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Insights and Solutions

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Meet the editor

Prof. Dr. Zulqarnain Baloch is a distinguished academic and researcher with expertise in microbiology and infectious diseases. He currently serves as a professor in the Faculty of Life Science and Technology at Kunming University of Science and Technology in China, where he engages in research focused on unravelling the complex interactions between pathogens and their hosts. Dr. Baloch has contributed significantly to the field through numerous publications in peer-reviewed journals, focusing on the epidemiology, prevention, and treatment of infectious diseases. His work has garnered recognition both nationally and internationally, and he is known for his commitment to advancing scientific knowledge and fostering collaboration among researchers. In addition to his research endeavors, Dr. Baloch is dedicated to mentoring the next generation of scientists and promoting interdisciplinary approaches to address global health challenges. He actively participates in conferences and workshops, sharing his insights and expertise with colleagues and students alike. With a passion for addressing pressing health issues, Prof. Dr. Zulqarnain Baloch continues to make significant contributions to understanding infectious diseases and their impact on public health.

Contents

Preface

Human papillomavirus (HPV) is a pervasive and persistent challenge in global health. It is responsible for a wide spectrum of diseases, from benign warts to life-threatening cancers. While significant strides have been made in understanding, treating, and preventing HPV, it continues to affect millions of lives each year. Given these ongoing challenges, there is a growing need for a new resource on HPV.

Confronting HPV – Insights and Solutions aims to thoroughly explore the most up-to-date research and strategies surrounding HPV epidemiology, treatment, and prevention. This book is designed for healthcare professionals, researchers, and public health advocates seeking to understand the virus's complex nature and its impact on public health. I have compiled the available scientific findings, practical treatment approaches, and preventive measures in this work.

This book goes beyond the medical and clinical aspects of HPV, delving into its epidemiology and the critical role of vaccination in prevention. By confronting HPV head-on, we can better equip ourselves with the knowledge and tools needed to combat its spread, reduce stigmatization, and empower individuals with prevention strategies. This book aims to offer actionable insights that can be used to drive positive change in both policy and practice, ultimately improving the lives of those affected by HPV.

I want to express my gratitude to the researchers, clinicians, and public health experts whose work has informed much of the content in this book. Their dedication continues to inspire advancements in HPV management. I hope that *Confronting HPV – Insights and Solutions* will contribute to the ongoing global fight against HPV, serving as a valuable resource for those dedicated to reducing its burden.

Thank you for joining me in exploring one of our time's most critical public health issues.

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Chapter 1

Basic Principles of Cervical Cancer Treatment

Li Yuanyue, Hu Qian, Liu Ling, Yuan Tao, Ge Jing and Wu Xiaomei

Abstract

This chapter delves into the fundamental principles governing the treatment of cervical cancer. It explores the foundational aspects essential for understanding the various modalities of treatment employed in managing this disease. Through a comprehensive examination, it elucidates the key principles guiding therapeutic decisions, encompassing aspects such as early detection, staging, and personalized treatment strategies. Additionally, the chapter discusses the importance of multidisciplinary approaches and emerging trends in cervical cancer management, offering insights into optimizing patient outcomes and advancing the field of oncology.

Keywords: HPV, principles, diagnosis, treatments, cervical cancer

1. Introduction

Cervical cancer, caused primarily by persistent infections with high-risk human papillomavirus (HPV) types, stands as a significant global health challenge. It ranks as the fourth most common cancer among women worldwide, with approximately 570,000 new cases and 311,000 deaths reported in 2018 alone [1]. This disease places a disproportionate burden on women in low- and middle-income countries, emphasizing the urgent need for comprehensive strategies, particularly in regions where healthcare resources may be limited [2]. It needs a multifaceted and principled approach to treatment. The basic principles of cervical cancer treatment serve as the foundation upon which therapeutic strategies are built, aiming to maximize efficacy while minimizing adverse effects. This chapter delves into the fundamental tenets that guide clinicians in their endeavor to combat cervical cancer, exploring key principles that encompass the realms of diagnosis, surgery, radiation therapy, chemotherapy, and holistic patient care.

As we navigate through the intricate landscape of cervical cancer therapeutics, this chapter will illuminate the essential principles that guide clinical decisions, encompassing the nuances of surgical interventions, the precision of radiation therapy, the systemic impact of chemotherapy, and the importance of supportive care. The intricate interplay of these principles underscores the dynamic nature of cervical cancer treatment, emphasizing the need for a holistic and collaborative approach to enhance both the clinical and emotional well-being of individuals facing this formidable diagnosis.

2. Early detection and diagnosis

Cervical cancer is a formidable global health concern, disproportionately affecting women across diverse socioeconomic and cultural landscapes. The imperative of early detection and precise diagnosis in this context cannot be overstated. This chapter endeavors to unravel the critical dimensions of early detection, examining the historical evolution of screening methods, the intricacies of diagnostic techniques, the challenges encountered, and the promising innovations that offer hope on the horizon.

2.1 Importance of early detection

The significance of early detection lies in its transformative potential for improving treatment outcomes and ultimately reducing mortality rates associated with cervical cancer. Early-stage diagnosis allows for less invasive treatment options, better preservation of fertility, and increased chances of successful intervention. Beyond the individual impact, early detection plays a crucial role in public health by curbing the spread of the disease and lessening the economic burden on healthcare systems.

2.2 Screening programs: A gateway to early detection

2.2.1 Pap smear (Pap test)

The historical trajectory of cervical cancer screening took a monumental turn with the introduction of the Pap smear, a cytological examination developed by Dr. George Papanicolaou in the early twentieth century. This simple yet revolutionary test involves collecting cells from the cervix to detect abnormalities. Pap smears have been instrumental in identifying pre-cancerous lesions and guiding early interventions. However, challenges such as false positives and the need for regular screenings persist.

2.2.2 HPV testing

The integration of HPV testing into screening programs has marked a paradigm shift in cervical cancer prevention. HPV, particularly high-risk types such as HPV-16 and HPV-18, is a primary causative factor for cervical cancer. Combining HPV testing with Pap smears enhances the accuracy of identifying high-risk individuals, allowing for targeted interventions and reducing unnecessary procedures for low-risk populations.

2.2.3 Emerging technologies

Recent years have witnessed the advent of advanced screening technologies, including liquid-based cytology and molecular testing. Liquid-based cytology improves specimen quality, reducing the likelihood of inadequate samples and enhancing the sensitivity of the test. Molecular testing, which can identify HPV DNA, RNA, or specific biomarkers, holds promise for increased accuracy and efficiency in detecting cervical abnormalities.

