the proportional rise in the number of cells optimistic for the senescence-associated beta-galactosidase (SA-beta-gal), a commonly used senescence indicator, varied depending on the chemotherapeutic agent and its mode of conduct. Further confirmation of these findings was obtained using a broader panel of senescence markers. TIS is currently regarded as a critical detrimental effect of anticancer therapies since, most significantly, the induction of tumor cell senescence following chemotherapy or radiation was seen in patient tumors in vivo [8, 9].

Unlike typical cells that experience replicative senescence, TIS cancer cells do not grow; however, they remain active in their metabolism and go through metabolic reconfiguration associated with senescence. Their biological process is set up for growth, which leads to the creation of a particular secretome known as SASP, as well as increased cell shape, which is one of the characteristics of senescence. TIS cancer cells are metabolically active and go through senescence-specific metabolic reprogramming, but they do not multiply like normal cells going through replicative senescence [10]. The cellular expansion has been directly associated with the induction of cyclin-dependent kinases (CDK) inhibitors p21 and p16, which cause cellular hypertrophy and geroconversion—the transition from a temporary cell cycle arrest to a permanent, senescence-like arrest—by arresting the cell cycle without blocking growth-promoting pathways. Accordingly, new research indicates that senescence may be caused by big cells rather than only a result of them. In actuality, proliferating cells' proteomes are remodeled into a senescence-like condition as their size increases. Several organelles in cells change in ways unique to senescence. Although recognized as distinctive characteristics of cell senescence, morphological changes in the nucleus have lately been employed as senescence indicators [11]. The senescence-associated beta-galactosidase, a characteristic unique to senescence, is expressed more when the lysosomal compartment enlarges. In senescent cells, mitochondrial dimensions also seem to rise in relation to cytoplasmic mass; this alteration is followed by significant mitochondrial proteome remodeling and mitochondrial disorders. Nevertheless, the raised burden of the mitochondria compensates for mitochondrial dysfunctions, increasing the overall bioenergetic performance of the mitochondria in SenCells while decreasing the bioenergetic output of mitochondria. The malignant microenvironment's biological and non-cellular constituents are impacted by the cytokines, chemokines, growth factors, and extracellular proteases secreted by TIS malignant cells when they obtain an SASP. While the content of SASP is diverse and differs among different kinds of cells, the senescence stimulant has less of an impact on it. The malignant microenvironment's biological and non-cellular constituents are impacted by the cytokines, chemokines, growth factors, and extracellular proteases secreted by TIS malignant cells when they obtain an SASP. While the content of SASP is diverse and differs among different kinds of cells, the senescence stimulant has less of an impact on it. p53 has the ability to significantly alter the SASP's activity and structure [12, 13].

While TIS and replicative senescence have many characteristics in common, the period required for senescence activation is distinct because TIS is quickly induced by a brief exposure to radiation or anticancer medications, whereas replicative senescence in human cells necessitates multiple population doublings in vitro. It is debatable whether a completely senescent phenotype should evolve in vitro and, thus, what temporal scale is best for experimental investigations. Over the course of many days, cellular senescence progresses progressively. Therefore, a drug-induced cell cycle halt nonetheless can be regarded as senescence since geroconversion needs latency

post-stimulation. These findings imply that in an effort to prevent partial, premature senescence in in vitro investigations and to determine the ideal cell culture parameters for clearly inducing TIS, interventional attempts should be undertaken. As a result, confirmation of main senescence indicators—which confirm the end of the cell cycle and characteristic morphological changes—as well as secondary markers, including the SASP, are necessary for a trustworthy evaluation of cellular senescence in vitro [14–16].

2. Genomic instability in chemotherapy-induced senescence

Damage to the genome can result from a variety of internal and external sources. For instance, endogenous genotoxic agents include endogenous alkylating factors, reactive nitrogen species, reactive oxygen species (ROS), and consequences of typical cellular breakdown, such as lipid peroxidation products. In addition to this, the exogenous agents are genotoxic substances, ionizing radiation, and ultraviolet (UV) radiation. Furthermore, genetic material is harmed by spontaneous processes like hydrolysis. Double-strand breaks (DSBs) and single-strand breaks (SSBs) can also be caused by those many kinds of DNA-damaging triggers [17].

The DDR route is a developmentally conserved mechanism found in cells that detects and fixes harm to DNA. When this mechanism is activated, the cell cycle can be stopped, and defective DNA cannot reach daughter cells. Special components detect single-strand and double-strand breaks at the genetic injury spot. These attract and activate two protein kinases, ATR and ataxia-telangiectasia mutant (ATM), accordingly, after detecting damage to DNA. The histone H2AX is immediately phosphorylated in cis when both main kinases attach to the disrupted citation. It seems that this step is necessary for the DDR to activate. The activity of local ATM and ATR must be raised over a certain threshold in order to include DDR elements that function distant from the genome injury region. ATM phosphorylation activates the checkpoint kinase (CHK)2 once genomic injury reaches above the threshold. Subsequently, by phosphorylating its substrates, CHK2 moves freely across the nucleoplasm and sends DDR signals into the nuclear space. Moreover, CHK1 dissipates after being phosphorylated, primarily by ATR, and fails to remain in the nucleus at the genome-injured site. ATM phosphorylation activates the checkpoint kinase (CHK)2 once genomic injury is above the threshold. Subsequently, by phosphorylating its substrates, CHK2 moves freely across the nucleoplasm and sends DDR signals into the nuclear space. Moreover, CHK1 dissipates after being phosphorylated, primarily by ATR, and fails to remain in the nucleus at the DNA-damaged sites. Toward the final stages, several elevated routes, such as p53 phosphatases and cell-division cycle 25 (CDC25), lead to checkpoint need. Since these phosphatases are essential for growth, CDC25 dysregulation-induced DNA damage starts an expedited cell cycle arrest. In contrast, its stability causes DDR kinases to phosphorylate it, which in turn causes p53 to be upregulated. In order to cause an eternal cell cycle arrest, p53 additionally serves like a transcription element that trans-activates the production of its intended genes, particularly p21. A number of mechanisms that initiate and sustain cell cycle arrest are activated by DDR [18–20].

Furthermore, it is essential for triggering the process of senescence. Growth factors, chemokines, matrix metalloproteases, extracellular matrices, inflammatory cell secretion, and other gene expressions are all universally altered in senescent cells and are referred to as SASP. Among these SASP components are matrix metalloproteinase (MMP)-1, -3, and -10, PAI-1, vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), plasminogen activator, interleukin (IL)-1β, IL-1α,

IL-8, IL-6, CCL-2, GROα, and GROβ. Interestingly, SASP acts as a senescence inducer as well as a result of senescence. SASP aids in both tissue healing and the recruitment of senescence in injured cells. On the other hand, it may result in age-related disorders, such as cancer and tissue malfunction. SASP is a sluggish and prolonged reaction in contrast to the quick internal DDD signals. SASP increases throughout the days, alongside related variables like IL-6 reaching its greatest production quantities from 5 to 10 days subsequent to the induction of DDR. In contrast, elementary DDR kinases like ATM turned active after a few moments as reactions to genome injury, and the subsequent DDR transcription reaction detected other transcription factors like p53 in hours [21–23]. **Figure 1** highlights the interplay between DNA damage, DDR, and senescence, emphasizing its implications in aging and disease.

A number of transcription elements are referred to as SASP controllers. One important regulator of the SASP is the transcription element NF-κB, which is linked to inflammation. The induction of the p65 (NF-κB subunit) and its chromatin fortification are dependent on the manifestation of many SASP factors, such as IL-8 and IL-6. Additionally, a number of studies showed that the interaction between active ATM and NF-kappa-B essential modulator (NEMO) can directly trigger NF-κB signaling amplification. NEMO functions as a regulatory component that inhibits the IκB kinase (IKK) complex and NF-κB signaling. DDR activation happens after the ATM/NEMO complex is exported into the cytoplasm. The resulting complex attaches and consequently triggers IKKα/β in the cytoplasm. Phosphorylation of IκB proteins and the start of NF-κB signaling follow this activation. Another transcription element referred to by the term—an inflammatory phase controller—is CCAAT-enhancer-binding proteins (C/EBPβ). Additionally, C/EBPβ aids in the induction of SASP in conjunction with NF-κB. Furthermore, PARP-1 (poly-ADP-ribose polymerase 1) is a crucial DDR regulator, a sensor of DNA damage, and a key player in NF-kB activation in senescent cells [24–26].

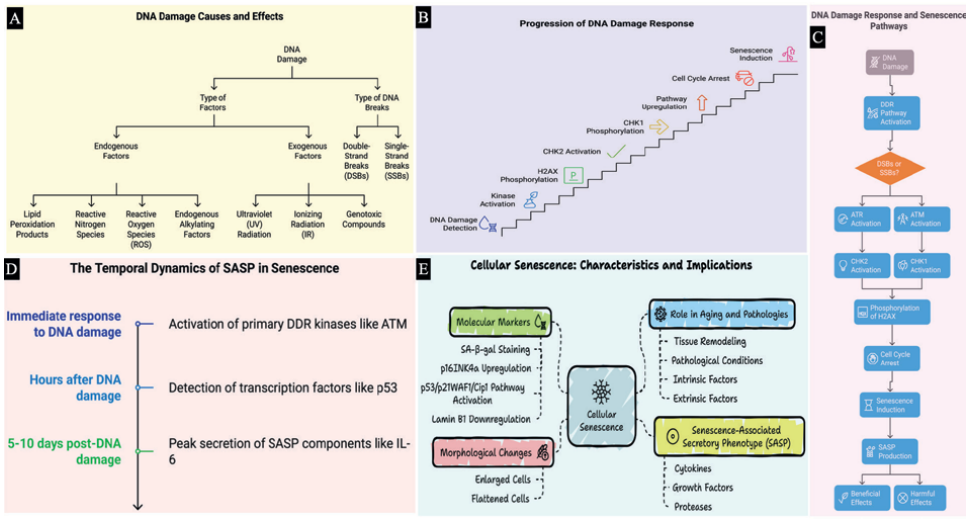

Figure 1.
Causes and effects of DNA damage leading to cellular senescence: (A) Presents DNA damage sources; (B) presents progression of DNA damage response; (C) presents DNA damage response leading to cellular senescence; (D) presents temporal dynamics of DNA damage response; and (E) presents cellular senescence markers and outcomes. The figure highlights the interplay between DNA damage, DDR, and senescence, emphasizing its implications in aging and disease.

2.1 Evidence for genomic instability in chemotherapy-induced senescence

Doxorubicin: Over almost four decades, the anthracycline doxorubicin has been extensively utilized as an anticancer medication. Nonetheless, there is ongoing debate on the doxorubicin mechanism of action. Indeed, there are a number of possibilities that could work, including intercalation into the genome resulting in interference in the synthesis of biological macromolecules, free radical formation that finally leads to genome damage or lipid peroxidation, binding to the genome and alkylation, genome cross-bridging, tampering with the unfolding of the genome or genome strand splitting and helices action, effect on membrane, induction of genome damage through suppression of topoisomerase II action, and initiation of cell death, which is the last cellular outcome to upstream signals [27]. Experiments demonstrated in Ref. [28] that the majority of HCT116 cells (human colon cancer cell line) ceased proliferating following therapy with a modest dosage of doxorubicin, as evidenced by SA-b-galactosidase production and the absence of Ki67 activity. There was additionally a rise in the levels of cyclin D1, p53, and p21, other classic senescence signs. Multiple nucleated cells made up a significant portion of the large, polyploid, and polymorphic cells. According to mitotic index studies, cyclin B1's mostly cytoplasmic distribution, and the absence of separated multiple centrosomes, the great majority of doxorubicin-treated cells did not undergo mitosis. This enabled us to determine that endoreduplication occurred in HCT116 cells treated with doxorubicin. Meanwhile, the cytogenetic study revealed few occurrences of aberrant polyploid cell mitoses that resulted in aneuploid offspring. Therefore, a therapy that caused senescence in a human colon cancer cell line had two effects: it prevented most of the cells from multiplying, but it also caused proliferating aneuploid cells to arise. These experiments show that doxorubicin essentially does not cause human colon cancer cells to undergo apoptosis. Most cells that receive doxorubicin reproduce DNA yet lack mitosis, suggesting that endoreduplication is the cause of the observed polyploidy. However, a small percentage of polyploid cells suffer abnormal mitoses, increasing the genomic instability of HCT116 cells that are driven to undergo senescence.

Cisplatin: Cisplatin is a common chemotherapeutic medication that may harm DNA by binding to it and creating intrastrand and interstrand cross-links between purine nucleotides. It was believed that cisplatin resistance in cancer treatment was related to improved DNA damage repair. Although toxicity and resistance are the main obstacles preventing cisplatin from being used effectively in cancer treatment, understanding the regulatory processes that govern various aspects of tumor senescence allows for the development of novel therapeutic strategies to increase the drug's effectiveness and lessen its adverse effects [29]. In order to examine the impact of cisplatin on hepatocellular cancer, administered varying doses of cisplatin to human hepatoma cell line (HepG2) cells expressing wild-type TP53 [30]. These cells experienced the G1 stage halt and permanent growth suppression as a result of cisplatin. HepG2 cells treated to a modest concentration of cisplatin showed a rise of senescence-associated β-galactosidase. After the cisplatin exposure, P19 activity surged right away and peaked at 48 hours, after which it rapidly dropped to the baseline level, while TP53 and P21 mRNA expression levels grew steadily. P53 and P21 expression alterations that mirrored their mRNA expressions during cisplatin-induced cellular senescence in HepG2 cells were validated by Western blotting. These findings demonstrated a functional connection between hepatocellular senescence and cisplatin. Further, given the wide utilization of cisplatin, individuals with locally aggressive head and neck squamous cell carcinoma are now routinely treated with

multimodality chemotherapy that uses cisplatin as a neoadjuvant or in conjunction with radiotherapy. In this line, studies suggest that Wee-1 suppression may have disrupted the G2 checkpoint, selectively exposed p53-deficient individuals to different DNA-damaging drugs, including cisplatin, and prevented tumor growth in in vivo experiments, according to MK-1775, a particular inhibitor of Wee-1, and siRNA-mediated reduction of this gene. In individuals who have metastatic solid tumors, it is currently in phase I and II clinical studies as a chemosensitizer in conjunction with cisplatin. It has low cytotoxicity and good tolerability. By preventing G2 arrest and accumulating cells with unfixed DNA lesions during mitosis, MK-1775 stimulates vulnerable p53 mutant head and neck squamous cell lines to cisplatin [31]. Cisplatin is also implemented in non-small cell lung cancer, where [32] the action of this drug in PC9 cell lines, which are non-small cell lung cancer cell lines was evaluated. The findings showed that PC9 cells were severely inhibited in their ability to develop by cisplatin. By increasing p21WAF1/CIP1 and decreasing cyclin D1, CDK4, and E2F-1 activity in PC9 cells, cisplatin caused G1 phase arrest. Additional investigation revealed that cisplatin-induced apoptosis is linked to an active caspase cascade, elevated ROS generation, and upregulated expression of Bak, tBID, Fas, and FasL. Further, cisplatin treatment resulted in a G1 stage halt and also observed initiation of senescence-associated beta-galactosidase activity.

Etoposide: Derived from *Podophyllum peltatum* or *Podophyllum emodi*, etoposide is a partially synthetic derivative of podophyllotoxin. This substance affects topoisomerase II, an enzyme that processes chromatin in transcription, recombination, and replication. The enzyme's ability to reversibly cleave either of the genomic strands is essential for both binding the molecule and modifying its topology, or superhelical architecture. Because it stabilizes cleavable enzyme-genome complexes, etoposide prevents topoisomerase from ligating those inconsistencies. When these components communicate with transcriptional compounds, replication forks, or additional enzymes involved in genome processing, permanent breaks in DNA strands may eventually develop [33]. The impact of low-dose etoposide exposure on the A549 cell line was assessed in Ref. [34] in light of certain cytoskeletal alterations linked to senescence development. Etoposide caused the A549 cell population to exhibit an appearance resembling senescence. However, additional senescence indicators such as SAHF formation, p21Cip1/Waf1/Sdi1 induction, or sustained cell cycle arrest were not directly associated with morphological changes. Instead, there was a noticeable increase in the frequency of polyploid, TUNEL-positive cells. Cyclin D1 was also shown to be upregulated. Experiments further provide early evidence—derived from microscopic analyses—that vimentin may play a part in the nuclear changes that accompany polyploidization-depolyploidization events that occur after genotoxic exposures. Experiments demonstrated that adrenocortical tumor cell proliferation was decreased by etoposide treatment at 10 μM via causing cellular senescence instead of apoptosis. A number of indicators of cellular senescence were noted, such as downregulated Lamin B1, increased p53 and p21 levels, expanded nuclei, and activated senescence-associated β-galactosidase activity. Additionally, we discovered that etoposide produced many centrosomes. Etoposide-induced senescence was mitigated by the suppression of several centrosomes, which was achieved by either exposing cells to roscovitine or centrinone or by overexpressing NR5A1/SF-1. This suggests that etoposide-induced senescence through many centrosomes. Additionally, primary cilia contributed to the senescence brought on by etoposide. Etoposide further triggered DNA-PK-Chk2 signaling; blocking this signaling cascade reduced cellular senescence after alleviating several etoposide-induced centrosomes and primary cilia. Etoposide

therapy for centrosomal abnormalities and senescence additionally induced autophagy alongside harming DNA transmission. Crucially, etoposide-triggered autophagy was decreased by DNA-PK-Chk2 pathway shutdown, whereas DNA-PK-Chk2 activation was unaffected by autophagy inhibition [35]. In order to investigate the fact that autophagy facilitates the beginning of DNA damage response (DDR)-related cellular senescence, experiments in Ref. [36] grew human keratinocytes (HaCaT) with or without etoposide therapy. Cells subjected to 5 μmol of etoposide showed higher levels of γH2AX, p53 binding protein1 (53BP1), and senescence-associated β-galactosidase (SA-β-Gal), indicating DDR-related cellular senescence, while cells treated with 1 μmol of etoposide showed no senescent alterations. Additionally, senescent cells expressed more pATM, pp53, and p21, which are factors linked to the activated ataxia-telangiectasia mutant (ATM) transmission cascade. Although no senescent-induced cells (1 μmol treated cell) exhibited higher levels of LC3 and Beclin-1, the 5 μmol etoposide-treated senescent cells displayed downregulated expression of these two proteins. However, the production of rubicon, an adverse regulator of autophagy, was raised. The cultures exposed using just 1 μmol etoposide and primed with N-acetylcysteine exhibited elevated levels of rubicon, p21, and senescent markers. According to this research, the ATM/p53/p21 signaling pathway is activated by rubicon-regulated autophagy, which in turn promotes etoposide-induced DDR-related cellular senescence. The occurrence of DDR-related cellular senescence is accelerated by impaired autophagy brought on by rubicon overexpression.