3. Diagnostic techniques: Precision in identification

3.1 Colposcopy

Colposcopy, an optical examination of the cervix, is often employed to further evaluate abnormal Pap smear results. This technique allows for the visualization of suspicious areas, guiding clinicians to targeted biopsies. Technological advancements, such as enhanced imaging modalities like colposcopic photography, aid in refining diagnostic accuracy.

3.1.1 Biopsy

Once abnormalities are identified, various biopsy types, including punch biopsies, cone biopsies, and endocervical curettage, play a pivotal role in confirming a diagnosis. Histopathological examination of biopsy specimens provides definitive insights into the nature and extent of cervical abnormalities, facilitating precise treatment planning.

3.1.2 Imaging modalities

For comprehensive staging and assessment of the extent of cervical cancer, imaging modalities such as magnetic resonance imaging (MRI) and computed tomography (CT) scans come into play. These technologies aid clinicians in determining the appropriate course of treatment based on the stage of the disease.

3.2 Role of biomarkers in early detection

Biomarkers associated with cervical cancer, including proteins, genetic markers, and HPV-specific markers, offer potential avenues for early detection and personalized treatment. Their identification and validation represent a promising frontier in improving diagnostic precision and tailoring interventions to individual patient profiles.

3.3 Challenges and innovations in early detection

3.3.1 Socioeconomic barriers

While advancements in screening and diagnostic technologies are promising, socioeconomic barriers persist, hindering access to these critical services. Disparities in healthcare access, education, and awareness contribute to delayed diagnoses and poorer outcomes. Addressing these barriers requires targeted strategies, community engagement, and policy initiatives to ensure equitable access to early detection and diagnostic services.

3.3.2 Ongoing research and technological advancements

The landscape of cervical cancer early detection is dynamic, with ongoing research exploring innovative methods. Artificial intelligence and machine learning applications in cervical cancer diagnostics show promise in enhancing accuracy and efficiency. These technological advancements have the potential to revolutionize early detection processes, making them more accessible and effective on a global scale.

3.4 Patient education and awareness

3.4.1 Empowering individuals

Patient education plays a pivotal role in promoting awareness of cervical cancer risk factors, symptoms, and the importance of regular screenings. Empowered individuals are more likely to seek timely medical attention, fostering a proactive approach to cervical health.

3.4.2 Cultural sensitivity

Cultural nuances and societal taboos can impact women's willingness to participate in screening programs. Tailoring awareness campaigns to address these cultural sensitivities and fostering open dialogs about cervical health contribute to increased screening rates and early detection.

4. Importance of multidisciplinary collaboration for dealing with cervical cancer

Cervical cancer, a significant global health challenge affecting women worldwide, demands a nuanced and multifaceted approach to effective treatment. The complexity of this malignancy, characterized by its diverse stages and treatment modalities, necessitates a collaborative effort among healthcare professionals across various specialties. Multidisciplinary collaboration in cervical cancer treatment brings together the expertise of gynecologic oncologists, radiation oncologists, medical oncologists, surgeons, pathologists, radiologists, nurses, and support staff, forming a cohesive team that collectively navigates the complexities of diagnosis, treatment planning, and ongoing care.

4.1 The multidisciplinary team: composition and roles in cervical cancer treatments

4.1.1 Gynecologic oncologists

Gynecologic oncologists are at the forefront of cervical cancer care, providing expertise in surgical and medical interventions specific to gynecologic cancers. Their leadership in treatment planning and coordination is pivotal in ensuring a comprehensive approach tailored to each patient's unique circumstances.

4.1.2 Radiation oncologists

Specializing in delivering targeted radiation therapy to cancerous tissues, radiation oncologists collaborate closely with other specialists. Their role is integral in determining the optimal radiation strategy, whether as a standalone treatment or in combination with surgery and chemotherapy.

4.1.3 Medical oncologists

Medical oncologists bring expertise in systemic therapies, including chemotherapy. Collaborating with other team members, they contribute to treatment decisions aimed at maximizing the effectiveness of chemotherapy while minimizing adverse effects.

4.1.4 Surgeons

Surgeons play a critical role in cervical cancer treatment, performing procedures such as radical hysterectomy or lymph node dissection. Their expertise is essential in determining the feasibility and extent of surgical interventions, collaborating with other specialists for comprehensive care.

4.1.5 Pathologists

Pathologists are instrumental in the diagnostic phase, conducting histopathological examinations to confirm the presence of cancer and characterize its specific features. Their findings guide treatment decisions and contribute to the overall understanding of the disease.

4.1.6 Radiologists

Imaging experts and radiologists contribute to the staging and treatment planning processes. Through technologies like MRI and CT scans, they provide crucial insights into the extent of the disease, aiding in collaborative decision-making.

4.1.7 Nurses and support staff

Nurses and support staff form the backbone of patient care, playing integral roles in education, emotional support, and overall coordination within the multidisciplinary team. Their contributions enhance communication and streamline the patient care experience.

5. Available treatment approaches

The integration of precision medicine into cervical cancer treatment involves analyzing genomic and molecular data to tailor interventions. Multidisciplinary collaboration is crucial in interpreting genetic information and implementing targeted therapies based on individual tumor profiles. An outline of how cervical cancer is managed and treated varies depending on the stage of the disease. The approach involves different strategies for early-stage and more advanced cases.

For early-stage cervical cancer, surgery is often a primary treatment method. Options include total hysterectomy (with or without salpingo-oophorectomy) for women who have completed childbearing, radical hysterectomy for larger lesions, and fertility-sparing surgeries such as loop electrosurgical excision procedure (LEEP), conization, and trachelectomy for women of childbearing age.

In cases where surgery may not be sufficient or appropriate, other treatments come into play. Radiation therapy, either alone or in combination with chemotherapy, is commonly employed. This is particularly relevant for cases where the cancer has spread beyond the cervix or if surgery is not a viable option.

As the disease progresses to more advanced stages, a combination of treatments is often recommended. Chemotherapy, either alone or in conjunction with radiation therapy, may be used to target cancer cells throughout the body. In some instances, targeted therapies and immunotherapy might be considered as part of the treatment plan.

The specific management and treatment plan are tailored to the individual's condition, taking into account factors such as the stage of the disease, overall health, and personal preferences. Regular follow-up and monitoring are crucial to assess the effectiveness of the chosen treatment and address any potential side effects or complications. Individuals diagnosed with cervical cancer need to work closely with their healthcare team to determine the most appropriate and effective course of action based on their unique circumstances.

Here, we are discussing available treatment approaches one by one.

5.1 Surgery

Surgery stands as a widely employed and effective method for addressing early-stage cancers, involving the physical extraction of cancerous tissue. Not only is it capable of removing the primary tumor, but it can also eliminate metastatic tissue [3]. In the context of treating cervical cancer, various surgical procedures are currently in use, each chosen based on the disease stage and the extent of its spread [4]. Details can be checked in **Figure 1**. Choices include total hysterectomy (**Figure 2**), radical hysterectomy, loop electrosurgical excision procedure (LEEP), conization, trachelectomy, and cryosurgery [6].

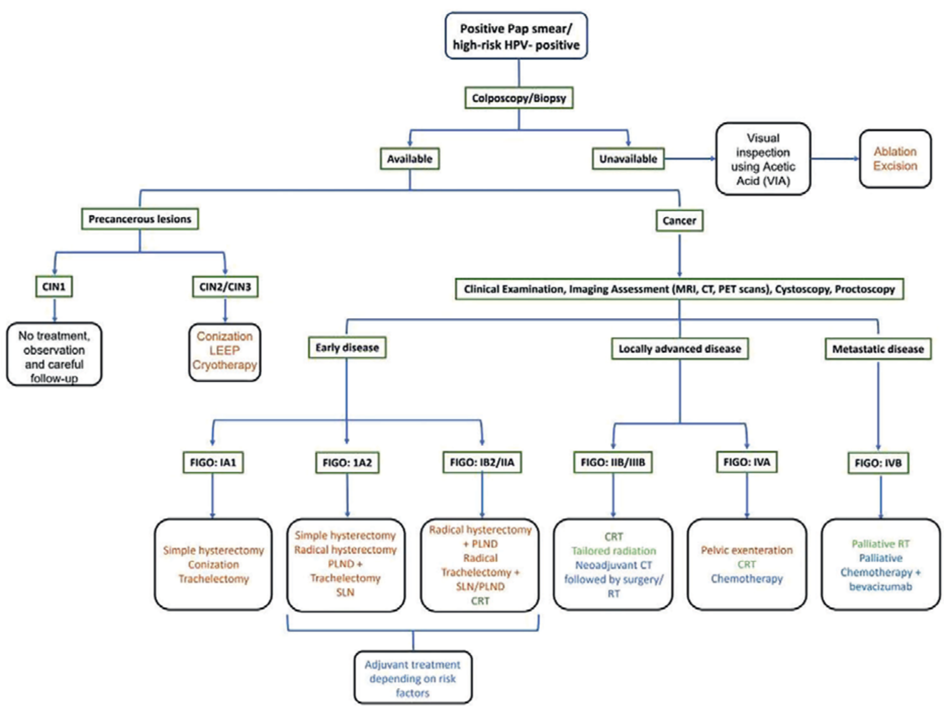

Figure 1.
Summary of cervical cancer patient's treatment [5].

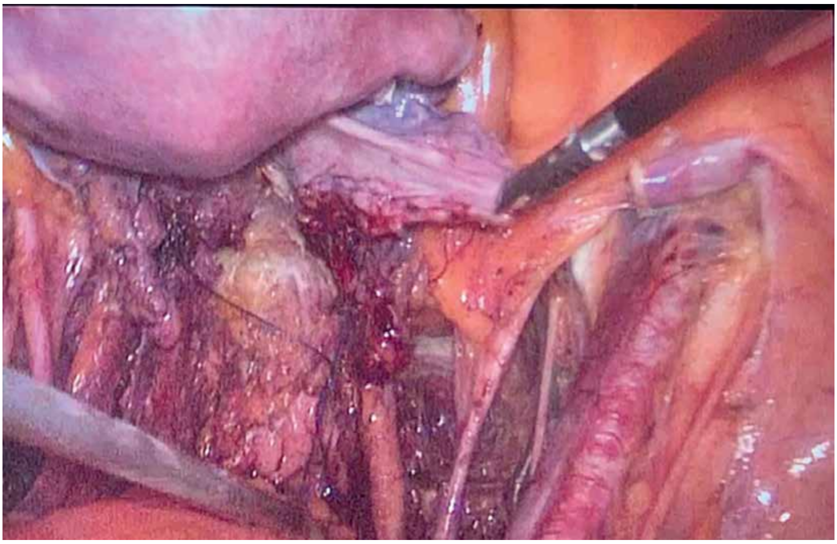

Figure 2.
Laparoscopic radical hysterectomy for cervical cancer.

For women who have completed childbearing, total hysterectomy with or without salpingo-oophorectomy (removal of one or both ovaries) remains the preferred treatment. Radical hysterectomy is more suitable for larger cervical cancer lesions (up to 4 cm) and involves the complete removal of the uterus, cervix, parametria, and upper vaginal cuff [7]. The Laparoscopic Approach to Cervical Cancer (LACC) trial found that performing radical hysterectomy *via* laparoscopy led to increased recurrence rates, fertility loss, and potential long-term urinary issues [8]. Consequently, the open technique is preferred for radical hysterectomy, especially for tumors exceeding 2 cm. For women of childbearing age with early-stage disease, a more conservative approach is necessary. Fertility-sparing surgeries such as LEEP, conization, and trachelectomy are options. LEEP employs a thin wire to remove abnormal cervical tissue and can be performed under local anesthesia in low-cost clinical settings, such as in LMICs. Conization, which entails excising a cone-shaped wedge from the cervix, involves hospital admission with higher associated costs [7]. Radical trachelectomy involves removing the cervix, surrounding tissue (parametrium), and the upper vagina through vaginal, laparoscopic, or robot-assisted methods [7, 8].

5.2 Chemotherapy

Chemotherapy stands as the standard treatment for locally advanced or metastatic cervical cancer. Chemotherapy plays a crucial role in the standard treatment protocol for cervical cancer, serving as an adjuvant therapy post-surgery in cases where poor prognostic tumor features elevate the risk of recurrence. It is also utilized in combination with radiotherapy, as previously mentioned, and as a standalone treatment for locally advanced disease (**Figure 1**). For the past three decades, the platinum-based chemotherapeutic, cisplatin, has been the most effective single agent in cervical cancer treatment [9]. However, despite initial positive patient responses, resistance often develops during the treatment course, diminishing the effectiveness of additional second-line platinum-based chemotherapeutics [10].