Busulfan: Experiments have investigated the processes by which busulfan, a typical cytotoxic drug, causes cellular senescence in human diploid fibroblasts and normal murine bone marrow blood cells. Busulfan is frequently employed to manage myeloablation before bone marrow transplantation and persistent myelogenous leukemia. Busulfan is frequently employed to manage myeloablation before bone marrow transplantation and persistent myelogenous leukemia. Nevertheless, a variety of typical injuries to tissues, including hemorrhagic cystitis, chronic lung fibrosis, liver veno-occlusive disease, and bone marrow repression, impair the curative effectiveness of busulfan. Through the linkage proteins and the genome, busulfan, a strong alkylating chemical, threatens the genome [37]. Protein p53 cascade may be activated as a result of the hyperlinks being transformed into lesions in the genome. Consequently, busulfan has been thought to cause senescence through the p53-genome degradation route. Experiments further demonstrated that busulfan induction of hematopoietic cell senescence is unrelated to p53 activation or p21Cip1/WAF1 (p21) upregulation. Furthermore, busulfan-stimulated senescence in diploid fibroblast cells is not prevented by p53-specific siRNA, which downregulates p53 expression. Such data implies busulfan might cause cellular senescence by a process that does not rely on p53. Experiments often conclude that busulfan-stimulated diploid fibroblast cell senescence is linked to a sustained stimulation of p38 mitogen-activated protein kinase (p38 MAPK) and extracellular signal-regulated kinase (Erk) and that busulfan-stimulated senescence in diploid fibroblast cells can be prevented by blocking the Erk-p38 MAPK pathway with a particular p38 or Erk inhibitor, lending credence to this theory [38].

Temozolomide: Cancerous gliomas are treated with temozolomide, a DNA-methylating medication that causes programmed cell death. One report in Ref. [39] looks into the processes that allow temozolomide to cause glioblastoma cells to undergo senescence. The specific DNA lesion O6-methylguanine (O6MeG) caused temozolomide-stimulated senescence, which was typified by cell arrest in the G2–M phase. The MRN component recognized injury, the ATR/CHK1 axis of the DNA

damage response pathway was activated, and CDC25c degradation facilitated the onset of temozolomide-stimulated senescence, according to antagonist assays. Temozolomide-stimulated senescence depended on persistent p21 upregulation and needed operational p53. Despite not expressing p21, p53-deficient cells continued to be able to cause a G2–M halt yet were unable to cause senescence. In the cellular framework, p14 and p16, which are p53 targets, disappeared and did not appear to be involved in temozolomide-stimulated senescence. Another study in Ref. [40] shows that temozolomide and carmustine enhance the quantity of heterochromatic proteins MeCP2 and HP1a attached to the genome and cause aggregation of pericentromeric heterochromatin areas. Additionally, temozolomide and carmustine raise the amounts of histone H3 trimethylated on lysine 9 (H3-triMeK9) and diminish the general degree of histone H3 acetylation. The senescence status is preceded by such occurrences. Conclusions were drawn that the effectiveness of temozolomide and carmustine in treating gliomas could be linked to an initial occurrence that is marked by modifications in the functioning of heterochromatin and its suppression, and this is subsequently followed by apoptosis and senescence.

Mitomycin C: Around 1974, mitomycin C, a cytotoxic drug, was first utilized to combat cancer. Adenocarcinoma of the GI tract and pancreas, anal, bladder, breast, cervical, colorectal, head and neck, and non-small cell lung cancer are just a few of the many malignancies that mitomycin C has been utilized to treat over the years. It functions as a DNA-bridging and bi-alkylating chemical. Mitomycin C is administered intravenously in hospitals and has a half-life of roughly 50 minutes in circulation. Metabolism of mitomycin C is completed in the liver. The typical intravenous frequency is 20 mg/m^2, and a plasma level of 2.4 μg/ml can be attained [41]. One experimental study in Ref. [42] investigated the circumstances whereby mitomycin C can cause senescence in human non-small cell lung cancer A549 cells. Being subjected to 10 or 20 nanograms per milliliter of mitomycin C for 6 days caused cell senescence and nearly complete (with a low dose) or complete (with a high dose) eradication of the growth capacity of A549 cells, whereas exposure to 100 or 500 nanograms per milliliter of mitomycin C caused their halt in the S phase of the cell cycle and afterward death. The cells showed signs of genome replication stress in response to such small amounts of mitomycin C, including γH2AX expression, p21WAF1, and a very low level of EdU incorporation into DNA. The findings support the idea that cells with active cancer genes undergo senescence as a result of prolonged genome replication stress. It makes sense that malignancies with continuous expression of cancer genes to induce mTOR signaling would be more likely to experience senescence after receiving low doses of medicines that damage DNA for an extended period of time.

Melphalan: Multiple myeloma is an incurable cancer that often relapses despite treatment advancements. Tumor dormancy, where cancer cells remain inactive and resistant to therapy, may play a role in relapse. Dormant multiple myeloma cells share similarities with senescent cells, which enter a growth arrest state due to stress [43]. High-dose melphalan, a chemotherapy drug used in multiple myeloma, was hypothesized to induce a senescent-like dormant state in surviving multiple myeloma cells. Experiments using mouse and human multiple myeloma cells treated with high-dose melphalan showed growth arrest, enlarged cell size, and increased markers of senescence, such as genomic damage and anti-apoptosis pathways. These effects were consistent across species. Genetic analysis revealed upregulation of senescence-related genes and downregulation of cell cycle and DNA repair genes in melphalan-treated cells [44].

Methotrexate: Experimental data in Ref. [45] illustrated that after receiving a high concentration of methotrexate, human colorectal adenocarcinoma C85 cells

experience surged senescence. This is demonstrated by the following traits in culture: halt in proliferation at the G1 and S phases of the cell cycle; SA-b-galactosidase positivity; induced expression of p21waf1/cip1 and decreased expression of p16INK4a; and continued DNA synthesis at a reduced level. Both untreated cells and cells that have undergone treatment retain the same percentage of human colorectal adenocarcinoma cells with DNA content greater than 4 N (14%). It is discovered that the primary karyotypic aberration is multinucleation.

5-Bromo-2-deoxyuridine (BrdU): 5-Bromo-2-deoxyuridine (BrdU) is a thymidine counterpart that is integrated into replicating DNA. Sublethal quantities of BrdU are known for decades to change the development and phenotype of a variety of cell types, despite the fact that the compound was initially intended as a chemotherapeutic agent [46]. Nevertheless, the processes behind these 5-Bromo-2-deoxyuridine-mediated actions are yet unclear. By looking at DNA damage responses, cell cycle impacts, and phenotypic modifications, research illustrated the effect of 5-Bromo-2-deoxyuridine on A549 lung cancer cells. These cultures are p16-null but exhibit wild-type p53. In these cells, sublethal levels of 5-Bromo-2-deoxyuridine trigger a DNA damage response that activates p53, Chk1, and Chk2. The modified populations exhibit a greater proportion of multinucleated cells and larger nuclei. Colonies in the S, G2/M, and G0 stages proliferate and accumulate less when cell cycle suppression takes place. When the nuclear localization of the nuclear antigen of cells that divide occurs, 5-Bromo-2-deoxyuridine causes a rapid suppression of p21 production. Nuclear antigen levels in cells that divide fall in comparison to control cells, but p21 levels rise. Additionally, p27 and p57 expression are upregulated. After 7 days of contact with 5-Bromo-2-deoxyuridine, affected cells exhibit a senescent-like phenotype, exhibiting increased β-galactosidase activity, granularity, and cell dimension. This research proposed that 5-Bromo-2-deoxyuridine causes A549 cells to undergo a DNA damage response, which leads to decreased growth, mitotic departure, and senescence-like phenotypic alterations [47].

Palbociclib: Clinical studies have demonstrated the efficacy of palbociclib, an FDA-approved cyclin-dependent kinase (CDK) 4/6 inhibitor, in treating breast cancer. Its application to oral cancer is not widely studied, though [48]. In 2020, as shown in Ref. [49], it was established that palbociclib can speed up cellular senescence and death while also dramatically reducing oral squamous cell carcinoma cells' capacity for proliferation, migration, and invasion. Researchers discovered that palbociclib inhibited the development of cell cycles by hindering c-Myc and CDC25A transcription through the p53-independent mechanism, which also caused injury to the genome and p21 production. Furthermore, this compound was found to inhibit the ability of OSCC cells to compensate for damaged genomes by downregulating RAD51 activity. Upon further examining Ref. [49], the process of suppression and the detrimental impact of palbociclib toward oral squamous cell carcinoma culture, palbociclib can hasten cellular senescence and death while also dramatically reducing oral squamous cell carcinoma cells' capacity for proliferation, migration, and invasion. Researchers discovered that palbociclib inhibited cell cycle progression by downregulating c-Myc and CDC25A expression through the p53-independent mechanism, which also caused DNA damage and p21 expression. Furthermore, palbociclib inhibited the ability of OSCC cells to repair DNA damage by downregulating RAD51 expression. At last, the study concluded that palbociclib had anti-oral squamous cell carcinoma activity, expedited cellular senescence and death, and concurrently caused DNA damage and inhibited its repair. Researchers in Ref. [50] further examined the combined effect of palbociclib with radiation

therapy (4Gy) and observed two times higher γH2AX, increased β-gal expression by around 95%, and decreased viability and synergy. Further, combined treatment decreased Rad51 and Ku80 activity, suggesting homologous recombination and non-homologous end-joining pathway impairment. The combination also decreased the size and proliferation of three-dimensional immortalized spheroids and organoids derived from patient tumors (conditionally reprogrammed oral cavity squamous cell carcinoma CR-06 and CR-18). Moreover, CR-06 and CR-18 showed three times lower organoid size and proliferation with concurrent therapy. They suggested that when paired with radiation therapy, targeting CDK4/6 can increase the effectiveness of oral cavity squamous cell carcinoma by causing senescence and DNA damage repair.

3. Conclusion

The study of chemotherapy-induced senescence and its relationship with genomic instability has unveiled a complex interplay between cellular aging, DNA damage, and tumorigenesis. Since the pioneering work of Hayflick and Morehead, our understanding of cellular senescence has evolved significantly, moving beyond the simplistic view of irreversible growth arrest to a more nuanced perspective that encompasses a dynamic and multifaceted phenotype. Senescent cells, characterized by metabolic activity, altered morphology, and the secretion of pro-inflammatory factors (SASP), play a dual role in both tissue repair and the progression of age-related diseases, including cancer.

Chemotherapeutic agents, such as doxorubicin, cisplatin, etoposide, busulfan, temozolomide, mitomycin C, melphalan, methotrexate, and palbociclib, induce senescence in both cancerous and non-cancerous cells, often through mechanisms involving DNA damage and the activation of pathways such as p53/p21 and p16INK4a. These agents trigger genomic instability, leading to polyploidy, aneuploidy, and other chromosomal aberrations, which can paradoxically promote tumorigenesis even as they halt cell proliferation. The induction of senescence by these drugs is accompanied by metabolic reprogramming, mitochondrial dysfunction, and the secretion of SASP factors, which can influence the tumor microenvironment and contribute to both anti-tumor and pro-tumor effects.

The DNA damage response (DDR) pathway plays a central role in mediating chemotherapy-induced senescence. Activation of ATM/ATR, CHK1/CHK2, and downstream effectors such as p53 and p21 leads to cell cycle arrest and the establishment of a senescent phenotype. However, the persistence of senescent cells, particularly in the context of cancer therapy, poses a significant challenge. While senescence can serve as a barrier to tumor progression, the accumulation of senescent cells can also promote inflammation, tissue dysfunction, and the emergence of therapy-resistant cancer cells.

Emerging evidence suggests that targeting senescent cells, either through senolytic therapies or by modulating the SASP, may enhance the efficacy of chemotherapy and reduce the risk of cancer recurrence. Additionally, understanding the temporal dynamics of senescence induction and the specific molecular pathways involved in chemotherapy-induced senescence could lead to more precise therapeutic strategies. For instance, combining chemotherapy with inhibitors of DDR components or SASP regulators may help mitigate the adverse effects of senescence while maximizing its anti-tumor potential.

In conclusion, chemotherapy-induced senescence represents a double-edged sword in cancer treatment. While it serves as a critical mechanism for halting the proliferation of cancer cells, it also contributes to genomic instability and the creation of a pro-tumorigenic microenvironment. Future research should focus on elucidating the molecular mechanisms underlying chemotherapy-induced senescence, identifying biomarkers for senescent cells, and developing therapeutic approaches that harness the beneficial aspects of senescence while minimizing its detrimental effects. By doing so, we may improve the outcomes of cancer therapy and reduce the burden of age-related diseases associated with the accumulation of senescent cells.

Acronyms and abbreviations

BrdU	5-Bromo-2-deoxyuridine
ATM	ataxia-telangiectasia mutant
CDC25	cell-division cycle 25
CHK2	checkpoint kinase
CDK	cyclin-dependent kinases
DDR	DNA damage response
DSBs	double-strand breaks
SSBs	single-strand breaks
HCT116 cells	human colon cancer cell line
HepG2	human hepatoma cell line
HaCaT	human keratinocytes
IL	interleukin
MMP	metalloproteinase
OIS	oncogene-induced senescence
OSCC	oral squamous cell carcinoma cells
PARP-1	poly-ADP-ribose polymerase 1
ROS	reactive oxygen species
TIS	therapy-induced senescence
TGF-β	transforming growth factor-beta
UV	ultraviolet
VEGF	vascular endothelial growth factor

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Chapter 6

Genome Duplication and Plant Evolution

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Abstract

One of the major contributory forces to plant evolution is polyploidy, also termed "whole-genome duplication (WGD)". Due to this phenomenon, most plant species exist as polyploids, which has provided sufficient explanation for hypothetical change of their contents, leading to different gene expressions. The aftermath of this event is often evident in meiotic episodes, which are well coordinated, such that a negligible change can be lethargic or result in the emergence of new species, depending on fitness and the adaptive advantages conferred. The contribution of these incidents to evolution is dependent on hybridizations and reproductive isolation mechanisms. The events can leave the genome in its original state or produce variable and complex consequences, where the effect of the changes can be investigated using cytogenetics and advanced tools of molecular techniques.

Keywords: polyploidy, hybridization, speciation, allopolyploidy, autopolyploidy

1. Introduction

Polyploidy, referred to as the "dead end of evolution," is a phenomenon in which an organism possesses more than two complete sets of a genome [1]. The transformation of plants to sessile organisms revealed series of events such as phenotypic adaptability, changes and challenges of ecological competitions for survival and fitness, which promote variabilities and diversification of forms from an evolutionary standpoint. However, some other contributory intrinsic factors to this transition have their basis on an increase in ploidy levels, genome sizes and numbers, called "polyploidy" [2]. This heritable process is more pronounced in the kingdom Plantae when compared to other kingdoms, with implications for species diversity and subsequent effects on plant domestication. It has played a tremendous role in providing a pool of genomic resources, capable of producing new attributes and phenotypic variations in modern-day plants to cope with the continually fluctuating physical conditions of the environment compared to their ancestral wild diploid progenitors [3]. About 30–80% of all flowering plant species are considered to be recent polyploids, with the belief of having undergone at least one cycle of polyploidization (**Table 1**) during their

Plant	Probable ancestral haploid number	Chromosome number	Ploidy level
Domestic oat	7	42	6n
Peanut	10	40	4n
Sugar cane	10	80	8n
Banana	11	22, 33	2n, 3n
White potato	12	48	4n
Tobacco	12	48	4n
Cotton	13	52	4n
Apple	17	34, 51	2n, 3n

Table 1.
Examples of common plants that have gone through polyploidy [4–6].

evolutionary history, thereby further highlighting the importance of whole-genome duplication in plant diversification [7]. Nonetheless, the action of transposable elements, that is, DNA factors, is also significant to shaping the course of evolution [8]. Cytological mechanisms that create room for exchange of genetic materials, be it through intra- or interspecific hybridization, genome change through meiotic and mitotic mechanisms, are of significant importance to polyploidization [2]. This study discusses notable events in plant evolution. It details the importance of this phenomenon to living organisms as a whole, and provides insight into the traditional, as well as the recently advanced tools of studying the mechanism.

2. Origin and sources of plant evolution

Plants are believed to have evolved from aquatic green alga protists, and have since developed key adaptations for terrestrial life, leading to their dominance on land, and are evident in their lack of curricular structural protections [9]. The evolution of plants is a remarkable journey, spanning hundreds of millions of years, from simple aquatic ancestors to the diverse and dominant terrestrial life forms we see today. This journey is marked by several key innovations that have shaped the course of life on Earth. However, this evolution was initially faced with challenges such as dryness, extreme temperatures, and strong sunlight, which constrained plant size and required adaptations for sexual reproduction. The transition from water to land is considered a pivotal event, leading to morphological, physiological, and developmental changes that resulted in plant biodiversity [10]. Using two early plants in the study of transition from aquatic to terrestrial habitat of plants, Wang et al. [10] established that adaptive traits for terrestrial habitations were found in the plants with variations in sizes, structures and gene complements while exchange of genes through sexual reproduction and meiotic processes, termed hybridization, was suggested. Researchers have reported that most flowering plants have undergone at least one gene doubling or whole-genome duplication (WGD); hence, they proposed it as a major source of evolutionary changes. Concomitantly, some other factors with significant contributions to this change, such as sexual polyploidization and hybridization, have also been identified [2, 5, 6].

Although polyploidy can result to the formation of new species, either through individual gene doubling and/or whole-genome duplication (WGD), however, WGD

has been proposed to be a more important phenomenon in evolutionary genomic variability and plasticity, with the biological consequence of producing observable physical changes in the affected species and generating reproductive isolation and as such, serving as an important mechanism to drive the process of plant evolution [2]. Dar and Rehman [11] noted that most angiosperms have experienced WGD, with their genomes retaining significant duplications, which expands the range of genetic diversity in them.

Sexual polyploidization, a reproductive process, strictly regulated for reduction of homologous chromosome segregation, resulting to haploid (n) male and female gametes in the process of meiosis, with instances of non-reduction in meiotic process, which leads to the production of diploid (2n) gametes, has also been identified as a major mechanism in plant evolution and speciation **Figure 1A** [2, 12]. Formation of tetraploids or triploids is dependent on subsequent gamete fusion, a major process that drives speciation through hybridization, when stable [2]. Similarly, somatic ploidy instability, causing whole-genome doubling, has been reported in somatic cells, with the ability to drive evolution through polyploidy, especially in asexual propagation (stolons, bulbs and rhizomes), leading to the establishment of stable polyploid lineages (**Figure 1B**) [2].

Interspecific hybridization, which refers to the mating between individuals from different species or genetically distinct populations, can result in the formation of new hybrid species, thereby leading to the production of new genetic variation and creation of novel trait combinations (**Figure 2**) [13]. Zhang et al. [14] reported that interbreeding of divergent species can result in hybrid speciation, where hybrid populations diverge from parental lineages, leading to entirely new species. Although this process is not straightforward, as it encompasses reproductive isolation and polyploidization; however, the latter has been identified as the potential driving phenomenon of plant speciation and evolution [13, 15, 16].

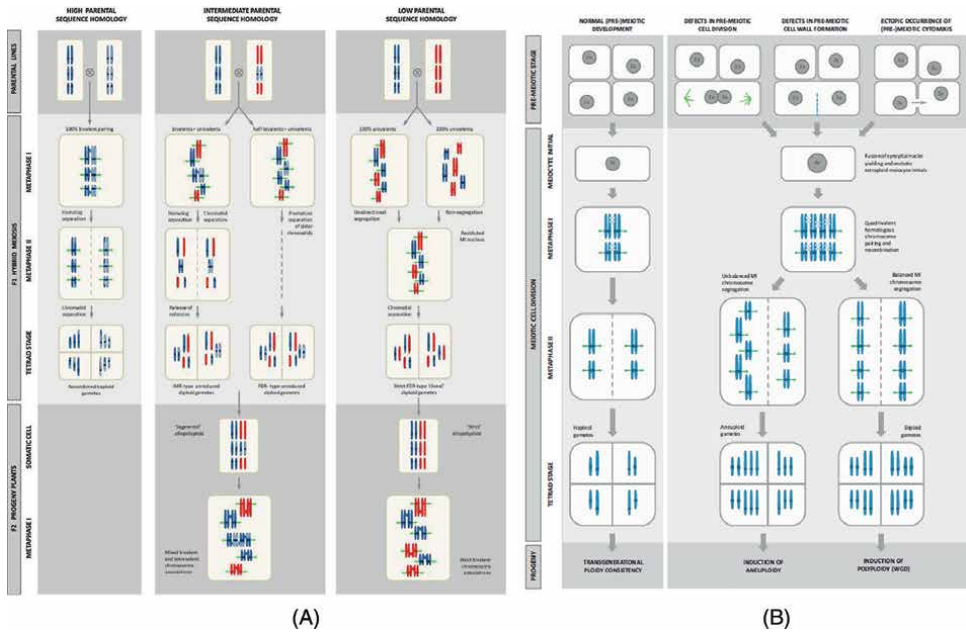

Figure 1.
A: the impact of sequence homology between parent progenitor genomes on meiotic restitution in their F1 progeny and B: mechanisms and outcomes of pre-meiotic endomitosis [2].

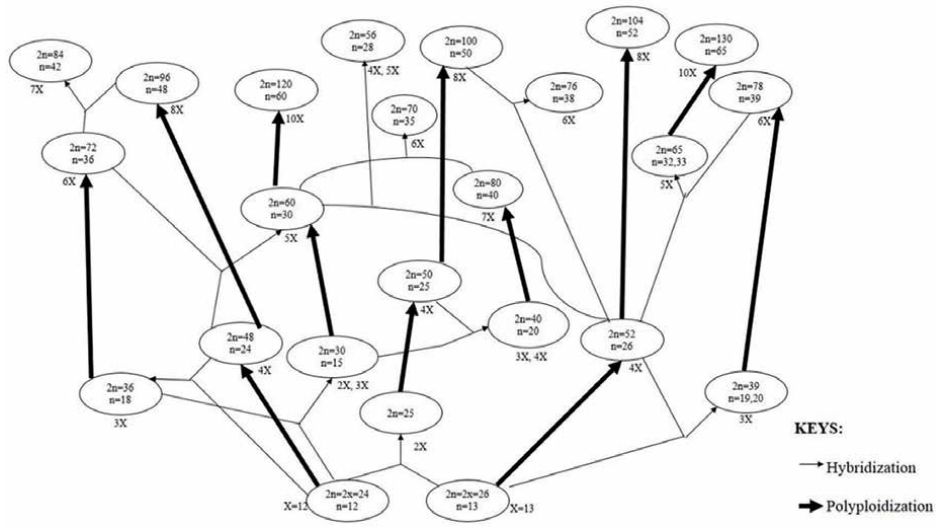

Figure 2.
Possible relationship among Ocimum *species using different studies [13].*

Due to this phenomenon, most plant species exist as polyploids, which has provided sufficient explanation for the hypothetical change of their contents, leading to different gene expressions. The aftermath of this event is often evident in meiotic episodes, which are well coordinated, such that a negligible change can be lethargic or result in the emergence of new species, depending on fitness and the adaptive advantages conferred. The contribution of these incidents to evolution is dependent on hybridizations and reproductive isolation mechanisms. The events can leave the genome in its original state or otherwise, where the effect of the changes can be investigated using cytogenetics and advanced tools of molecular techniques.

3. Types of polyploidy

The study on polyploidy has gained researchers' attention because of the realization of the significance of the phenomenon to human livelihood improvement, through adaptive advantage conferment on organisms, especially plants, where it is common. Madlung and Wendel [17] expressed it as a phenomenon of co-occurrence of 2 or more chromosomes set in a nucleus (**Figure 3**), shedding light on the frequently neglected aspect of aneuploidy and euploidy.

Aneuploidy is a form of polyploidy, involving gains or losses of genomes, thereby creating an imbalanced chromosomal arrangement. It is the variation of haploid chromosome number by more or few chromosomes, which arises mostly as a result of errors in chromosomal segregation. It is an irreversible process during meiotic segregation [19], giving rise to a number of chromosomes in a cell or organism that deviates from multiples of the haploid genome. Although there is a paucity of studies on plant aneuploidy, Yali [20] noted that plants with this meiotic aberration are mainly characterized by low vigor. In addition, the explicit report of Guerra [18] on polyploidy showed that aneuploidy was actually detrimental and lethargic in any organism. However, Dar and Rehman [11] reported that the gain of extra

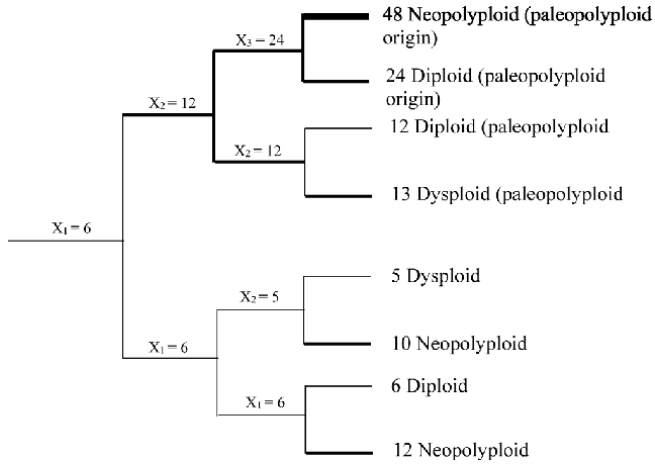

Figure 3.
Schematic relationship among different chromosome numbers and ploidy levels in a cladogram. The lines of the cladogram were drawn twice as thick after each new polyploidization event [18].

chromosomes (ascending aneuploidy) has better chances of tolerance at any ploidy level. Furthermore, while some authors use aneuploidy and dysploidy interchangeably, others differentiate the two. Dysploidy, which is also a phenomenon that gives rise to an increase or decrease in species chromosome numbers, but without an appreciable loss of genetic material, has been widely reported in some plant species such as *Dioscorea* spp. [21] and *Ocimum* spp. **Figure 4** [22], which might have been stabilized evolutionarily.

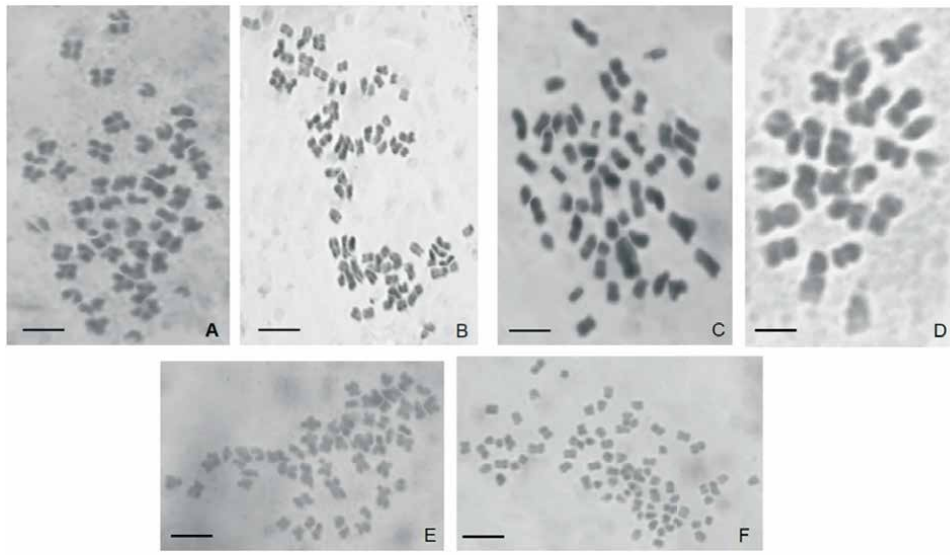

Figure 4.
Mitotic chromosomes of Ocimum *species studied. A. Metaphase in* O. basilicum *(b_1) (2n = 4x = 52). B. Metaphase in* O. basilicum *(b_2) (2n = 6x = 72). C. Metaphase in* O. canum *(c_1) (2n = 4x = 52). D. Metaphase in* O. canum *(c_2) (2n = 2x = 24). E. Metaphase in* O. americanum *(2n = 4x = 52). F. Metaphase in* O. kilimandscharicum *(2n = 6x = 76) [22].*

A chromosome complement that is an exact multiple of the haploid number is referred to as euploidy. When the chromosome sets are two, it is referred to as 2n (diploid), three chromosome sets are a triploid (3n), four chromosome sets are a tetraploid (4n), and so on. Autopolyploids are species that occur from the duplication of a given organism's chromosome, which has a homologous genome, that is, having more than two copies of the genome. They are believed to have arisen through a polyploidization event within or between populations or varieties of a single species, often intraspecific [2]. The effects of chromosome doubling in autoploids are dependent on the concerned species. In forage and grasses, spontaneous chromosome doubling was reported to have resulted in increased cell vigor and size, a condition that is likely to be advantageous in a given environment [2, 13, 23, 24]. Most crops have evolved through the process of genome duplication of autopolyploids; however, this is often accompanied by the loss of sexual capability [13, 25].

Allopolyploid results when two distinct species hybridize, after which the resulting hybrid's chromosome number is doubled through a phenomenon synonymously referred to as amphiploidy [26]. The genetic material of both parent species is combined in this process, resulting in a new organism that is distinct in nature through the process of interspecific hybridization [13, 27]. Allopolyploidy is of paramount interest in plant evolution because it is likely to allow new genetic variation and traits that would be advantageous in enabling adaptability in different environments [28, 29]. Examples of allopolyploid organisms of particular interest include cultivated food crops such as cotton and wheat, which exhibit complex evolutionary backgrounds that consist of multiple instances of hybridization [28].

It is noteworthy that the processes of evolution, especially in plants, are continuous, with polyploidy being instrumental to this process. However, regardless of the genome properties, newly established polyploids, whose diploid relative can be found, are termed neo-polyploids. This might be ambiguous evolutionarily, and as such has paved the way for some other definitions, where Guerra [18] stated that neo-polyploids are all polyploids, either intraspecific or intrageneric, whose diploid relative can be found, while others were grouped as paleopolyploids. For clarity, the presence or absence of diploid relatives differentiates neopolyploid and paleopolyploid, respectively [18].

4. Importance of polyploidy to plant evolution

The pathways of polyploidy are complex and multifaceted, with studies constantly conducted in a bid to experimentally explain it [30, 31]. Some of the notable consequences of polyploidy in plants are their effect on plant size, morphology, physiological traits, and growth pattern [32]. Polyploid individuals may exhibit novel phenotypic traits such as increased cell size and larger organs than their diploid counterparts, in the form of larger roots, leaves, increased pollen size, larger stomata, tubercles, fruits, flowers and seeds. They also have altered growth rates and tend to flower later or over a longer period of time than their diploid counterparts. The effects of polyploidy also extend to the structure of plants, such that characters like penetration of roots, leaf size, and resistance to drought are affected.

Using transcriptomic approach, the molecular basis for these observable outcomes through polyploidization has been widely studied, which has established significant differences between the genomes of plants of higher ploidy levels and those from lower ploidy levels, resulting to polyploidization-driven advantages by interplay of

variable gene networks, involved in differentially expressed genes, which triggers polyploid plant to evolve novel phenotypic traits. These special traits contribute to tolerance of such plants to biotic and abiotic stress factors, allowing polyploids to invade new environmental niches or survive extreme conditions [33]. Mu et al. [34] opined that significant up-regulation of indole acetic acid (IAA) and ethylene signaling transduction hormones in autotetraploid *Betula platyphylla* may have been the probable reason for its exhibition of increased breast-height diameter, volume, leaf, fruit, and stomata size. To decipher the changes in gene expression between diploid *Brassica rapa* and autotetraploid *B. rapa*, Braynen et al. [35] used comparative transcriptome analysis, which showed notable differences between key genes within plant hormone signal transduction and flowering time pathways, resulting in a down-regulation for most genes and up-regulation of some for the polyploid plants. Wang et al. [36] reported the differential expression of 1395 transcripts between *Phragmites australis* tetraploids and octoploids, classified into several functional categories and associated with reproduction and resistance to abiotic stresses.

As such, polyploidy is increasingly viewed as a significant force of ecological resilience and adaptability in plants. Empirical evidence has shown that polyploid plants generally display more developed morphological and physiological characters that enable their survival in varied ecological contexts [37]. Baniaga et al. [38] stated that polyploidy introduces more genetic diversity, capable of enabling adaptation to different ecological niches, which is of more importance in unstable ecological contexts, where new ecological niches and new resources can be utilized more easily, using new polyploid species in place of their diploid relatives. Ramsey [39] shows that tolerances to different ecological contexts can be developed by polyploids to enable survival in more harsh situations. Quadrana et al. [40] stated that changes in the activity of transposable elements probably play a key role in the adaptation of polyploids to different stresses, by modification of the expression of stress-related genes. In addition, Banerjee et al. [41] noted that polyploid plants generally display higher physiological plasticity, which is useful in dealing with ecological stressors.

Polyploids also take advantage of the phenomenon of heterosis in plants through the fixing of divergent parental genomes in allopolyploids, unlike in diploid F_1 hybrids, where heterozygosity and heterosis decay in subsequent progeny generations (**Figure 5**). The enforced pairing of homologous chromosomes in allopolyploids prevents intergenomic recombination, effectively maintaining the same level of heterozygosity through the generations. In addition, autopolyploids, formed by hybridization, followed by chromosome doubling, show stronger heterosis than their corresponding diploid hybrids, resulting in improved adaptive traits such as viability, productivity, resistance to biotic and abiotic stressors, among others [42].

Polyploidy is also the primary driving force of speciation, responsible for the diversification of plant species and for increasing biodiversity. Otto and Whitton [43] documented how this phenomenon leads to reproductive isolation, a primary process of speciation. By introducing genetic variance and enabling new traits to evolve, polyploidy facilitates the adaptation of plants to new ecological niches, thus increasing biodiversity. The process is most frequently encountered in instances of sympatric speciation, in which new species arise from a common ancestor in their geographic distribution, often resulting in a direct product of polyploidy [44]. The genetic and phenotypic consequences of polyploidy often result in reproduction barriers, such as flowering time or pollen vector preferences, leading to isolation and speciation [44]. Han et al. [45] presented a detailed examination of the role of polyploidy in the diversification of the *Allium* genus, highlighting how polyploid species have adapted

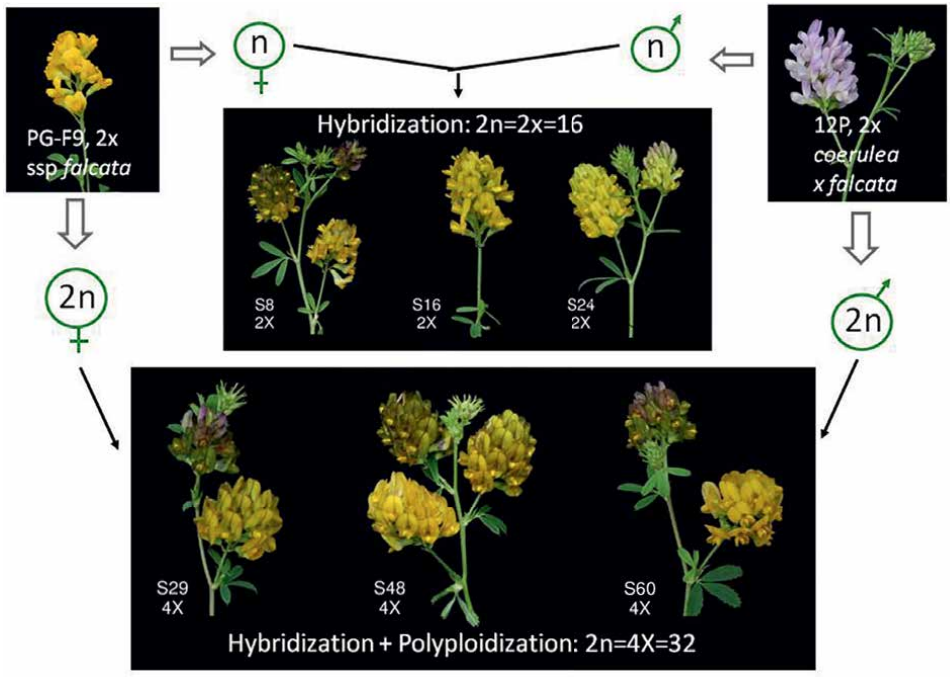

Figure 5.
The study of Rosellini et al. [12] sexual polyploidization in Medicago sativa *L. for heterosis and increased biomass.*

to distinct ecological conditions, enhancing species richness in certain regions. Similarly, Ramsey [39] provided evidence of polyploidy-induced speciation in various plant groups, demonstrating that polyploidy can catalyze rapid evolutionary change. These case studies underscore the importance of polyploidy in shaping plant biodiversity. Furthermore, Wood et al. [46] observed that vascular plant polyploid speciation is more common than previously estimated, indicating a high percentage of angiosperms that contain more than one ploidy level. The continued efforts on polyploidy and its impact on speciation continue to expose the nature of plant evolution and the dynamic positive contribution to biodiversity.