Studies indicate that combining cisplatin with other agents may enhance efficacy compared to single-drug treatments [9, 11]. For instance, a study by Long et al. demonstrated that while the response rate for cisplatin alone was 20%, combining it with topotecan increased the response rate to 39% [12]. Similar results were reported when cisplatin was combined with paclitaxel [13]. Currently, topotecan, paclitaxel, and other non-platinum-based chemotherapeutics such as 5-fluorouracil and bleomycin are commonly used in combination with cisplatin for treating cervical cancer, leading to a significant and clinically meaningful improvement in median survival duration [9].

5.3 Chemoradiation and radiation therapy

Chemotherapy is frequently combined with radiotherapy (chemoradiotherapy), particularly for locally advanced cervical cancer, aiming to reduce disease recurrence. While this approach improves overall and progression-free survival and reduces the risks of local and distant cervical cancer recurrences, it can also result in adverse events and chronic morbidity, as revealed by a systematic review and meta-analysis [14]. Additionally, palliative chemotherapy is employed to enhance the quality of life and alleviate disease symptoms, even though its effectiveness in reducing tumor size may be limited [15, 16]. Recognizing the importance of discovering and developing new therapies are crucial, especially in addressing multidrug resistance in cancer cells, which can impact the success of chemotherapy [17].

Radiotherapy plays a crucial role in the overall treatment of cervical cancer, serving as a critical component with essential roles in both locally advanced disease and metastatic disease. Radiotherapy, utilizing high-energy X-rays, plays a crucial role in the comprehensive treatment of cervical cancer [5]. A gynecologist named Amand Routh, working at Charing Cross Hospital in London during 1906, released the pioneering case report that outlined the application of radiation in the treatment of cervical cancer. The benefits of radiation therapy, such as reduced periprocedural mortality rates and its efficacy in addressing locally advanced malignancies, were swiftly recognized. Over the subsequent decade, several case series were published, offering documented evidence of successful cures in situations where surgery was deemed impractical or unsuitable [18, 19]. Collaborative approaches between surgeons and radiation oncologists aim to optimize the benefits of surgery and radiation therapy. This coordination is particularly significant for enhanced treatment efficacy, while also managing potential side effects and complications.

The established treatment for locally advanced cervical cancer (LACC) involves concurrent chemoradiotherapy (CCRT), combining external beam radiotherapy (EBRT) with subsequent brachytherapy (BT) [20]. Since 1999, CCRT has been the standard care for LACC, following the outcomes of five Phase III randomized controlled trials (RCT) that demonstrated a 30–50% improvement in survival by incorporating cisplatin-based chemotherapy alongside radiation (GOG 85, GOG 120, GOG 123, SWOG 8797/Intergroup 0107, RTOG 9001) [21–26]. Over the last two decades, there has been a notable shift in radiation treatment approaches, transitioning from a two-dimensional (2D) method, relying solely on anatomical bony landmarks, to an image-guided three-dimensional (3D) strategy. This updated approach considers variations in tumor size and position, aiming to deliver doses more precisely to clinical targets while minimizing exposure to organs at risk (OARs). This evolution in treatment paradigms signifies a commitment to improving the effectiveness and precision of radiation therapy for LACC.

5.4 Immunotherapy

Treatment options for recurrent cervical cancer are indeed limited, and they are generally regarded as non-curative [27]. Therefore, exploring immunotherapy as a novel treatment for cervical cancer, specifically targeting HPV oncoproteins, has demonstrated significant promise [5]. One notable advantage of this approach lies in its specificity, as it precisely targets dysplastic precancerous and malignant cervical epithelial cells expressing HPV oncoproteins.

Numerous innovative immunotherapeutic strategies are currently under investigation for targeting cervical cancer, with one notable approach focusing on the programmed death 1 (PD-1) pathway and its ligands, programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2) [27]. PD-1 serves as an immune checkpoint receptor that binds to its ligands (PD-L1 and PD-L2), often expressed on neoplastic cells, allowing them to evade detection by the immune system. Blocking the PD-1 receptor or its ligands, PD-L1 and PD-L2, is designed to reinvigorate T cell function and enhance T cell-mediated killing [27]. In recent years, there has been a growing interest in understanding the role of the PD-1/PD-L1 pathway in the development of virally driven cancers. PD-L1 expression is believed to contribute to the initiation and persistence of HPV infection by progressively suppressing T cell activity [28]. Given the strong association between HPV infection and nearly all cervical malignancies, the investigation into the PD-1/PD-L1 axis has become particularly relevant in understanding the pathogenesis of HPV-driven oncogenesis, spanning from cervical dysplasia to invasive cervical cancer [29].

6. Follow-up care and survivorship planning

6.1 Surveillance and monitoring

Post-treatment surveillance for cancer recurrence requires ongoing collaboration among the multidisciplinary team. Regular monitoring ensures early detection of potential complications or side effects, facilitating timely interventions.

6.2 Survivorship care plans

Creating comprehensive survivorship care plans involves input from various specialties within the multidisciplinary team. These plans address the long-term physical and emotional aspects of survivorship, emphasizing ongoing support and monitoring.

7. Innovations and research collaborations

7.1 Clinical trials

Participation in collaborative clinical trials is a testament to the multidisciplinary team's commitment to advancing cervical cancer treatment. These trials explore innovative treatments, contributing to the evolution of evidence-based practices through collaborative research.

7.2 Technological advancements

The integration of advanced technologies, such as genetic approaches including CRISPR/Cas9, artificial intelligence, and machine learning, in treatment planning and delivery is an evolving aspect of cervical cancer care. Multidisciplinary collaboration ensures that emerging technologies are adapted and implemented effectively.

8. Challenges and solutions

8.1 Communication and coordination

Effective communication and coordination among multidisciplinary team members are paramount. Challenges in communication are addressed through regular meetings, shared electronic health records, and the implementation of technologies that facilitate seamless collaboration.

8.2 Resource allocation

Optimizing resource allocation for comprehensive and equitable care poses challenges. Multidisciplinary collaboration involves strategic planning to ensure that resources are utilized efficiently, addressing disparities and improving overall patient care.

9. Patient-centered care and shared decision-making

9.1 Informed decision-making

Facilitating informed decision-making is a collaborative effort that involves all members of the multidisciplinary team. Patient education, open communication, and shared decision-making empower individuals to actively participate in their treatment journey.

9.2 Emotional and psychosocial support

Addressing the emotional and psychosocial needs of patients is an ongoing collaborative endeavor. Integrating support services within the multidisciplinary framework ensures a holistic approach to patient care.