5. Studies of polyploidy and plant evolution

Due to the importance of polyploidy to adaptiveness and evolution, its study is imperative, where Cytotaxonomy is the earliest and the oldest technique employed to achieve this. Using cytotaxonomical analyses in the study of polyploidy involves chromosome counting, as well as observation of the arrangement of the concerned chromosomes in descending order, according to their sizes, respectively referred to as cytogenetics and karyotyping (**Figure 6**). Despite the technique being antiquated, it still remains the quickest, cheapest, and easiest way to get any substantial information about the genome of a species, while complementing all other modern, refined techniques [18, 48–51]. The use of this technique had been employed in the study of many species which are paleoploids and neoploids but the associated major impediment is the limitation with respect to the acquisition of the necessary skills needed in growing and harvesting of meristematic tissues, as well as counting of chromosomes,

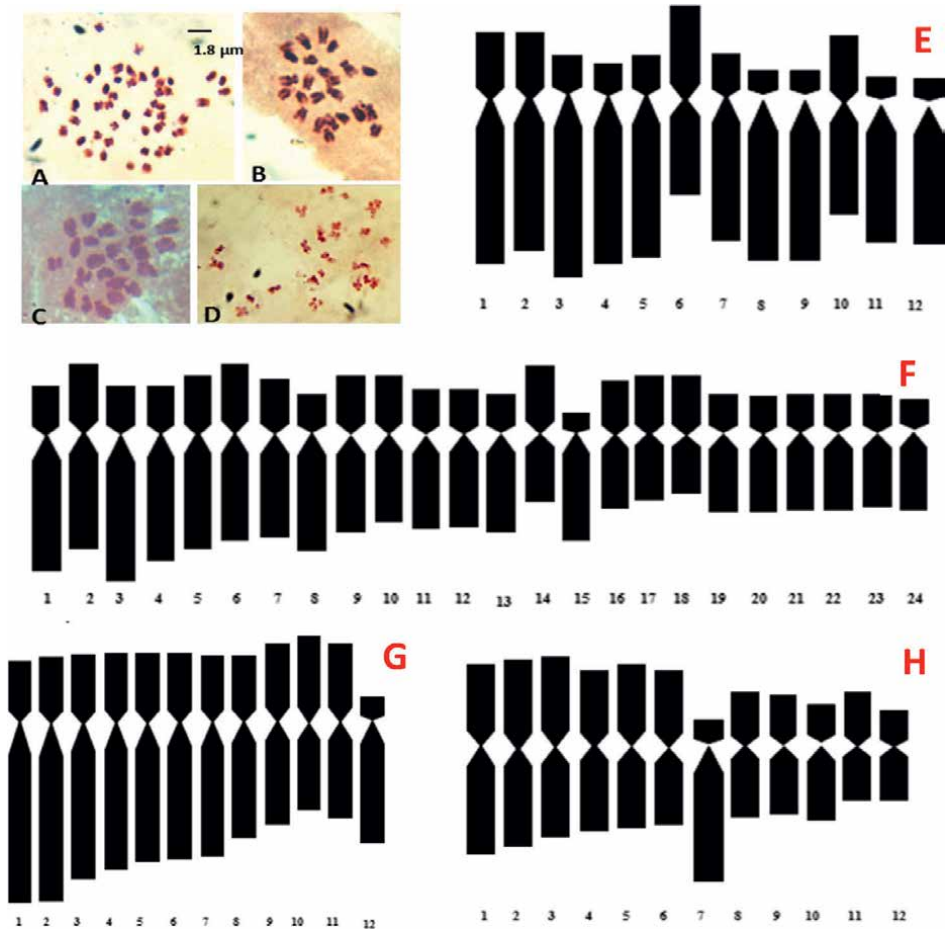

Figure 6.
Mitotic chromosome (A–D) and Karyogram (E–H) of four Physalis *species. A.* P. angulata *(2n = 48); B.* P. pubescens *(2n = 24); C.* P. micrantha *(2n = 24); D.* P. peruviana *(2n = 24); E.* P. pubescens *(2n = 24); F.* P. angulata *(2n = 48); G.* P. micrantha *(2n = 24); H.* P. peruviana *(2n = 24) [47]. Copyright by Azeez S.O. and Faluyi, J.O. (2020). https://www.researchgate.net/profile/Sekinat-Azeez/publications*

especially in organisms with small and/or many chromosomes, which can result to miscounts [21, 22].

In recent time, molecular cytogenetics techniques, such as Fluorescence *in situ* hybridization (FISH) and Genomic *in situ* hybridization (GISH) can be explored to study polyploidy, due to advances in technology. These diagnostic tools can locate gene and DNA sequences on chromosomes by staining with the use of fluorescence molecules. However, while FISH utilizes probing of small DNA sequence, GISH probes whole genomic sequences. These techniques have been used in the analyses of genomic structure and function, chromosome constituents, recombination patterns, alien gene introgression, genome evolution, aneuploidy, and polyploidy [52, 53].

Another technique developed to study polyploidy on the basis of DNA content is flow cytometry. The technique has been used for the study of plant genome sizes, analyses of genome-size derived information, such as ploidy level and comparison of plant genome sizes of different cell populations. The technique has been useful, especially in crops that have small chromosomes, which ordinarily would make

chromosome counts difficult [21, 54, 55]. This is because it could circumvent challenges, such as special specimen preparation, rapid sample preparation, use of live sample and bulk sample analysis. It is noteworthy that the use of flow cytometry is dependent on prior determination of diploid relative with the use of the conventional chromosome count technique. Studies have shown that the use of chromosome count is still the most effective and recommended technique in organism ploidy determination while other techniques can be used in the provision of additional information [56–59].

Also, several other techniques, such as phenotypic, histology and hybridization approaches have been used in the study of polyploidy, knowing that the phenomenon confers adaptiveness and gigantism on organisms, even plants [60–62]. The study on different *Ocimum* species that have speciated through polyploidy, hybridization and reproductive mechanisms showed that polyploid species are significantly bigger morphologically, compared to their diploid relatives [13, 22, 63]. However, the study of Sanders [64] revealed that this varied in different organisms, while selection, DNA content and ploidy level interplay in organ and organisms' sizes in a non-correlated manner. On the other hand, hybridization has been used to study polyploidy in many plant species, which include *Ocimum* [13], while conclusions are drawn based on hybrid sterility, incompatibility, loss of sexual capability and speciation [13, 62]. Results of these techniques can be more useful as a check rather than in drawing conclusions.

5.1 Polymerase chain reaction (PCR) and qPCR

Polymerase chain reaction (PCR) and quantitative PCR (qPCR) are basic techniques in polyploidy studies, specifically in measuring gene duplication. Conventional PCR can amplify the genes of interest to enable researchers to check for duplicate copies in polyploid genomes. This method successfully identifies homologous genes, evolutionarily derived from another ancestral genome and thus contributes to the understanding of the influence of polyploidy on genetic diversity [65]. Several molecular markers such as restriction fragment length polymorphism (RFLP), single sequence repeats (SSR) or microsatellites can detect genetic variation within polyploid genomes by analyzing differences in DNA fragment lengths. RFLPs exploit variations in homologous DNA sequences, so that researchers can distinguish between homologous genes with varying ancestries. For instance, it was used to identify genetic variation among polyploid cotton species and demonstrate how variation in fragment size between genomes reveals the intricacies of polyploid inheritance [66]. Mapping these variations enables the production of genetic markers that are beneficial for breeding and diversity research and are instrumental in the effective management and conservation of polyploid taxa. RFLP has also been used in characterizing the genetic constitution of polyploids by identifying allelic differences that may confer specific phenotypic traits.

Simple sequence repeats (SSRs) define regions of repeated DNA, which serve as good markers for tracking genetic diversity in polyploid species. With microsatellite markers, allele frequencies are investigated according to ploidy levels and inferences are made regarding population genetic structure [67]. Such a method can interrogate relationships of interest to the incidence of polyploidization, due to changes in the markers of microsatellites for indicating polyploidy-infused genetic difference. Microsatellites measure genetic diversity and can contribute to conservation by establishing genetically distinct populations and informing breeding programs for

specific environmental adaptations. On the contrary, qPCR provides quantitative data regarding the comparative content of a gene copy with information regarding expression levels in altered conditions. This feature is particularly useful in investigating genes potentially impacted by polyploidy through modifying dosage effects and allelic diversity. For example, studies in polyploid wheat showed that qPCR was capable of quantifying the levels of expression of stress-responsive genes and identifying the impact of gene duplication on abiotic stress tolerance of the plant [68].

5.2 Genome sequencing

Whole-genome sequencing (WGS) of polyploid organisms is a comprehensive method of describing genetic structure. Sequencing the whole genome allows scientists to assess the degree and nature of gene duplications, allowing for a refined appreciation of the degree of heterogeneity among different genome copies. For instance, studies have shown that the allopolyploid plant, *Brassica napus*, contains complex genomes from the composition of homologous gene duplications, borne out of hybridization processes. This complexity could bring about diversified gene functions and expressions, thus revealing how evolutionary adaptations are present [69]. WGS also aids in the labeling of structural variations and also chromosomal rearrangements, brought about by polyploidization. The ability to detect such changes is valuable in showing how polyploid genomes develop and influence phenotypic traits. WGS can also analyze genetic variation in polyploid populations, which will play a vital role in breeding programs to enhance crop traits and resistance [70].

At the moment, statistical tools for the construction of linkage maps for polyploids are underdeveloped and the few available ones are mostly for autotetraploids [71, 72]. Linkage mapping in polyploid plants is essential to trace patterns of inheritance of different alleles from each set of chromosomes. By constructing genetic maps, scientists comprehend how genes from different genomes interact, thus providing a better understanding of the genetic control of traits controlled by polyploidy. The mapping method is particularly beneficial for identifying loci related to traits and gene interactions [73]. To perform linkage mapping in polyploids, software packages which have been developed include Tetraploid Snap [74]; PolyGembler [75]; MAPpoly [76] and MSTMAP [77], among others.

Genome-wide association studies (GWAS) enable researchers to associate specific traits with particular genomic regions in polyploid organisms. By genotyping some individuals for genetic variants and correlating these variants with phenotypic traits, GWAS have mapped significant genetic determinants associated with key agronomic traits in polyploid crops [78]. This approach contributes to the understanding of the influence of polyploidy on the expression of traits, thus assisting breeders in developing crops with enhanced performance by making selections based on identified genetic markers.

5.3 Gene expression

RNA sequencing (RNA-Seq) is a valuable technique for gene expression research in polyploid species. RNA-Seq allows researchers to compare how gene expression differs across genomes or alleles in polyploid species. For example, transcriptome analysis of the polyploid Gossypium species has revealed significant differences in gene expression associated with stress response, which are often controlled by gene dosage effects due to polyploidy [79]. RNA-Seq provides insights into the regulatory roles of

duplicates, along with the potential for differential gene expression brought about by polyploidization. It provides a close-up of the way multi-copy genes are expressed in the environment, which will have a bearing on phenotypic traits of polyploids [80]. This detailed information is invaluable in explaining the adaptive function of polyploidy in nature and agriculture. Transcriptomic analysis allows researchers to investigate gene expression levels across polyploid species to understand phenotypic expressions regulated by more than one gene dose. RNA-Seq has emerged as the most dominant method that yields high-resolution information regarding differential patterns of gene expression within sets of duplicated copies found in the form of a polyploid genome. For instance, comparative transcriptome analysis between wild and cultured *Chrysanthemum* species demonstrated extensive gene expression change by whole-genome duplications [80]. This change is responsible for rapid adaptation and evolutionary success, which emphasizes the significance of transcriptomics in understanding the role of polyploidy in regulating conductive pathways and phenotypic impacts in plants.

Proteomic analysis enables researchers to investigate the functional consequences of polyploidy by examining the protein complement of polyploid organisms. By protein profiling, researchers can discern how gene copy changes are translated into phenotypic disparities, including developmental traits and stress responses. Proteomic pattern studies in polyploid *Solanum* species revealed protein differential expression associated with polyploidization, introducing valuable knowledge regarding the biological effects of polyploidy [81]. Transcriptomic and proteomic studies contribute significantly toward building an exhaustive image of the effect of polyploidy at the molecular level, directing crop improvement strategies and biodiversity conservation. In conclusion, molecular methods for examining polyploidy include several approaches, each of which provides its own distinctive insight into polyploid organisms. Techniques such as DNA sequencing, PCR, RFLP, genetic mapping, microsatellite analysis, comparative genomics, bioinformatics, and transcriptomic-proteomic analyses collectively uncover how polyploidy affects genetic diversity, gene expression, and evolutionary processes within and among taxa. As these technologies continue to evolve, they will undoubtedly enhance our capacity to explore and manipulate the complexities in polyploid genomes, with potential implications for agricultural uses and biodiversity conservation.

6. Conclusion

Polyploidy plays a major role in the evolution of plant species, through differences in chromosome number, ploidy level, genome size, transposable elements, expression of genes and their regulatory mechanisms, among others. Knowledge of the advantages of polyploidization events abounds, ranging from the exhibition of novel phenotypes by polyploids, diversification of species following reproductive isolation, generation of new gene combinations with enhanced capacity for environmental resilience, adaptability, etc. There is the potential for polyploids to consistently contribute significantly to plant evolution, even in situations of extreme stresses, hence, necessitating continuous study of their pathways to evolution, using various approaches, which may be fundamental, advanced or combinative in nature.

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Chapter 7

Numerical and Structural Chromosomal Rearrangements and their Influence on the Evolution of the Human Karyotype

Humberto Ossa Reyes, Rafael Humberto Ossa Trujillo and Claudia Esperanza Ossa Trujillo

Abstract

This chapter will present a broad overview of the diversity of all numerical and structural anomalies after analyzing 40.320 karyotypes performed at the Genetics and Molecular Biology Laboratory of Bogotá, Colombia, from January 2014 to December 2024. The standard classical cytogenetics technique was used to perform karyotypes, and software analyzed images and organized karyotypes. We found 17 chromosomal anomalies in the Colombian population in particular, and in general to all human beings, because they belong to the same species that transmits, from generation to generation, both molecular mutations and numerical and structural rearrangements to their offspring. We appreciate the diversity of visible mutational mechanisms in our genome. That, in one way or another, forms the basis and raw material on which natural selection acts to drive the chromosomal evolution of our species. We present the frequency of all visible chromosomal abnormalities in the Colombian population.

Keywords: cytogenetics, evolution, chromosomes, translocations, purpose, chromosomal rearrangements

1. Introduction

Classical cytogenetics began to establish itself as a fundamental technique for the study of chromosomal abnormalities visible under a microscope and their relationship with genetic diseases thanks to the pioneering work of Joe Hin Tjio and Albert Levan [1] in the early 1950s who introduced the G-banding technique and contributed, together with Patricia Jacobs, the first correctly organized karyotype with its respective homologous pairs [1, 2]. In addition, they demonstrated that human beings have 46 chromosomes. Thanks to these contributions, Langdon Down demonstrated

the first association between clinical genetics and chromosomal abnormalities in 1866. Had clinically characterized Down syndrome, and almost 100 years later, it was possible to demonstrate that this syndrome is caused by the presence of an extra chromosome 21 [3, 4].

From these discoveries, classical cytogenetics emerged with all its strength as a branch of medicine that became the gold standard for the diagnosis of numerical and structural anomalies of human chromosomes, promoting the birth of a new medical specialization, which began with Down syndrome and continued with all other trisomies, fulfilling the dream of many scientists who promoted the development of medical genetics when they discovered new syndromes that would bear their names for posterity: Henry Turner [4], Harry Klinefelter [5], Klaus Patau [6], John Hilton Edwards [7, 8], etc. This is the case of Professor Emilio Yunis from the Faculty of Medicine of the National University of Colombia, who introduced classical cytogenetics not only in Colombia but also in Latin America and was the first to offer cytogenetic diagnostic services with great success to the entire country, fulfilling the contribution of his name given to the syndrome he discovered and published [9, 10].

Cytogenetics, when there was no such thing as current software for assembling karyotypes, had to be done manually by taking photographs of the metaphases on rolls of Kodak film that were then developed on photographic paper, and the chromosomes had to be cut with scissors, which made the work very tedious and time-consuming since it could easily take 20 to 30 days to have a karyotype assembled, with the drawback that as many photos had to be taken as there were overlapping chromosomes in the metaphase [11].

It was not until 1980 that Dr. Thomas Lörch developed a system for automatically detecting metaphases as part of his doctoral thesis at the Institute of Applied Physics at the University of Heidelberg. He called the new system Metafer and founded MetaSystems in 1986 [11]. Other companies also began distributing these software programs that allowed the acquisition and improved processing of images, the separation of chromosomes and the assembly of karyotypes, all digitally, significantly reducing the workload. By 2010, our laboratory had acquired this software and has since performed all the karyotypes, the statistical analysis we are presenting.