10. Research and innovation

Here, we aim to unravel the groundbreaking developments in cervical cancer treatment, spotlighting the pivotal role of research and innovation in shaping the future of care. From targeted therapies to precision medicine and emerging technologies, the evolving landscape offers hope for more effective and personalized treatment strategies.

10.1 The genomic revolution

Advancements in genomic research have ushered in a new era in cervical cancer treatment. Comprehensive genomic profiling allows for a deeper understanding of the molecular characteristics of tumors, enabling clinicians to identify specific genetic mutations and alterations. This information forms the basis for tailoring treatment strategies, a paradigm known as precision medicine.

10.2 Targeted therapies

Targeted therapies, designed to selectively interfere with specific molecules involved in cancer growth, have emerged as a result of precision medicine approaches. For cervical cancer, targeted therapies may focus on blocking pathways implicated in the progression of the disease, offering a more nuanced and effective treatment option.

10.3 The immune landscape

Immunotherapy has garnered attention as a transformative approach in cancer treatment. Cervical cancer, known for its association with persistent human papillomavirus (HPV) infections, provides a unique immunological context. Research in immunotherapy explores ways to harness the body's immune system to recognize and eliminate cancer cells.

Checkpoint inhibitors, a class of immunotherapeutic agents, have shown promise in cervical cancer treatment. These drugs release the brakes on the immune system, enabling it to mount a more robust response against cancer cells. Ongoing research delves into optimizing the use of checkpoint inhibitors and exploring combinations with other treatment modalities.

10.4 Artificial intelligence (AI) and machine learning

Artificial intelligence and machine learning have become indispensable tools in cervical cancer research. From improving diagnostic accuracy to aiding in treatment planning, AI offers a range of applications. Machine learning algorithms analyze vast datasets, identifying patterns and assisting clinicians in making more informed decisions.

10.5 Robotic surgery

In the realm of surgical interventions, robotic surgery has gained prominence. With enhanced precision and minimally invasive techniques, robotic-assisted surgeries offer advantages in terms of reduced recovery times and improved patient outcomes. Ongoing research explores the optimal integration of robotic surgery into the treatment paradigm for cervical cancer.

11. Challenges and future directions

11.1 Overcoming resistance

One of the challenges in cervical cancer research involves addressing resistance mechanisms that can limit the effectiveness of treatments. Understanding the factors contributing to resistance and developing strategies to overcome them are critical areas of ongoing investigation.

11.2 Accessibility and equity

Ensuring that innovative treatments are accessible globally is a pressing concern. Research efforts extend beyond developing new therapies to devising strategies for equitable distribution, particularly in regions with limited healthcare resources.

12. Conclusion

The management of cervical cancer is a dynamic and evolving field that integrates established principles with ongoing research to consistently improve patient outcomes. By adhering to these foundational principles and staying informed about advancements, healthcare professionals can provide the highest quality care to individuals affected by cervical cancer.

The past decades have seen significant changes in cervical cancer prevention, primarily driven by the introduction of human papillomavirus (HPV) vaccination. Some countries have notably witnessed a considerable reduction in HPV-mediated cervical diseases, representing a significant advancement in the global effort against cervical cancer. However, the widespread adoption of HPV vaccination encounters challenges such as economic limitations, differing healthcare priorities among nations, and instances of vaccine hesitancy among both the population and governments.

Precise diagnosis of cervical cancer and its related lesions is crucial for establishing the foundation of its treatment. In this dynamic landscape, it is clear that cervical screening will maintain a central role in preventing cervical cancer, requiring adjustments to accommodate the changing incidence of HPV-associated neoplasia. Traditionally, cervical screening relied on Papanicolaou staining of cytology samples. However, with an improved understanding of HPV's role in the progression of cervical cancer and the availability of sensitive detection systems, HPV testing has become an integral component of modern screening protocols.

Although HPV testing improves disease detection, it faces a limitation in specificity, as it cannot distinguish high-grade from low-grade lesions. This challenge has led to the development of effective triage approaches to categorize HPV-positive individuals based on their risk of cancer progression. While cytology triage remains a fundamental aspect of screening, ongoing research is exploring innovative strategies, including DNA methylation, biomarker detection, and the integration of artificial intelligence systems. These endeavors aim to identify cervical abnormalities with greater precision, enhancing the overall accuracy of cervical cancer screening.

In treatment aspects: chemotherapy, surgery, radio-chemotherapy radiotherapy, and immunotherapy serve as the cornerstone treatments for cervical cancer. A combination of aforesaid therapeutics is also used according to patients. Despite the relatively high 5-year survival rate, women with cervical cancer require ongoing, person-centered supportive care. Patient-reported outcomes (PROs) can offer valuable insights into the advantages and drawbacks of cervical cancer treatments, making a compelling case for considering the impact on patients' quality of life when making decisions about their treatment [19].

In this chapter, we delve into the diagnosis of cervical cancer and related lesions, emphasizing its significance. We also provide details about the medical teams involved in the diagnosis and subsequent treatment process. Furthermore, we discuss available treatment options in a step-by-step manner. Navigating this transformative era, a thorough comprehension of these advancements is crucial to advancing toward a future where cervical cancer is not just preventable but also effectively managed.

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Chapter 2

Incidence of HPV, Cervical Cancer in China, and Its Potential Risk Factors and Protocols for Remedy

Wu Xiaomei and Li Yuanyue

Abstract

Cervical cancer ranks as the fourth most prevalent cancer globally among women, recording approximately 604,127 new cases and 341,831 fatalities in 2020. High-risk human papillomavirus (HPV) genotype infection is a significant risk factor for cervical cancer, one of the leading causes of cancer-related mortality among women worldwide. In China, the prevalence and impact of HPV and cervical cancer have garnered increasing attention due to its substantial burden on public health. This chapter provides an in-depth exploration of the incidence, prevalence, risk factors, screening methods, prevention strategies, and challenges associated with HPV and cervical cancer in China. Drawing upon epidemiological data, clinical studies, and public health reports, this chapter aims to offer a comprehensive understanding of the current landscape and future directions for combating HPV-related cervical cancer in China.