After more than 70 years of providing diagnostic services worldwide, classical cytogenetics has proven to be the gold standard in recognizing diseases associated with numerical and structural abnormalities of human chromosomes. For this reason, cytogenetics refuses to disappear and continues to do so because it is the simplest and most economical way. Its diagnosis is in the field of what is visible through light microscopy, and its protocols are standardized worldwide in millions of laboratories that bring together countless expert professionals [12].

In order to refine the sample, it was necessary to create criteria for the inclusion or rejection of data, which made it necessary to exclude all karyotypes that present small chromosomal fragments or markers that have not yet been identified, in addition to the karyotypes obtained from bone marrow for leukemia studies, including FISH, since they belong to molecular cytogenomics, which together add up to 9104 data to be analyzed in another future study. Logically, karyotypes with expected results are also excluded, corresponding to 14.344 women and 14.181 men, which in the end reduces the sample to 2691 data, which are those that present numerical and structural anomalies, the reason for this work, which represents all the diversity found at the chromosomal level. This is where the importance of classical cytogenetics as a screening or selection test is evident since it groups the essential data in a fast, precise and low-cost way so that the technology can now be applied to molecular tests like

next-generation sequencing (NGS), massive parallel sequencing (MPS), multiplex li gation-dependent p robe application (MLPA) and fluorescent *in situ* hybridization (FISH) among others. With these technologies, the rupture sites of abnormalities can be detected with complete precision structural features of chromosomes involved in rearrangements, as well as much more information on tiny mutations which reach the nucleotide sequence level.

In order to provide further support to the concept where we affirm that the numerical and structural anomalies currently present in the Colombian population by suggesting that they are also found in the global human population, we present molecular evidence at the level of human population genetics that scientifically demonstrates that Colombia is a megadiverse country at the genetic level, in the Colombian mestizo population, since it is the product of the fusion of the three ethnic groups with the most excellent representation throughout the planet: Native Americans (Asians), Europeans who already have a long history of migratory waves and miscegenation and Africans brought as slaves to the American continent beginning in 1492 with the discovery of America that generated the most significant migration and mixture of populations in human history. Thus, currently, Colombia is made up, in most of the national territory, of 60% European ancestry, 30 to 32% native ancestry (Asian) and 8 to 10% African ancestry. For this reason, it is possible to draw a parallel that relates the diversity of numerical and structural anomalies found with the ancestry of the Colombian population as far as our origin is concerned [13].

2. Enumeration of each of the 17 anomalies found in the Colombian population

We prefer to use sketches to present the facies of the syndromes instead of photographs of the purposes because, in addition to being original, they offer us the possibility of explaining biological individuality, where we are all genetically different, and we are only similar to ourselves, excluding monozygotic twins. For this reason, a photograph would not present a specific syndrome's entire range of facies because photographs are variable. At the same time, with sketches, we can do so, allowing it to be easier to understand the phenomena of penetrance and expressivity, an intrinsic characteristic of genes, where penetrance reflects the clinical severity of each facies while expressivity shows how florid each syndrome can be in terms of the facies it manifests. **Table 1** presents the frequencies of the 17 different anomalies found in the study. We also present the frequencies of each syndrome and its types. In those rearrangements that did not present different types, the frequencies found in the population are presented.

2.1 Down syndrome

As can be seen in **Table 2**, found nine were types of chromosomal rearrangements were found in Down syndrome, which is caused by trisomy of chromosome 21: 93.18% of cases present universal free trisomy, which is caused by meiotic nondisjunction during gamete formation; 2.37% of cases result from mosaicism due to nondisjunction, the most common being postzygotic loss of chromosome 21 from a trisomic zygote (trisomic rescue); 2.02% result from a Robertsonian translocation 14;21, most of which are inherited from one of the parents who is the carrier of the balanced translocation; 1.85% of Down cases are due to a Robertsonian translocation

Total sample of syndromes and other rearrangements		
Down syndrome	1731	64.33%
Turner Syndrome	369	13.71%
XXX Syndrome	32	1.19%
Klinefelter syndrome	107	3.98%
XYY and XXYY syndrome	43	1.60%
Edwards syndrome	111	4.12%
Patau syndrome	52	1.93%
Autosomal trisomies	21	0.78%
Isochromosomes not related to classical syndromes	3	0.11%
Non-Robertsonian translocations	56	2.08%
Robertsonian translocations	54	2.01%
Additions	32	1.19%
Rings	15	0.56%
Duplications	14	0.52%
Investments not related to classic syndromes	6	0.22%
Mosaics not related to classical syndromes	6	0.22%
Deletions not related to classical syndromes	39	1.45%
Total syndromes	**2691**	**100.00**

Table 1.
Total sample of syndromes and other rearrangements.

21;21, in which case the event is hereditary; 0.17% correspond to the 13;14 translocation and Down syndrome in the same purpose (double syndrome); 0.12% correspond to the Robertsonian translocation Y;21; 0.12% to non-Robertsonian translocations, which also present both events in the same purpose; 0.12% correspond to a double syndrome in the same purpose: Down and Klinefelter; 0.06% corresponds to the non-Robertsonian translocation 13;21 with two chromosomal rearrangements in the same purpose. It should be noted that the frequencies were not calculated against the sample (N) of all the syndromes together (2691) but against the total population found in each syndrome, for example, Down syndrome (1791), since the aim is to assess the distribution panorama of the various types or forms that we observe in the syndromes that manifest them (**Figure 1**).

This whole range of different chromosomal rearrangements to generate the same syndrome demonstrates the great diversity that is present at this level in the genome, allowing only those that are compatible to be maintained, while the incompatible rearrangements, when aborted in most pregnancies, cease to be visible because they are incompatible with life, and this information is lost. The most important thing is that we maintain the diversity necessary and admitted by natural selection and thus continue to operate to favor the rearrangements with more excellent adaptation, as occurs with universal free trisomy 21 present in 93.18% of patients with Down syndrome.

If we look at the chromosomal makeup of our ancestor, the chimpanzee, which has 48 chromosomes, most of them phenotypically similar to ours and after five million years of isolation and a constant process of speciation, which undoubtedly, through

Universal Down Syndrome		
47,XX,+21	690	93.18%
47,XY,+21	923	
Total	1613	
Mosaic Down syndrome		
mos 47,XX,+21/46,XX	17	2.37%
mos 47,XY,+21/46,XY	20	
mos 45,X/47,XY,+21	2	
mos 45,X/47,XX,+21	1	
mos 46,XX,der(14;21)(q10;q10),+21/46,XX	1	
Total	41	
Down syndrome due to Robertsonian translocation		
46,XY,der(14;21)(q10;q10),+21	35	2.02%
46,XX,der(14;21)(q10;q10),+21		
46,XY,+21,der(21;21)(q10;q10)	32	1.85%
46,XX,+21,der(21;21)(q10;q10)		
46,XY,der(13;14)(q10;q10),+21	3	0.17%
46,X,der(Y;21)(q10;q10),+21	2	0.12%
Total	72	
Double syndrome: Down and Klinefelter		
48,XXY,+21	2	0.12%
Total	2	
Double syndrome: Down syndrome and non-Robertsonian translocation		
47,XX,t(10;11)(q24;p15),+21	1	0.12%
47,XX,t(7;15)(q36;q22),+21	1	
46,XY,der(13;21)(q10;q10),+21	1	0.06%
Total	3	
Total Down syndrome	1731	64.32%
Total sample of the syndrome population	2691	

Table 2.
Down syndrome and its variants.

the diversity generated by the different genomic rearrangements, has evolved to a karyotype of 46 chromosomes similar to those of the chimpanzee not only in their phenotypic and molecular form but also at the level of the distribution of chromosomal banding.

Maternal age is a factor linked to the appearance of Down syndrome, as with all other trisomies 13, 18, X and Y, since at 30 years of age, the risk of having a child with Down syndrome increases from 1/1000 at 30 years to 1/100 at 40 years. Down syndrome has an incidence of one case per 650 to 1000 live births.

Cytogenetic diagnosis of Down syndrome allows us to demonstrate trisomy 21 in its different phenotypic forms and causal mechanisms. It can be performed by

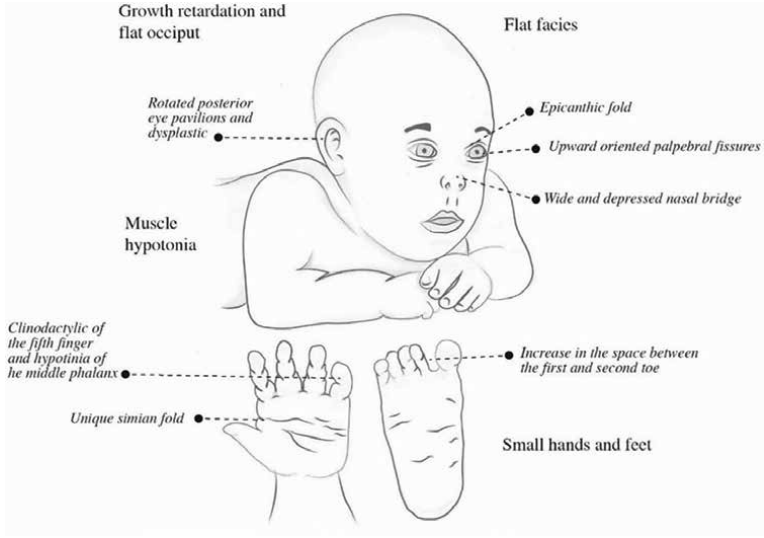

Figure 1.
Phenotypic characteristics of Down syndrome determined by free trisomy, mosaic and Robertsonian translocations 21;21, 21;13, 21;14 and 21;Y.

chorionic villus biopsy, amniocentesis, fluorescent *in situ* hybridization (FISH), or conventional karyotype taken from peripheral blood.

The life expectancy of people with Down syndrome has increased significantly. It is currently 49 years in the United States, with survival rates up to age 60 in 44.4% of cases and up to age 68 in 13.6%. The factors that most severely affect the life expectancy of people with Down syndrome are congenital heart defects and myelodysplastic syndromes.

Genetic counseling depends on the causal mechanism of trisomy 21 (free or translocation) and the other nine forms that characterize this syndrome. In the case of universal free trisomy, the risk of recurrence corresponds to that of the general population. If one of the parents carries a Robertsonian translocation that includes chromosome 21, for example, a rob(14q)(21q), the risk is less than 1%. The risk increases to 10 or 15% if the mother carries this translocation. If a woman with universal trisomy 21 achieves a pregnancy, the risk of having an affected child is the same as that of the general population. In the case of parents with both chromosomes 21 translocated t(21:21), the risk of recurrence is 100%, and in this case, Down syndrome is hereditary.

2.2 Turner syndrome

As can be seen in **Table 3**, they were found seven different types of chromosomal rearrangements have been found in Turner syndrome, the most frequent alteration being the complete or partial absence of an X chromosome, frequently accompanied by mosaicisms. It affects about 1 in 2500–3000 female newborns, and 1% of female fetus conceptions carry this pathology. Between 95 and 99% of affected fetuses may spontaneously abort. The karyotype of Turner syndrome subjects showing a universal X monosomy (45,X) has a frequency of 59.89%, followed by mosaicisms of 21.41% and isochromosomes of 21.41%; long arm of the X in universal form [46,X,i(X)(q10)] of 8.94%; Turner syndrome by deletions was found in 7.98%; followed by translocation in 1.63%; by inversion in 0.27%

Universal Turner Syndrome		
45,X	220	59.89%
Total	**220**	
46,X,psu idic(X)(p21)	1	0.27%
Total	**1**	
Mosaic Turner syndrome		
mos 45,X/46,XX	34	21.41%
mos 45,X/46,X,i(X)(q10)	15	
mos 46,X,i(X)(q10)/45,X	10	
mos 47,XXX/45,X	4	
mos 45,X/47,XXX	2	
mos 45,X/46,X,inv(X)(p21q24)	1	
mos 45,X/46,X,del(X)(q12)	1	
mos 46,X,i(X)(q10)/46,XX	1	
mos 46,XX,pseu dic(X;X)(p22.3;p22.3)/45,X	1	
mos 46,X,del(X)(q11.2)/46,XX	1	
mos 45,X/46,X,del(X)(q11)	1	
mos 45,X/46,X,del(X)(p11)	1	
mos 45,X/46,X,del(X)(p21)	1	
mos 45,X/46,X,del(X)(q22)	1	
mos 45,X/46,X,del(X)(q12)	1	
mos 46,X,i(X)(q10)/45,X	1	
mos 46,X,i(X)(q10)/45,X/46,XX	1	
mos 45,X/47,XXX/46,XX	2	
mos 45,X/46,X,del(X)(q12)/46,XX	1	
Total	**80**	
Turner syndrome due to isochromosome		
46,X,i(X)(q10)	33	8.94%
Total	**33**	
Turner syndrome by deletion		
s46,X,del(X)(q13)	13	7.98%
46,X,del(X)(q25)	1	
46,X,del(X)(p11)	4	
46,X,del(X)(p21)	4	
46,X,del(X)(q11)	1	
46,X,del(X)(q22)	4	
46,X,del(X)(q25)	2	
Total	**29**	
Turner syndrome due to translocation		
46,X,t(X;10)(q22;p15)	1	1.63%

46,XX,t(X;12)(p22.2;q22)	1	
46,X,t(X;13)(q21.2;p12)	1	
46,X,t(X;17)(q22;q25)	1	
46,XX,t(X;20)(p11.4;q13.1)	1	
46,X,t(X;7)(q13;p12)	1	
Total	**6**	
Turner syndrome by inversion		
mos 45,X/46,X,inv.(X)(p21q24)	1	0.27%
Total	**369**	**13.71%**
Total population sample of syndromes	**2691**	

Table 3.
Turner syndrome and its variants.

and finally by iso-dicentric in 0.27%. It should be noted that the frequencies were not calculated against the sample of all the syndromes together (2691) but against the total population that was found in Turner syndrome (369) since the aim is to have an overview of the distribution of the various forms that make up this syndrome (**Figure 2**).

The mother transmits 80% of the X chromosomes in 45,X females. Due to meiotic errors, both the father and the mother can generate Xq isochromosomes. In individuals with Turner syndrome, various structural abnormalities affect the X chromosome, including deletions, inversions, translocations and iso-dicentric chromosomes. The preferential inactivation of the abnormal X chromosome ensures a relatively mild phenotype in most cases.

There is considerable phenotypic variation in Turner syndrome. Some girls have few manifestations, and only short stature suggests the diagnosis. The phenotype may be so subtle that only routine karyotyping of short girls allows a definitive diagnosis since chromosomal damage is visible under a microscope. The presence of mosaicism modifies the phenotype to the point of decreasing the risk of some characteristics, such as coarctation of the aorta. Almost all purposes with Turner syndrome present short stature and gonadal dysgenesis with an average final height without treatment of less than 147 cm; today, hormonal management is available in the early stages [14].

The diagnosis relies primarily on confirmation through conventional karyotype, but in some cases, an increase in the karyotype is necessary to rule out a possible low-frequency mosaic. FISH should be considered in cases that present structural abnormalities in an X chromosome.

The life expectancy of the Purpose-Adults may be reduced compared to their peers due to the high frequency of obesity and cardiovascular disease, mainly coronary heart disease and aortic dissection, which is the most common among those with a pre-existing aortic coarctation. They may experience problems with social integration because their physical characteristics make them look different from their peers. Most have independent lives and are self-sufficient like other adults.

The cytogenetic study should include at least the analysis of 30 to 100 metaphase cells to establish whether there is a mosaic because, despite being controversial, the mosaic cannot be declared if there are not at least two or more metaphases with the anomaly in a count of 30 to 100 metaphases. A single metaphase may be artifactual, but if a specific syndrome is suspected in the clinical setting and two metaphases are found in the count, the carrier of the syndrome should be reported

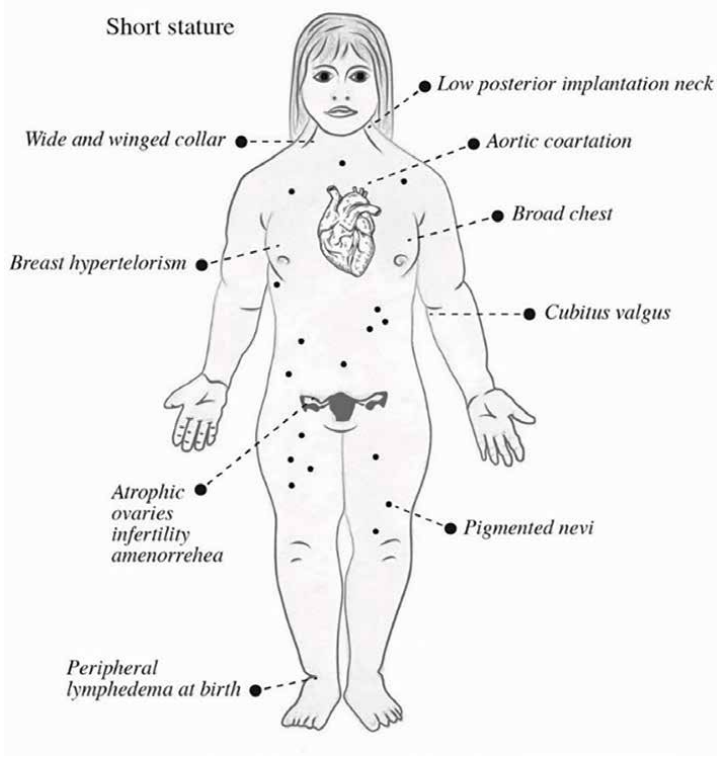

Figure 2.
Phenotypic characteristics of Turner syndrome determined by free monosomy, isochromosome, mosaics, deletions, translocations and inversions.

due to low-frequency mosaicism. A karyotype is not required of the parents in cases of universal monosomy 45,X. Maternal karyotyping is indicated if the woman has a 46,XX karyotype with an X chromosome with a structural abnormality. The risk of recurrence in universal monosomy is very low. Females who are carriers of a structural rearrangement of an X chromosome have a high risk of recurrence, and the phenotype can be severe and even lethal in pregnancies with a male fetus.