Keywords: human papillomavirus, cervical cancer, incidence, prevalence, China

1. Introduction

Cervical cancer ranks as the fourth most prevalent cancer globally among women, recording approximately 604,000 new cases and 342,000 mortalities in 2020 [1]. Total mortality rates associated with cervical cancer are notably lower than their incidence [1]. HPV infection is one of the most common sexually transmitted infections globally [2]. High-risk human papillomavirus (HR-HPV) genotype infection is a significant risk factor for cervical cancer, one of the leading causes of cancer-related mortality among women worldwide [3, 4]. HPV is a diverse group of viruses, with over 200 different HPV genotypes identified, among which some are classified as high-risk genotypes due to their association with the development of cervical cancer [5]. Cervical cancer arises from the abnormal growth of cells in the cervix, primarily caused by persistent infection with high-risk HPV types (**Figure 1**).

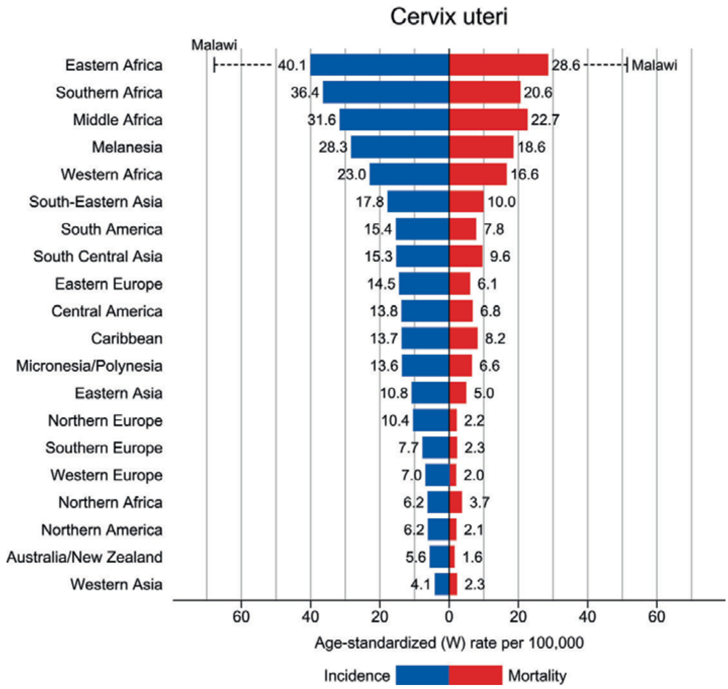

Figure 1.
Age-standardized rates for the incidence and mortality of cervical cancer varied by region in 2020 [1].

2. Epidemiology of cervical cancer and HPV in China

China, the world's largest and most populous, contends with a considerable challenge of cervical cancer, witnessing around 109,741 new cases each year within its borders, constituting roughly a quarter of the world's total. Without the implementation of effective measures, projections indicate that this number could escalate to 187,000 new cases by the year 2050 [6].

In China, cervical cancer holds the position of the sixth most prevalent form of cancer among women. Additionally, it stands as the third most common type of cancer among women aged 15–44 years in the country [7]. Data from the ICO/IARC HPV Information Center 2020 estimates 59,060 deaths attributed to cervical cancer. In China, cervical cancer ranks as the seventh leading cause of cancer-related deaths among females. Furthermore, it stands as the third leading cause of cancer deaths among women aged 15–44 years in the country [7]. Alarmingly, over the past two decades, both the incidence and mortality rates of cervical cancer have shown a steady increase across China [8].

Age is a significant contributing factor to HPV infection and the development of cervical cancer. The age-specific incidence rates of cervical cancer in China are shown in **Figure 2**, which shows how the occurrence of this cancer varies across different age groups within the population [6]. The temporal trends in cervical cancer incidence in China, as indicated by data from cancer registries, illustrate how the rates have changed over time (**Figure 3**) [7].

Cervical cancer screening plays a crucial role in effectively preventing both the incidence of cervical cancer and deaths associated with the disease. However, the

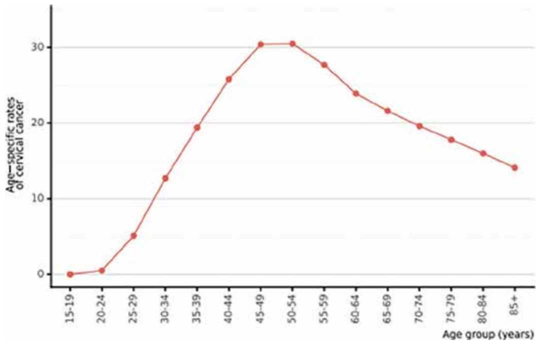

Figure 2.
The age-specific incidence rates of cervical cancer in China, providing estimates for the year 2020 [6].

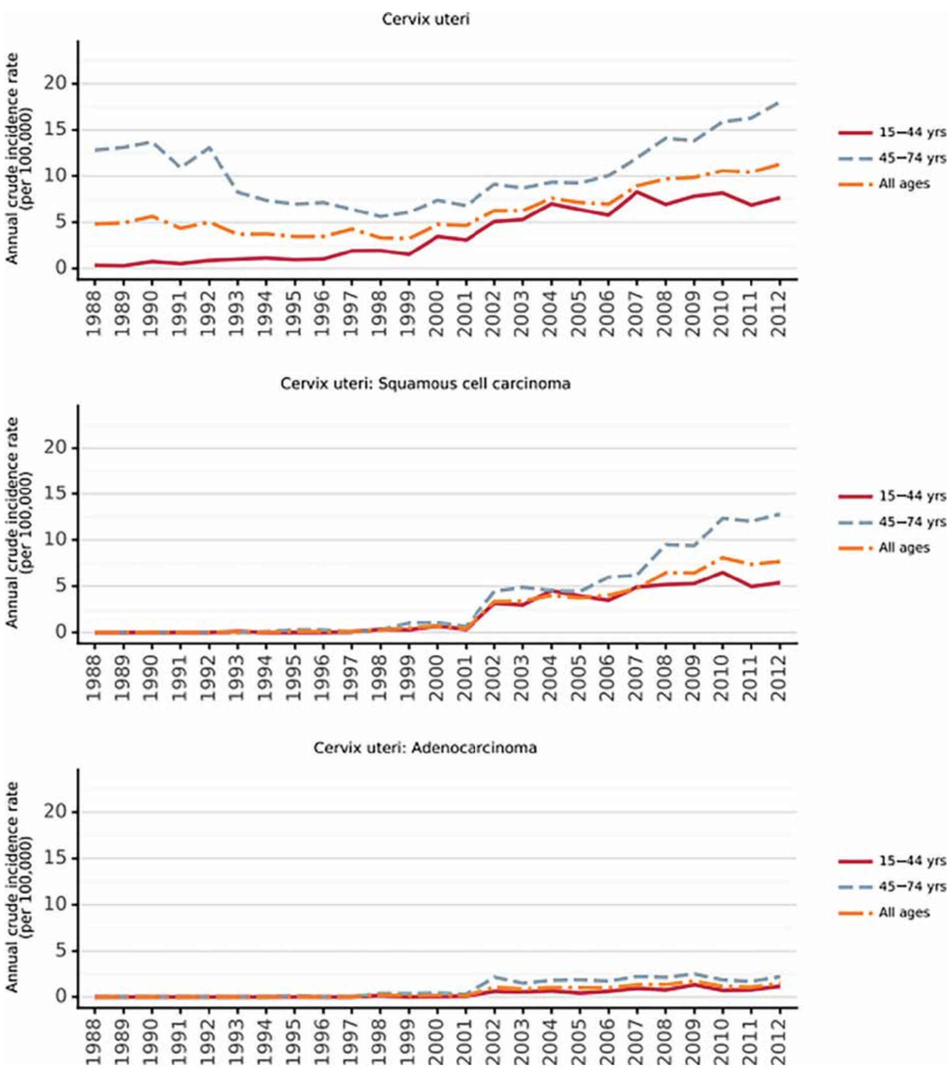

Figure 3.
The time trend of cervical cancer incidence among different age groups in China varies over different periods [7].

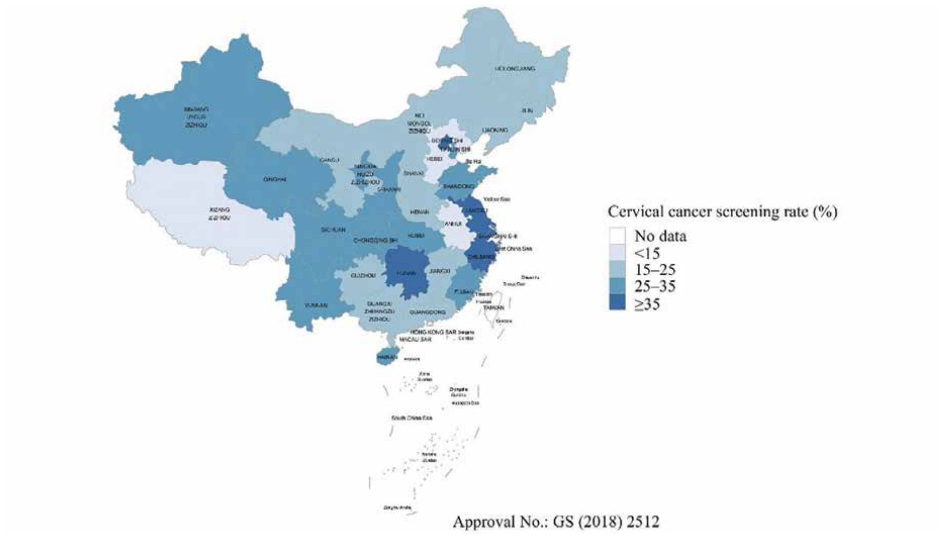

Figure 4.
Cervical cancer screening rates among Chinese women at the provincial level in China in 2015 [9].

cervical cancer screening rates among Chinese women at the provincial level in 2015 varied significantly **Figure 4**. Each province had its own screening programs and initiatives, resulting in disparities in screening rates across regions. Generally, more developed provinces with better healthcare infrastructure tended to have higher screening rates compared to less developed regions [9].

The prevalence of HPV infection in China varies across different regions and populations [10–13]. Numerous studies conducted in various provinces have reported varying prevalence rates among different ethnicities [11, 12], age [10–13], and demographic backgrounds [10–13]. Generally, the prevalence of HPV infection in China ranges from 10–29%, with higher rates observed in certain high-risk populations [10–13]. Studies have shown that HPV prevalence is highest among women aged 15–24 years, with an estimated prevalence exceeding 20% in some regions. However, prevalence rates tend to decline with age, with lower rates observed in older age groups. Additionally, HPV prevalence varies by geographical region, with higher rates reported in urban areas compared to rural areas, possibly due to differences in lifestyle, access to healthcare, and sexual behavior.

A review article published in 2019 investigated 198 studies, which included 3,177,080 women with symptomatic outpatient or general health checkups and 85,743 cases of cervical lesions, among which 24,925 cases were cervical cancer. They found that the high-risk HPV infection rate among women in mainland China stood at 19.0%. Similarly, the infection rate among symptomatic outpatients and healthy women was 25.7 and 12.9%, respectively. Interesting HPV infection rate was high in rural women (15.7%) compared to rural women (14.1%) [14]. Further, they also found that overall, the HR-HPV infection rate was higher in women from northern China compared to other regions. A study conducted in 2015 reported the nationwide prevalence of HPV infection and its genotype distribution across 37 cities in China. The study revealed an HR-HPV prevalence of 21.07%, with notable variations observed among different cities [15].

HPV genotype distribution in China exhibits unique epidemiological patterns influenced by factors such as geographical location, population demographics, and evolving vaccination strategies. Understanding the prevalence of different HPV genotypes is essential for guiding cervical cancer prevention efforts, vaccination strategies, and screening programs. High-risk HPV genotypes, notably HPV-16 and HPV-18, are responsible for the majority of cervical cancer cases worldwide. In China, HPV-16, 52,58, and 33 genotypes are the most prevalent genotypes associated with cervical cancer and precancerous lesions. However, geographical and ethnicities, etc. variations in HPV genotype distribution have been observed within China, with certain provinces reporting higher prevalence rates of specific genotypes compared to others [10–13]. Another study showed that HPV16 (4.82%) and HPV52 (4.52%) emerged as the most prevalent genotypes, with HPV58 (2.74%) following closely behind. Among the low-risk HPV (LR-HPV) types, HPV6 (4.01%) and HPV11 (2.29%) predominated. Notably, mixed genotypes, such as HPV16 + 52 and HPV52 + 58, were frequently detected in women with multiple infections [15]. Factors, such as climate, population mobility, and local health policies, may influence the transmission dynamics of HPV genotypes in different regions. Understanding these regional variations is essential for designing targeted vaccination and screening programs tailored to the specific needs of each region.