Nature accepts the gain of genetic material more than loss. The gain is reflected in trisomies such as 21, 13 and 18 at the autosomal level and is very noticeable at the level of the X chromosome. The loss of genetic material is generally lethal, more so in the autosomes than in the X chromosome. The only monosomy where the entire X chromosome is lost, and which is compatible with life is Turner syndrome. At this level, it is seen that gaining genetic material is more tolerable than losing it since, in the case of the 47,XXX and 48,XXXX syndromes, where women who carry it present fewer side effects on their faces than Turner syndrome.

2.3 XXX syndrome

According to **Table 4**, four different types of trisomy X were found. 47,XXX women have a frequency of 81.25%. Unlike Turner syndrome, these women have a taller-than-average height and fertility equal to women in the general population. The other variant is the 48,XXXX women, who have a frequency of 3.125%. However, in this case, their phenotype is more affected since they may have more marked intellectual

Syndrome XXX y XXXX		
47,XXX	26	81.25%
48,XXXX	1	3.125%
Total	**27**	
Syndrome XXX y XXXX in mosaic		
mos 47,XXX/45,X	4	12.5%
mos 47,XXX/45,X/48,XXXX/46,XX	1	3.125%
TOTAL	**5**	
Total syndrome XXX y XXXX	**32**	**1.19%**
Total population sample of syndromes	**2691**	

Table 4.
Syndrome XXX y XXXX.

disabilities, as well as different facial characteristics with the presence of dysmorphia. Cardiac and renal complications and fertility problems have been observed. Mosaics 47,XXX/45,X were also found with a frequency of 12.5% and the extreme mosaic 47,XXX/45,X/48,XXXX/46,XX with a frequency of 3.125%. Again, the natural rule that gaining genetic material is more favorable than losing it becomes palpable [15].

2.4 Klinefelter syndrome

In the **Table 5** were found six different types of rearrangements were found that cause Klinefelter syndrome and define a group of chromosomal disorders in which there is at least one extra X chromosome about the normal male karyotype, 46,XY. Genotype 47,XXY is found with a frequency of 95.33%, being one of the most common disorders of the sexual chromosomes with a prevalence of 1/600 to 1/800 live male newborns, increasing its incidence from 1/25.000 live newborns at 33 years to 1/300 at 43 years, a

Klinefelter syndrome		
47,XXY	102	95.33%
Total	**102**	
Variants of Klinefelter syndrome		
48,XXXY	1	0.93%
49,XXXXY	2	1.87%
Total	**3**	
Mosaic Klinefelter syndrome		
mos 47,XXY/46,XX	1	0.93%
mos 47,XXY/48,XXYY	1	0.93%
Total	**2**	
Total Klinefelter syndrome	**107**	**3.97%**
Total population sample of syndromes	**2691**	

Table 5.
Klinefelter syndrome, XYY, XXYY. XXXY.

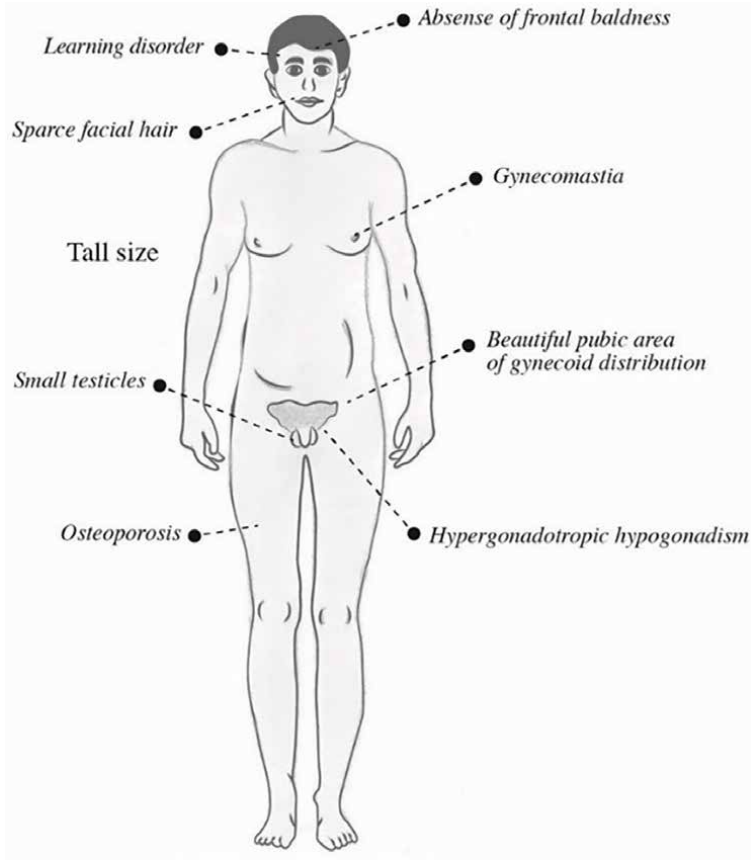

Figure 3.
Phenotypic features of Klinefelter syndrome determined by 48,XXXY; 49,XXXXY and mosaics.

phenomenon associated with maternal age. It is usually diagnosed prenatally as an incidental finding during amniocentesis, chorionic villus sampling or in adult life during the study of male infertility. Most Klinefelter men are probably never diagnosed. Other variants of the syndrome include disorders with more severe clinical features and an additional X or Y chromosome in the karyotype: 49,XXXXY in 1.87%; 48,XXXY in 0.93%; in mosaic mos 47,XXY/46,XX 0.93%; mos 47,XXY/48XXYY in 0.93%. This mosaic was placed within the Klinefelter because it has more XXY metaphases (**Figure 3**).

Higher degrees of aneuploidy for the X or Y chromosomes cause a more severe phenotype. Syndromes 48,XXYY (1 in 25.000 births) and 48,XXXY (1 in 100.000 births). It should be noted that the frequencies were not calculated against the sample of all the syndromes together (2.691) but against the total population found in Klinefelter syndrome (107) since the aim is to have an overview of the distribution of the various types that make up this syndrome [16, 17].

2.5 XYY and XXYY syndrome

In the same way that it happens in the case of Turner syndrome and XXX syndrome, when genetic material is added, the phenotypic and fertility conditions

Syndrome XYY y XXYY		
48,XXYY	7	16.28%
47,XYY	34	79.07%
Total	**41**	
Syndrome XYY in mosaic		
mos 47,XYY/46,XY	2	4.65%
Total	**2**	
Total syndrome XYY y XXYY	**43**	**1.6%**
Total population sample of syndromes	**2691**	

Table 6.
Syndrome XYY, XXYY.

improve. The same happens with Klinefelter syndrome and XXYY syndrome. The latter presents a phenotype opposite to Klinefelter since they are strong, rough, aggressive and normally fertile men. As expected, this is another clear example where nature favors the gain of genetic material more than its loss. According to **Table 6**, three different types of this syndrome were found: the 47,XYY with a frequency of 79.07%; while the 48,XXYY variant, whose frequency is 16.28%, presents with greater severity in some of its phenotypic aspects. Mosaics were also found: mos 47,XYY/46,XY with a frequency of 4.65% tending toward normality. It should be noted that the frequencies were not calculated against the sample of all the syndromes together (2.691) but against the total population found in the XYY, XXYY syndrome (43 individuals) since the aim is to have an overview of the distribution of the various types that make up this syndrome [18].

2.6 Edwards syndrome

As you can see in **Table 7**, they were found three different types of chromosomal rearrangements were found in Edwards syndrome. Universal trisomy 18 with a frequency of 97.3%, a mosaic with a frequency of 1.80% and a double syndrome: Edwards and XXX in 0.9%. About 80 to 90% of cases are due to nondisjunction during maternal meiosis, more frequent in older women. Its population incidence is 1/7900 live births (**Figure 4**).

The literature claims that there is a predominance of affected girls, with a sex ratio of three affected girls to one affected boy. This event is not reproduced in our study since we observed that this value is much lower, with no significant difference between both sexes with Edwards syndrome. Trisomy 18 is associated with a high rate of spontaneous abortions and a poor survival prognosis in newborns. It should be noted that the frequencies were not calculated against the sample of all syndromes together (2.691) but against the total population found in Edwards syndrome (111 individuals) since the aim is to have an overview of the various types that form this syndrome.

In the case of free and universal trisomy, the risk of recurrence is less than 0.55%, corresponding to that of the general population. In cases of translocation, the risk of recurrence may be higher depending on whether one of the parents is a carrier. It should be noted that this syndrome was not found to be associated with mosaics [19].

Universal Edwards syndrome		
47,XX,+18	63	97.3%
47,XY,+18	45	
Total	**108**	
Mosaic Edwards syndrome		
mos 47,XY,+18/46,XY	2	1.80%
Total	**2**	
Double syndrome: Edwards syndrome with XXX syndrome		
48,XXX,+18	1	0.9%
Total	**1**	**0.9%**
Total Edwards syndrome	**111**	**4.12%**
Total population sample of syndromes	**2691**	

Table 7.
Edwards syndrome.

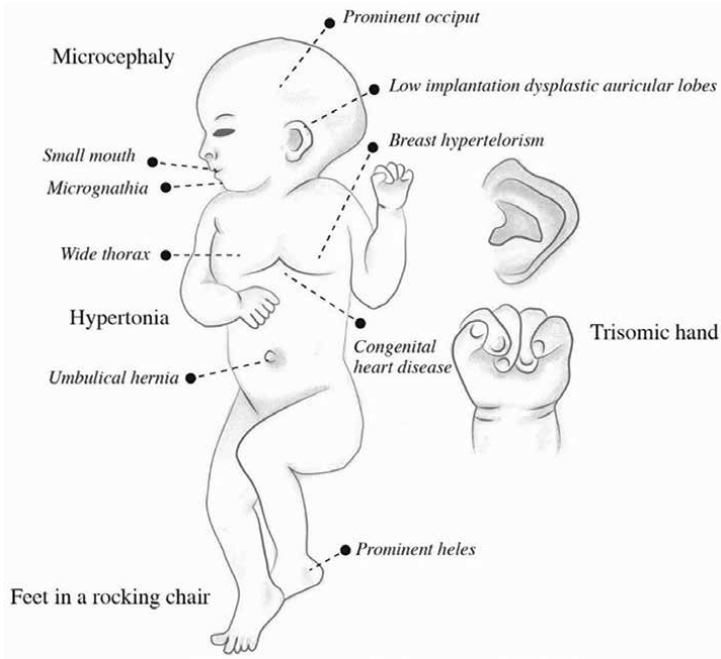

Figure 4.
Phenotypic characteristics of Edwards syndrome determined by free trisomy and mosaics.

2.7 Patau syndrome

In the **Table 8** were observed three different forms of chromosomal rearrangements were found to give rise to Patau syndrome. Trisomy 13 is associated with a high rate of spontaneous abortions and an inferior prognosis in newborns who survive.

Universal Patau syndrome		
47,XX,+13	22	88.46%
47,XY,+13	24	
Total	**46**	
Variants of Patau syndrome		
46,XX,+13,der(13;14)(q10;q10)	2	9.62%
46,XX,+13,der(13;13)(q10;q10)	2	
46,XY,+13,der(13;13)(q10;q10)	1	
Total	**5**	
46,X,der(X)t(X;13)(p22;q12) + 13	1	1.92%
Total	**1**	
Total Patau syndrome	**52**	**1.93%**
Total population sample of syndromes	**2691**	

Table 8.
Patau syndrome and variants.

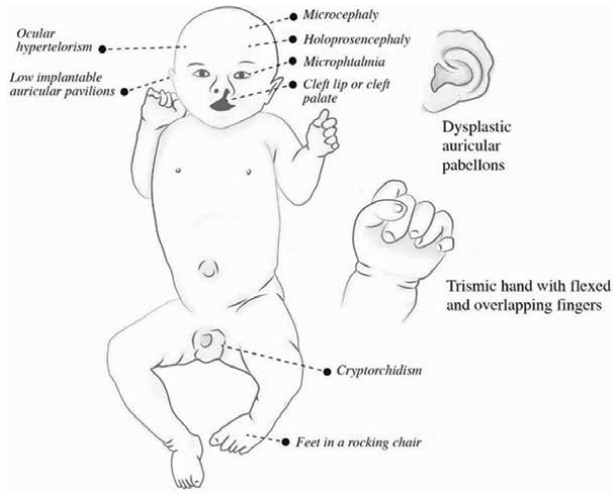

Figure 5.
Phenotypic characteristics of Patau syndrome determined by free trisomy, translocations and derivatives.

The second-trimester spontaneous abortion rate reaches 64%. Trisomy 13 has an incidence of 1/9500 live births [19]. About 88.46% of Patau syndrome cases have a universal trisomy 13, while 9.62% are associated with a Robertsonian translocation: 13;14 and 13;13, very rarely with non-Robertsonian translocations, for example, X;13 with a frequency of 1.92%. In the case of free and universal trisomy, the risk of recurrence is less than 0.5% concerning the general population. As with Edwards syndrome, Patau was not associated with mosaics. It should be noted that the frequencies were not calculated against the sample of all the syndromes together (2.691) but against the total population found in Patau syndrome (n = 52) since the aim is to have an overview of the distribution of the various types that make up this syndrome (**Figure 5**).

2.8 Autosomal trisomies

In **Table 9**, we found the trisomies not associated with traditional trisomies are presented since they involve the exchange of genetic material between chromosomes other than the acrocentric ones. Trisomy 8 is the most frequent, with a frequency of (47.62%) followed by trisomy 22, with a frequency of (28.58%) and finally, trisomy 9, 14, 15 and 16, with a frequency of (4.76%). It should be noted that trisomy 8 and 22 have a significant frequency, indicating that natural selection positively favors them throughout generations [20].

Autosomal trisomies		
47, XY,+8	10	47.62%
47,XX,+22	6	28.58%
47,XY,+22		
47,XY,+15	1	4.76%
47,XX,+14	1	4.76%
47,XX,+16	1	4.76%
47,XY,+9	1	4.76%
47,XY,+der(9)(p24q32)	1	4.76%
Total other trisomies	**21**	**0.78% %**
Total population sample of syndromes	**2691**	

Table 9.
Autosomal trisomies.

2.9 Non-Robertsonian translocations

According to **Table 10**, the following high diversity of non-Robertsonian translocations is associated with their low frequency since, in most cases, only one representative of each of them is found, which may indicate that they are de NOVO mutations and may represent, in the future, the base of the pyramid of diversity where natural

Non-Robertsonian translocations		
46,XX,t(1;2)(p36.3;q32)	1	1.79%
46,XX,t(1;22)(p35;q13)	1	1.79%
46,XX,t(1;5)(p34;q35)	1	1.79%
46,XX,t(1;5)(p36.3;q34)	1	1.79%
46,XX,t(1;7)(q42;p22)	1	1.79%
46,XX,t(1;8)(q12;p23.2)	2	3.57%
46,XX,t(1;13)(p32;q34)	1	1.79%
46,XY,der(1)t(1;13)(p13;p11.2)	1	1.79%
46,XY,t(1;11)(p34;q25)	1	1.79%
46,XY,t(1;11)(q42.2;q21)	1	1.79%
46,XY,t(1;8)(q12;p23.2)	1	1.79%
46,XX,t(1;6),(p36.3;p24)	1	1.79%
46,XY,t(2;17)(p25;q21)	1	1.79%

46,XY,t(2;4)(q22;p16)	1	1.79%
46,XY,t(2;7)(q31;p22)	1	1.79%
46,XX,t(2;12)(p12;p13)	1	1.79%
46,XX,t(2;3)(q24;q25)	1	1.79%
46,XY,t(2;14)(p13;q32)	1	1.79%
46,XX,t(2;4)(p25;q31)	1	1.79%
46,XX,t(2;5)(q11.2;q35)	1	1.79%
46,XX,t(2;15)(q21;q26.3)	1	1.79%
46,XY,t(3;14)(p21;q32)	1	1.79%
46,XY,t(3;4)(p22;q21)	1	1.79%
46,XY,t(3;8)(q25;q24)	1	1.79%
46,XX,t(3;5)(q23;q35)	1	1.79%
46,XX,t(3;5)(q26;q31)	1	1.79%
46,XY,der(4;6)(p16;p21),+6	1	1.79%
45,XX,der(7)t(7;22)(p22;q11.2)	1	1.79%
46,XX,+7,der(7)t(7;12)(q21;q12)	1	1.79%
46,XX,t(7;18)(q32;q23)	1	1.79%
47,XX,t(7;15)(q36;q22),+21	1	1.79%
46,XY,t(7;10)(q11.2;q26)	1	1.79%
46,XY,t(7;17)(q36;q25)	1	1.79%
46,XX,t(14;22)(p13;p13)	1	1.79%
46,XY,der(16)t(16;17)(p13.3;q21)	1	1.79%
46,XY,t(17;19)(p13;q13.2)	1	1.79%
46,XY,+9,t(9;15)(p10;q10)	1	1.79%
45,X,t(Y;4)(q11.2;p16)	1	1.79%
45,XY,dic(14;18)(q11.2;q11.2)	1	1.79%
Total non-Robertsonian translocations	**56**	**2.08%**

Table 10.
Non-Robertsonian translocations.

selection will act since they are just beginning their evolutionary process where natural selection will favor the best adapted and eliminate the less adapted. The vast majority are cases of single appearance with a fundamental frequency of (1.79%). It is important to note that only two rearrangements of this type have doubled their presence in the population with a frequency of 3.57%, which indicates that they have begun their evolutionary path; being notable that one of them involves chromosome 8 which, as we saw in autosomal trisomies, this chromosome also presents the highest frequency. We must emphasize that this chromosome is involved in most anomalies studied [21].

2.10 Robertsonian translocations

As seen in **Table 11**, the Robertsonian translocations that are not associated with any syndrome are shown but generating individuals carrying the balanced translocation that restart the tetravalent meiotic cycle and its distribution when they have offspring. This must be why they do not disappear from the population, but rather,

Robertsonian translocations		
45,XX,der(13;14)(q10;q10)	38	70.38%
45,XY,der(13;14)(q10;q10)		
45,XX,der(14;21)(q10;q10)	6	11.11%
45,XY,der(14;21)(q10;q10)		
45,XX,der(14;15)(q10;q10)	5	9.26%
45,XY,der(14;15)(q10;q10)		
45,XY,der(13;22)(q10;q10)	2	3.70%
45,XY,der(13;15)(q10;q10)	1	1.85%
45,XY,der(21;22)(q10;q10)	1	1.85%
46,XY,+22,der(22;22)(q10;q10)	1	1.85%
Total Robertsonian translocation	**54**	**2%**
Total population sample of syndromes	**2691**	

Table 11.
Robertsonian translocations.

their frequency is increasing with the passing of generations since they are the basis for the appearance of the derived chromosomes [21].

2.11 Additions

As seen in **Table 12**, the additions found in the study are presented and as expected, they comply with the rule of gain of genetic material and, therefore, have a more significant presence in the Colombian genome, highlighting the additions to chromosome 12 with a higher frequency of 12.5%; followed by additions to 15 and 9 with 9.375%. Chromosomes 8, 18 and 3 have a frequency of 6.25%. Finally, chromosomes 1, 4, 9, 10, 12, 13, 14, 16, 18, 21, 22 and X appear, with a minimum frequency of 3.125%, indicating that they are de *NOVO* mutations just beginning their natural selection process.

Additions		
46,XX,add(12)(p13)	4	12.5%
46,XX,add(15)(p11)	3	9.375%
46,XY,add(9)(p24)	3	9.375%
46,XX,add(9)(p24)		6.25%
46,XX,add(8)(p23)	2	6.25%
46,XY,add(8)(p23)	2	6.25%
46,XY,add(18)(q23)	2	6.25%
46,XX,add(3)(p21)	2	6.25%
46,XY,add(9)(q13)	1	3.125%
46,XX,add(1)(q44)	1	3.125%
46,XX,add(13)(p13)	1	3.125%

46,XX,add(16)(q24)	1	3.125%
46,XY,add(22)(p13)	1	3.125%
46,XX,add(21)(q22)	1	3.125%
46,XX,add(22)(q13)	1	3.125%
46,X,add(X)(q28)	1	3.125%
46,XY,add(10)(p12)	1	3.125%
46,XY,add(12)(q13)	1	3.125%
46,XY,add(14)(q11.2)	1	3.125%
46,XY,add(16)(p13.3)	1	3.125%
46,XY,add(18)(p11.3)	1	3.125%
46,XY,add(4)(q35)	1	3.125%
Total additions	**32**	
Total population sample of syndromes	**2691**	**1.19%**

Table 12.
Additions.

2.12 Rings

According to **Table 13**, it was observed that the rearrangements that lead to the formation of rings are presented, which are complex and are not associated with any

Rings		
46,XX,r(5)(p15q35)	2	0.074%
46,XX,r(1)(p36.2q43)	1	0.037%
46,XX,r(15)(p11.2q26.1)	1	0.037%
46,XX,r(20)(p13q13.3)	1	0.037%
46,XY,r(13)(p13q34)	1	0.037%
46,XY,r(18)(p113q23)	1	0.037%
46,XY,r(3)(p26q29)	1	0.037%
46,XY,r(4)(p16q35)	1	0.037%
46,XY,r(9)(p24q34)	1	0.037%
Total	**10**	6.67%
Mosaic ring		
mos 45,XX,-18/46,XX,r(18)(p11q23)	1	0.037%
mos 46,XX,r(10)(p15q26)/45,XX,-10	1	0.037%
mos 46,XY,r(3)(p26q39)/45,XY,-3	1	0.037%
mos 46,XX,r(13)(p11q34)/45,XX,-13	1	0.037%
mos 45,X/46,X,r(Y)(p11.3q12)	1	0.037%
Total	**5**	6.675%
Total rings	**15**	**0.56%**
Total population sample of syndromes	**2691**	

Table 13.
Rings.

of the genetic anomalies studied and all of them are manifested in different autosomal chromosomes except for chromosome 5, where we found the highest frequency of 0.074%. In the other eight casess, they were found with minimum frequencies of 0.037%, which indicates that this anomaly is not very involved in the evolution of the human karyotype and can be classified as random mutation rearrangements.

2.13 Duplications

In duplications, implicit genetic commitment is directly related to the amount of DNA and the genes involved, since this is directly related to protein synthesis. In **Table 14**, we list all the duplications observed in the sample, where most have a fundamental frequency of 0.037%, except for the two duplications found on chromosome 8, with a frequency of 0.074%. It is important to note that chromosome 8 is involved in most of the genetic anomalies found and, more importantly, at a higher frequency than the others. The fact that its frequency has doubled may indicate that natural selection favors it, while the other duplications are de *NOVO* mutations. It is also important to note that the duplications are not associated with other syndromes studied or analyzed.

Duplications		
46,XX,dup(16)(q11.2)	1	0.037%
46,XX,dup(12)(q22q24.33)	1	0.037%
46,XX,dup(13)(q12)	1	0.037%
46,XX,dup(18)(q12)	1	0.037%
46,XX,dup(4)(p16)	1	0.037%
46,XX,dup(4)(q26q35)	1	0.037%
46,XX,dup(7)(p11p13)	1	0.037%
46,XX,dup(9)(p21)	1	0.037%
46,XY,dup(8)(p21p23)	2	0.074%
46,XY,dup(4)(q22q26)	1	0.037%
46,XY,dup(4)(q26q35)	1	0.037%
46,XY,dup(5)(p14)	1	0.037%
Mosaic Duplications		
mos 46,XY,dup(17)(q21q25)/46,XY	1	0.037%
Total	**1**	
Total duplications	**14**	**0.52%**
Total population sample of syndromes	**2691**	

Table 14.
Duplications.

2.14 Investments

One of the adverse effects induced by inversions is the change in the reading frame, which affects the regulation and function of genes. They can also induce genetic variability, which is important for natural selection. An important fact to highlight is that inversions are not associated with any of the rearrangements

Investments not related to classic syndromes		
46,X,inv.(Y)(p11q11)	1	0.037%
46,XY,inv.(9)(p12q13),add(18)(p23)	1	0.037%
46,XY,inv.(3)(p21q13),t(14;17)(p11.2;p11.2)	1	0.037%
46,XX,dup(X)(p22.1),inv.(X)(p22.3p22.1)	1	0.037%
46,XX,inv.(3)(p21q12)	1	0.037%
Total	**5**	
Mosaic investment		
mos 45,X/46,X,inv.(X)(p21q24)	1	0.037%
Total	**1**	
Total investments not related to classical syndromes	**6**	**0.22%**
Total population sample of syndromes	**2691**	

Table 15.
Investments not related to classical syndromes.

analyzed, except for a single case of Turner syndrome, which only appears in mosaic form (mos 45,X/46,X,inv.(X)(p21q24)). As can be seen in **Table 15**, no case exceeded the fundamental frequency of 0.037%, which indicates that most are lethal.

2.15 Deletions

Deletions are not associated with any syndrome analyzed, except for Turner syndrome, where 29 deletions are found, all involving one of the two X chromosomes. According to **Table 16**, the highest frequency observed, 0.371%, is associated with a deletion in chromosome 18, followed by chromosome 5 with 0.29%, chromosome 13 with a frequency of 0.074% and finally, chromosome Y with the same frequency.

Deletions not related to classical syndromes		
46,XX,del(18)(p11.1)	10	0.371%
46,XX,del(18)(q21)		
46,XY,del(5)(p13)	8	0.297%
46,XX,del(5)(p13)		
46,X,del(Y)(q11.2)	2	0.074%
46,XX,del(13)(q31)	2	0.074%
46,XX,del(6)(q25)	1	0.037%
46,XX,del(10)(q25)	1	0.037%
46,XX,del(15)(q22)	1	0.037%
46,XX,del(5)(q31)	1	0.037%
46,XX,del(7)(p15)	1	0.037%
46,XX,del(7)(q32)	1	0.037%
46,XX,del(8)(q21)	1	0.037%
46,XY,del(12)(p12)	1	0.037%

46,XY,del(14)(q23)	1	0.037%
46,XY,del(17)(q12)	1	0.037%
46,XY,del(6)(q23)	1	0.037%
47,XY,+14,del(14)(q24)	1	0.037%
47,XY,+22,del(22)(q12)	1	0.037%
Total	**35**	
Mosaic deletions		
mos 46,XX,del(9)(q10)/46,XX	1	0.037%
mos 46,XY/46,XY,del(18)(p11.1)	1	0.037%
mos 47,XX,+9,del(9)(q34)/46,XX	1	0.037%
mos 46,XX,del(5)(p13),der(16)t(5;16)(p13;p13)/46,XX	1	0.037%
Total	**4**	
Total deletions not related to classical syndromes	**39**	**1.44%**
Total population sample of syndromes	**2691**	

Table 16.
Deletions not related to classical syndromes.

The other deletions presented a fundamental frequency of 0.037%, including four cases where mosaics are presented.

2.16 Mosaics

As expected, mosaics are involved in most of the numerical and structural anomalies analyzed as follows: Turner = 80; XXX and XXXX syndrome = 5; rings = 5; deletions = 4; Klinefelter = 2; XYY and XXYY syndrome = 2; duplications = 1 and inversions = 1. This indicates that any chromosomal anomaly is more compatible with life if found in mosaic form. On the other hand, we found mosaics without any involvement with the analyzed syndromes where only the mosaic 47,XY,+8/46,XY (2 individuals) and 47,XX + 8/46,XX (1 individual) with a frequency of 0.11% all other mosaics are found with a minimum frequency of 0.037%. It is important to highlight that chromosome 8 is involved in mosaics and most of the anomalies analyzed. As evidenced in **Table 17**, this may indicate that this chromosome has remarkable plasticity, since it is present in most chromosomal rearrangements.

Mosaics not related to classical syndromes		
mos 47,XY,+8/46,XY	3	0.111%
mos 47,XX,+8/46,XX		
mos 47,XX,+der(?Y)/46,XX	1	0.037%
mos 45,XY,-22/46,XY	1	0.037%
mos 46,XX,t(6;9)(p22;q34)/46,XX	1	0.037%
Total mosaics not related to classical syndromes	**6**	**0.22%**
Total population sample of syndromes	**2691**	

Table 17.
Mosaics not related to classical syndromes.

2.17 Isochromosomes

Isochromosomes are only involved in Turner syndrome, where we found 33 cases of X isochromosomes. Three isochromosomes were associated with chromosomes 8, 18 and 17, all with a minimum frequency of 0.37%. It is worth noting that for these cases, as evidenced in **Table 18**, we also found chromosome 8 involved, which warrants that future studies should work with chromosome 8.

Isochromosomes not related to classical syndromes		
46,XY,i(18)(q10)	1	0.037%
46,XY,i(8)(q10)	1	0.037%
mos 46,XY,i(17)(q10)/46,XY	1	0.037%
Total isochromosomes not related to classical syndromes	**3**	**0.11%**
Total population sample of syndromes	**2691**	

Table 18.
Isochromosomes not related to classical syndromes.

3. Conclusion

In **Table 1**, we present the global landscape of the main numerical and structural findings from this analysis of 40,320 karyotypes conducted on the Colombian population in general. Seventeen different anomalies were identified, along with the variability of existing rearrangements among each of them, which greatly enhances the variability present in the human genome with respect to these types of chromosomal rearrangements that play a role, one way or another, in the evolution and speciation of the karyotype of all diploid species.

In the 18 attached tables, we show the frequencies of all the combined diversity found in this study, which represents a significant contribution to the literature in this medical-scientific field.

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Chapter 8

Integrating Genetic Counseling in the Management of Mucopolysaccharidoses

Chung-Lin Lee, Ya-Hui Chang, Chih-Kuang Chuang, Huei-Ching Chiu, Yuan-Rong Tu, Yun-Ting Lo, Hsiang-Yu Lin and Shuan-Pei Lin

Abstract

This article explores the critical role of genetic counseling in managing mucopolysaccharidoses (MPS), a group of rare inherited metabolic disorders. It outlines the genetic basis of MPS and discusses how genetic counseling is integrated into various aspects of patient care, from pre-diagnostic stages through long-term management. The study highlights key components of genetic counseling, including medical history collection, genetic testing interpretation, and psychosocial support. It examines the application of genetic counseling in treatment decisions, family planning, and developing long-term management strategies. Specific genetic counseling interventions for MPS, such as carrier testing and prenatal diagnosis, are discussed. The role of genetic counselors in multidisciplinary teams as well as their contribution to patient education and support group coordination is emphasized. The article also addresses current challenges and opportunities in the field, including the effect of emerging technologies, ethical considerations, and importance of culturally sensitive counseling. It concludes by underscoring the significance of genetic counseling in improving outcomes for individuals with MPS, potentially leading to earlier diagnosis, better treatment adherence, and improved quality of life for patients and their families.

Keywords: mucopolysaccharidoses, genetic counseling, rare genetic disorders, multidisciplinary care, personalized medicine

1. Introduction

1.1 Brief overview of mucopolysaccharidoses

Mucopolysaccharidoses (MPS), a group of rare inherited metabolic disorders, are characterized by the body's inability to break down complex sugar molecules called glycosaminoglycans (GAGs) [1]. These disorders result in the progressive GAG accumulation in various tissues and organs, leading to a wide range of clinical

manifestations and complications. Given the complex nature of MPS and its genetic origin, integrating genetic counseling into their management is crucial for providing comprehensive care to patients and their families [2].

MPS is a rare group of disorders, with a combined incidence estimated at 1 in 25,000 live births [1, 2]. While individually rare, collectively they represent a significant burden on affected individuals, families, and healthcare systems. Early diagnosis and intervention are crucial in MPS management, as they can significantly impact disease progression and quality of life. Genetic counseling plays a pivotal role in this process, facilitating early detection, informed decision-making, and comprehensive care planning.

2. Genetic basis of MPS

2.1 Inheritance patterns of MPS

MPS have autosomal recessive inheritance, except for MPS II (Hunter syndrome), which follows an X-linked recessive inheritance pattern [3]. This means that for most MPS types, a child is infected if both parents are carriers of a mutated gene.

2.2 Relevant genes and their mutations

Each type of MPS is associated with mutations in genes specifically encoding lysosomal enzymes responsible for GAG degradation (**Table 1**). For example, MPS I (Hurler syndrome) is caused by IDUA mutations, whereas MPS II is caused by IDS mutations [4]. Understanding these genetic foundations is essential for accurate diagnosis, prognosis, and genetic counseling.

To illustrate the impact of these genetic mutations, consider the case of Sarah, a 3-year-old girl diagnosed with MPS I. Sarah's parents, both carriers of a mutated

MPS type	Common name	Affected gene	Enzyme deficiency	Inheritance pattern	Key clinical features
MPS I	Hurler/Scheie syndrome	IDUA	α-L-iduronidase	Autosomal recessive	Coarse facial features, developmental delay, skeletal abnormalities, cardiac issues
MPS II	Hunter syndrome	IDS	Iduronate-2-sulfatase	X-linked recessive	Similar to MPS I, but unique to males; milder facial features
MPS IIIA	Sanfilippo A	SGSH	Heparan N-sulfatase	Autosomal recessive	Severe behavioral problems, sleep disorders, developmental regression
MPS IIIB	Sanfilippo B	NAGLU	α-N-acetylglucosaminidase	Autosomal recessive	Similar to MPS IIIA
MPS IIIC	Sanfilippo C	HGSNAT	Acetyl-CoA:α-glucosaminide N-acetyltransferase	Autosomal recessive	Similar to MPS IIIA
MPS IIID	Sanfilippo D	GNS	N-acetylglucosamine-6-sulfatase	Autosomal recessive	Similar to MPS IIIA

MPS type	Common name	Affected gene	Enzyme deficiency	Inheritance pattern	Key clinical features
MPS IVA	Morquio A	GALNS	N-acetylgalactosamine-6-sulfate sulfatase	Autosomal recessive	Severe skeletal dysplasia, short stature, normal intelligence
MPS IVB	Morquio B	GLB1	β-galactosidase	Autosomal recessive	Similar to MPS IVA, but typically milder
MPS VI	Maroteaux–Lamy syndrome	ARSB	Arylsulfatase B	Autosomal recessive	Short stature, corneal clouding, cardiac valve disease
MPS VII	Sly syndrome	GUSB	β-glucuronidase	Autosomal recessive	Variable presentation, from mild to severe, often with hydrops fetalis
MPS IX	Natowicz syndrome	HYAL1	Hyaluronidase	Autosomal recessive	Very rare, joint pain and swelling, short stature

Table 1.
Types of mucopolysaccharidoses (MPS). Overview of different types of MPS, including their common names, affected genes, enzyme deficiencies, and inheritance patterns. MPS III (Sanfilippo syndrome) and MPS IV (Morquio syndrome) are further subdivided into their respective subtypes.

IDUA gene, were unaware of their carrier status until Sarah began showing symptoms of developmental delay and coarse facial features. Genetic testing revealed two mutated copies of the IDUA gene, confirming her diagnosis [5]. This case highlights the importance of understanding the genetic basis of MPS for early diagnosis and family planning.

3. Role of genetic counseling in MPS management

3.1 Pre-diagnostic counseling

Pre-diagnostic genetic counseling takes on a vital role in preparing individuals and families for the possibility of an MPS diagnosis. It involves discussing the reasons for genetic testing, explaining the testing process, and preparing the family for potential outcomes [6]. For example, in a case where a child presents with symptoms suggestive of MPS, a genetic counselor might discuss the following with the family:

- The purpose of genetic testing in diagnosing MPS
- The different types of MPS and their inheritance patterns
- The potential implications of a positive diagnosis
- The emotional and practical support available throughout the testing process

3.2 Postdiagnostic counseling

Once a diagnosis is confirmed, postdiagnostic counseling becomes crucial. This stage involves explaining the diagnosis, its implications, and available management options. It also includes providing emotional support and connecting families with

appropriate resources [7]. For instance, after Sarah's MPS I diagnosis, a genetic counselor might:

- Explain the specific genetic mutation causing Sarah's condition
- Discuss the prognosis and potential complications of MPS I
- Outline available treatment options, including enzyme replacement therapy and stem cell transplantation
- Provide information about support groups and resources for families affected by MPS

3.3 Family risk assessment

To identify other family members who may be at risk of carrying or being affected by MPS, genetic counselors perform comprehensive family risk assessments. This process involves constructing detailed pedigrees and offering testing to relevant family members (**Figure 1**) [8]. Family risk assessment is a critical component of genetic counseling for

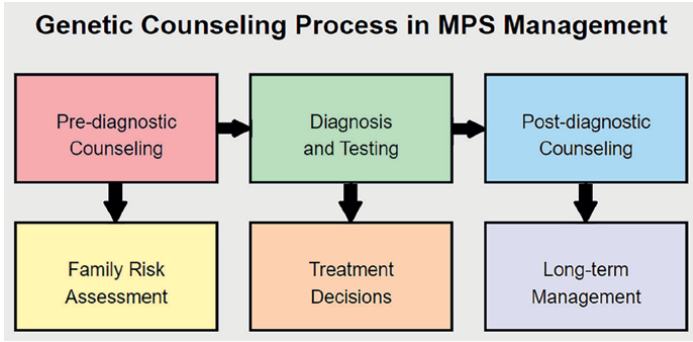

Figure 1.
The Genetic Counseling Process in MPS Management. Flowchart illustrating the genetic counseling process in MPS management, from pre-diagnostic counseling through long-term management.

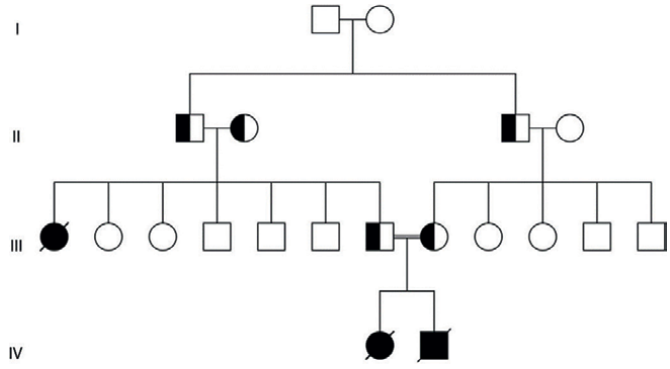

Figure 2.
Pedigree of a family affected by MPS I. Squares represent males, and circles represent females. Shaded symbols indicate affected individuals, half-shaded symbols represent carriers, and unshaded symbols show unaffected individuals. Double lines between parents indicate consanguinity. Roman numerals (I-IV) designate generations. The proband (IV-3) is indicated by an arrow.

MPS. By constructing detailed pedigrees, genetic counselors can identify patterns of inheritance and assess the risk for other family members. **Figure 2** illustrates the pedigree of a family affected by MPS I, demonstrating the autosomal recessive inheritance pattern [9]. In this pedigree, we can see that the proband (IV-3, indicated by an arrow) is affected with MPS I. Both parents (III-2 and III-3) are carriers, as are some other family members (II-1, II-2, III-1, IV-2). This pedigree also shows consanguinity in the first generation (I-1 and I-2), which increases the risk of autosomal recessive disorders like MPS I in subsequent generations. By analyzing such pedigrees, genetic counselors can identify at-risk individuals who may benefit from carrier testing or early diagnostic screening, facilitating early intervention and informed family planning decisions.

4. Key components of genetic counseling

4.1 Collection of medical and family history

A thorough medical and family history is the cornerstone of genetic counseling. It helps identify patterns of inheritance, assess risk, and guide genetic testing strategies [10].

Component	Description	Importance	Examples
Medical and family history collection	Gathering comprehensive information about the medical background of the patients and their families	Helps identify inheritance patterns and assess risk	• Constructing a three-generation pedigree • Documenting specific MPS symptoms and their onset in family members
Genetic testing interpretation	Explaining complex genetic information and test results to patients and families	Aids in making informed decisions and understanding of the condition	• Explaining the significance of identified mutations in MPS-related genes • Discussing the implications of carrier status
Psychosocial dupport	Providing emotional support and coping strategies	Helps families deal with the psychological effect of MPS diagnosis	• Using active listening techniques to address parents' concerns • Providing referrals to support groups or mental health professionals
Treatment option discussion	Explaining available therapies and emerging treatments	Assist in making informed treatment decisions	• Discussing pros and cons of enzyme replacement therapy • Explaining potential benefits and risks of stem cell transplantation
Family planning guidance	Discussing reproductive options and risks	Helps families make informed decisions about future pregnancies	• Explaining options like preimplantation genetic testing • Discussing the recurrence risk for future pregnancies
Long-term management planning	Developing strategies for ongoing care and support	Ensures comprehensive, long-term care for patients with MPS	• Creating a timeline for regular check-ups with various specialists • Discussing potential future needs like educational support or home modifications

Table 2.
Key components of genetic counseling in MPS management. Overview of the key components of genetic counseling in MPS management, including their descriptions and importance in patient care.

4.2 Interpretation and discussion of genetic testing

Genetic counselors play a crucial role in explaining complex genetic information to patients and families. They interpret test results, discuss their implications, and help families make informed decisions based on this information [11].

4.3 Psychosocial support

An MPS diagnosis can have significant psychological effects. Genetic counselors provide essential emotional support and help families cope with the challenges associated with living with a rare genetic disorder [12] (**Table 2**). Specific techniques used by genetic counselors may include:

- Active listening and empathy to validate the family's emotions
- Cognitive-behavioral techniques to help manage anxiety and depression
- Family systems therapy to address relationship dynamics affected by the diagnosis
- Mindfulness and relaxation techniques to manage stress

5. Application of genetic counseling in MPS treatment decisions

5.1 Discussion of treatment options

Genetic counselors work alongside other healthcare professionals to discuss available treatment options, including enzyme replacement therapy, hematopoietic stem cell transplantation, and emerging therapies such as gene therapy [13]. **Table 3** provides a comprehensive comparison of current treatment options for MPS, including both established and emerging therapies.

5.2 Family planning

Genetic counseling is crucial in discussing reproductive options for families affected by MPS. This may include preimplantation genetic testing, prenatal diagnosis, or discussion of alternative family-building options [14]. The psychological impact of these decisions can be profound, and genetic counselors play a key role in providing support. They may:

- Discuss the emotional aspects of family planning decisions
- Provide information about support groups for families facing similar decisions
- Offer strategies for coping with uncertainty and guilt
- Facilitate communication between partners who may have differing views on family planning

Treatment	Description	Benefits	Limitations
Enzyme Replacement Therapy (ERT)	Intravenous infusion of recombinant enzyme to replace the deficient enzyme	• Improves organ function and quality of life • Reduces urinary GAG levels • Non-invasive and widely available	• Does not cross the blood-brain barrier • Requires lifelong weekly or biweekly infusions • High cost • May induce antibody formation
Hematopoietic Stem Cell Transplantation (HSCT)	Transplantation of healthy stem cells to provide a continuous source of enzyme	• Can cross the blood–brain barrier • Potential for long-term correction • May improve cognitive outcomes in some MPS types	• High-risk procedure with potential mortality • Limited availability of suitable donors • Risk of graft-versus-host disease • Most effective when performed early
Gene Therapy	Introduction of functional genes to correct the genetic defect	• Potential for long-term or permanent correction • May address both systemic and neurological symptoms • Could be a one-time treatment	• Still experimental for most MPS types • Long-term effects unknown • Potential for off-target effects • High cost of development and treatment
Substrate Reduction Therapy (SRT)	Reduces the production of GAGs to balance the impaired degradation	• Oral medication (easier administration) • May cross the blood–brain barrier • Could be used in combination with ERT	• Still in clinical trials for most MPS types • May not be effective for all MPS types • Potential side effects are not fully known
Supportive Care	Symptomatic treatment addressing specific complications of MPS	• Addresses immediate quality of life issues • Can be tailored to individual patient needs • Often necessary regardless of other treatments	• Does not address the underlying cause of MPS • May require multiple specialists and interventions • Ongoing and potentially burdensome for patients and families

Table 3.
Comparison of Treatment Options for Mucopolysaccharidoses (MPS).

5.3 Long-term management strategies

Genetic counselors assist in formulating long-term management strategies, considering the progressive nature of MPS and the need for ongoing medical care and support [15].

6. Genetic counselors in multidisciplinary teams

6.1 Collaboration with other specialists

MPS management requires a multidisciplinary approach. To provide comprehensive care, genetic counselors collaborate with various specialists, including metabolic

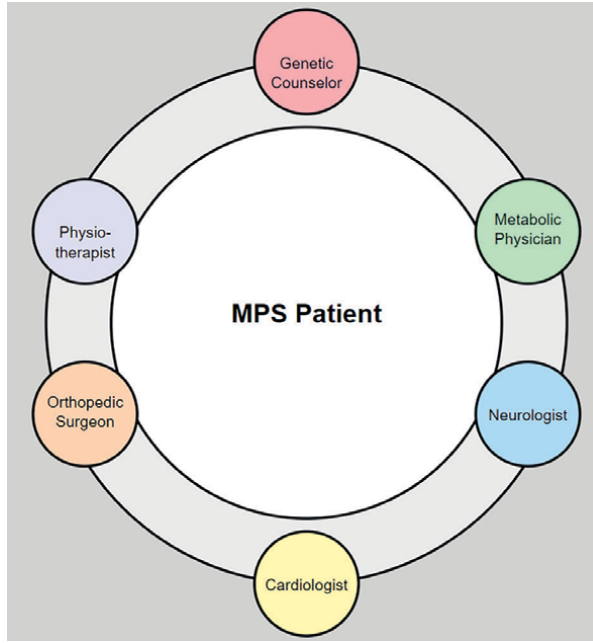

Figure 3.
Multidisciplinary Team Approach in MPS Management. Illustration of the multidisciplinary team approach in MPS management, showcasing the various specialists involved in patient care.

physicians, neurologists, cardiologists, and orthopedic surgeons [16] (**Figure 3**). For example, a genetic counselor might:

- Coordinate with a metabolic physician to explain enzyme replacement therapy options
- Work with a neurologist to help a family understand the cognitive impacts of MPS
- Liaise with a cardiologist to discuss cardiac monitoring and management
- Collaborate with an orthopedic surgeon to explain potential surgical interventions

6.2 Coordination of patient education and support groups

Genetic counselors often coordinate patient education initiatives and facilitate support groups, which are essential for empowering patients and families affected by MPS [17].

7. Challenges and opportunities in genetic counseling

7.1 Effects of emerging technologies and treatments

The rapid advancement of genetic technologies and emerging treatments presents opportunities and challenges for genetic counseling in MPS. Staying updated

with these developments is crucial for providing accurate and current information to families [18]. Recent advances in gene editing technologies, such as CRISPR-Cas9, offer potential new avenues for MPS treatment. While still in the early stages for MPS, these technologies may eventually allow for correction of the genetic mutations causing the disorder. Genetic counselors must stay informed about these developments to:

- Provide accurate information about potential future treatments
- Discuss the ethical implications of gene editing technologies
- Help families navigate decisions about participating in clinical trials

7.2 Ethical considerations

Genetic counselors must navigate complex ethical issues in their practice. Some key ethical challenges include [19]:

- Genetic testing in minors: Balancing the potential benefits of early diagnosis and intervention with the child's right to an open future and autonomy in decision-making.
- Disclosure of incidental findings: Deciding when and how to disclose unexpected genetic information that may have health implications for the patient or their family members.
- Balancing individual privacy with family risk: Navigating the tension between maintaining patient confidentiality and the potential need to inform at-risk family members.
- Prenatal testing and selective termination: Addressing the ethical implications of prenatal testing for MPS and the subsequent decisions families may face.
- Resource allocation: Considering the high cost of some MPS treatments and how to ethically allocate limited resources.
- Genetic discrimination: Helping patients navigate concerns about potential discrimination based on genetic information, particularly in insurance and employment contexts.
- Duty to recontact: Determining when and how to recontact patients with updated genetic information or newly available treatments.

Real-world ethical dilemmas genetic counselors might face include: [19].

- A teenage MPS patient who does not want their siblings to know about their carrier status, despite the potential reproductive implications for the siblings.
- Parents who disagree about whether to pursue experimental gene therapy for their child with MPS.

- Discovering that a patient's genetic test results reveal non-paternity, with potential implications for inheritance patterns and family dynamics.

7.3 Cultural sensitivity and personalized counseling

Recognizing and respecting cultural differences is essential in genetic counseling. Tailoring counseling approaches to the needs and cultural backgrounds of each family enhances the effectiveness of genetic counseling in MPS management [20].

8. Conclusion

8.1 Summary of the importance of genetic counseling for patients with MPS and their families

Genetic counseling plays a pivotal role in the comprehensive management of mucopolysaccharidoses (MPS) by providing essential support from diagnosis through long-term care. It empowers patients and families with knowledge, support, and tools to make informed decisions [21]. Key aspects of genetic counseling in MPS management include:

- Facilitating accurate and timely diagnosis
- Providing clear, understandable information about MPS and its implications
- Offering psychosocial support to patients and families
- Assisting in treatment decision-making and long-term management planning
- Addressing family planning and reproductive options
- Coordinating multidisciplinary care

8.2 Future outlook and research directions

The future of genetic counseling in MPS management looks promising, with potential advancements in several areas that could significantly enhance patient care and outcomes. These advancements are likely to reshape the practice of genetic counseling for MPS in the coming years [22].

8.2.1 Personalized medicine and targeted therapies

Future research is likely to focus on developing more personalized treatment approaches for MPS patients based on their specific genetic profiles.

8.2.2 Advanced diagnostic techniques

Improvements in genetic testing technologies, such as whole genome sequencing and advanced bioinformatics, may lead to earlier and more accurate diagnosis of MPS.

8.2.3 Gene therapy and gene editing

Ongoing research into gene therapy and gene editing techniques, such as CRISPR-Cas9, may lead to new treatment options for MPS.

8.2.4 Newborn screening

Efforts to include more MPS types in newborn screening programs may lead to earlier diagnosis and intervention.

8.2.5 Psychosocial research

Further research into the long-term psychosocial impacts of living with MPS and the effectiveness of various counseling interventions could improve support for patients and families.

8.2.6 Telemedicine and digital health

Advancements in telemedicine and digital health technologies may change how genetic counseling services are delivered.

8.2.7 Artificial intelligence and machine learning

The integration of AI and machine learning in genomics and healthcare may provide new tools for risk assessment and treatment planning.

8.2.8 Collaborative international research

Increased international collaboration in rare disease research may lead to larger datasets and more robust findings about MPS.

As these research directions progress, genetic counselors will play a crucial role in translating new scientific advancements into practical, understandable information for MPS patients and their families. They will need to continually update their knowledge and skills to provide the best possible care in this rapidly evolving field.

The future of genetic counseling in MPS management holds great promise for improving patient outcomes through more precise diagnosis, targeted treatments, and comprehensive support. As our understanding of MPS and our technological capabilities advance, the role of genetic counselors will become increasingly vital in helping patients and families navigate the complex landscape of genetic medicine.

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Conflict of interest

The authors declare that they have no competing interests or potential conflicts of interest in this study.

Other declarations

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of MacKay Memorial Hospital (approval number: 21MMHIS109e, approval date: 2021/10/01).

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Nomenclature

GAGs	Glycosaminoglycans
MPS	Mucopolysaccharidoses
ERT	Enzyme Replacement Therapy
HSCT	Hematopoietic Stem Cell Transplantation
IDUA	α-L-iduronidase
IDS	Iduronate-2-sulfatase
SGSH	Heparan N-sulfatase
NAGLU	α-N-acetylglucosaminidase
HGSNAT	Acetyl-CoA:α-glucosaminide N-acetyltransferase
GNS	N-acetylglucosamine-6-sulfatase
GALNS	N-acetylgalactosamine-6-sulfate sulfatase
GLB1	β-galactosidase
ARSB	Arylsulfatase B
GUSB	β-glucuronidase
HYAL1	Hyaluronidase

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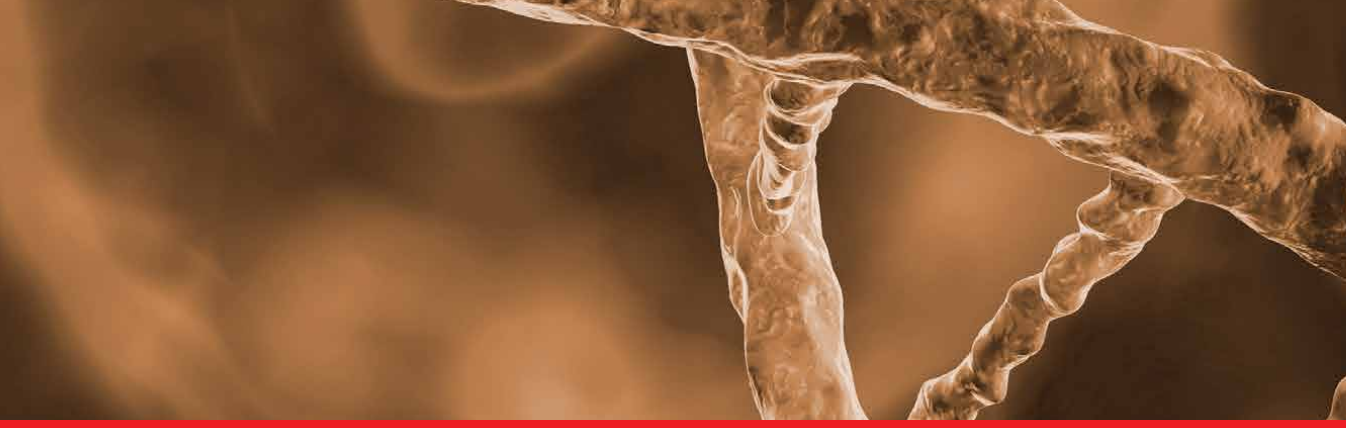

Edited by Sonia Soloneski

This book highlights the importance of various types of chromosomal aberrations and their profound impact on genomic stability, disease development, evolutionary processes, and clinical practice. In doing so, this book builds an understanding of the origins and implications of various chromosomal abnormalities and offers guidance for future investigations. By combining classical cytogenetics with modern molecular insights, this book provides readers with a multidisciplinary perspective on genome integrity, disease mechanisms, and therapeutic innovation.

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