3. Ethnic variation in HPV prevalence

Our research group conducted a study on HPV prevalence and genotype distribution in Yunnan province, revealing notable variations among different ethnic groups. Among Tibetan women, the overall HPV infection rate was 27.4%, while rates were 12.8% for Hani, 11.9% for Naxi, and 9.9% for Dai women [11, 12, 16]. Additionally, the prevalence of overall HPV, HR-HPV, and single-genotype HPV infections was significantly higher ($P = 0.001$) among Tibetan women compared to Naxi, Han, Hani, and Dai women [11, 12, 16]. Moreover, our investigation uncovered intriguing trends in HPV genotype distribution across different ethnicities. While HPV16 predominated across all groups, there were distinct variations in the distribution of other genotypes. For instance, HPV-33 (5.88%) was notably more prevalent among Tibetan women than among Naxi and Han women. In the Mojiang area, Han women exhibited a distribution featuring HPV-52 (1.3%), HPV-33 (0.6%), HPV-44 (0.6%), and HPV-54 (0.6%) alongside HPV-16 [12]. HPV prevalence among Uyghur women was 7.3%, while Han which is approximately 90% of the total Chinese population has HPV prevalence ranging from 6.7 to 29.6% [11–13, 15–19].

4. Age-related high-risk HPV infections in mainland China

It is well-accepted all over the world that the infection rates of HPVs are different in different ages women. Age-related high-risk HPV infections in mainland China exhibit distinctive patterns. A recently reported study found that the HR-HPV infection rate in women aged <25, 25–45, and > 45 years was 24.3, 19.9, and 21.4%, respectively [14]. Similarly, recent studies have highlighted variations in HPV prevalence across different age groups [14, 20–22], shedding light on the dynamics of HPV transmission and infection. While younger age groups often show higher rates of HPV infection due to increased sexual activity and less immune protection, older age groups are not immune to HPV acquisition [20–22]. Factors, such as changes in sexual behavior over time and immune system weakening with age, contribute to the complexity of age-related HPV infections. Understanding these age-related trends is crucial for informing effective prevention strategies and screening programs tailored to different age demographics.

5. Risk factors for HPV infection and cervical cancer

In China, several risk factors contribute to HPV infection and the development of cervical cancer, highlighting the multifaceted nature of this public health challenge. Understanding these risk factors is crucial for implementing effective preventive strategies and interventions. Below are some key risk factors identified in the Chinese context:

5.1 Early age of sexual debut

Early initiation of sexual activity is associated with an increased risk of HPV infection [23, 24]. In China, societal changes, urbanization, and evolving cultural norms may contribute to a trend of younger individuals engaging in sexual activity [10–12]. Women who initiate sexual activity at a younger age may have a higher likelihood of exposure to HPV, increasing their risk of infection and subsequent cervical cancer development.

5.2 Multiple sexual partners

Having multiple sexual partners is a significant risk factor for HPV infection [25]. In China, shifting societal attitudes toward relationships and sexual behavior, coupled with increased mobility and globalization, may contribute to higher rates of multiple sexual partnerships [26]. Individuals with multiple sexual partners have a higher probability of encountering HPV-infected partners, thereby increasing their risk of HPV transmission and cervical cancer.

5.3 Lack of condom use

Inconsistent or lack of condom use during sexual intercourse can facilitate the transmission of HPV [27]. Factors, such as misconceptions about contraceptive methods, cultural barriers, and discomfort in discussing sexual health, may contribute to low condom usage rates in certain populations in China. Failure to use condoms consistently can increase the risk of HPV infection and cervical cancer, particularly among sexually active individuals [28].

5.4 Low socioeconomic status

Socioeconomic factors play a significant role in HPV infection and cervical cancer risk [29]. Individuals from lower socioeconomic backgrounds in China may face barriers to accessing healthcare services, including HPV vaccination, screening, and treatment [30]. Limited education, low health literacy, and financial constraints may also impact preventive healthcare-seeking behaviors, leading to delayed diagnosis and treatment of HPV-related diseases.

5.5 Co-infection with other STIs

Co-infection with other sexually transmitted infections (STIs), such as Chlamydia trachomatis and herpes simplex virus (HSV), may increase the risk of HPV transmission and cervical cancer development [31]. The presence of concomitant STIs can exacerbate inflammation and compromise the immune response, creating an environment conducive to HPV persistence and oncogenic progression.

5.6 Smoking

Cigarette smoking has been identified as a modifiable risk factor for cervical cancer [32]. Smoking may impair the immune response, increase oxidative stress, and promote HPV persistence and progression to cervical neoplasia. In China, where smoking rates remain relatively high, particularly among men, tobacco use contributes to the burden of HPV-related diseases, including cervical cancer, among both smokers and nonsmokers through secondhand smoke exposure.

5.7 Lack of HPV vaccination

The availability and uptake of HPV vaccination play a critical role in preventing HPV infection and cervical cancer [33]. However, in China, low awareness about HPV vaccination, vaccine hesitancy, and limited access to vaccination services may contribute to low vaccination coverage rates. Failure to vaccinate susceptible individuals, particularly adolescent girls, leaves them vulnerable to HPV infection and increases their risk of developing cervical cancer later in life.

Addressing these risk factors requires a comprehensive approach that combines health education, promotion of preventive behaviors, expansion of HPV vaccination programs, and improving access to cervical cancer screening and treatment services [34]. By addressing the underlying determinants of HPV infection and cervical cancer risk, China can reduce the burden of these diseases and improve the overall health outcomes of its population.

6. Genetic factors

Genetic factors play a significant role in both HPV infection and the development of cervical cancer [35]. Understanding the genetic determinants associated with HPV and cervical cancer susceptibility can provide valuable insights into disease pathogenesis, risk stratification, and personalized prevention and treatment strategies [36]. Here are some key genetic factors relevant to HPV infection and cervical cancer:

6.1 Host genetic variations

Individual genetic variations within the host can influence susceptibility to HPV infection and subsequent progression to cervical cancer [36]. Polymorphisms in genes involved in immune response pathways, such as human leukocyte antigen (HLA) genes, cytokines, and innate immune receptors, may impact the ability to clear HPV infection and modulate the risk of developing cervical neoplasia. For example, certain HLA class II alleles have been associated with increased susceptibility to persistent HPV infection and cervical cancer progression.

6.2 Viral genetic variability

HPV is a genetically diverse virus, with multiple genotypes classified into high-risk (oncogenic) and low-risk (non-oncogenic) types based on their association with cervical cancer [37]. Viral genetic variations, particularly within the viral oncogenes E6 and E7, can influence HPV virulence, oncogenic potential, and persistence within the host epithelium. High-risk HPV genotypes, such as HPV-16 and HPV-18, harbor specific genetic mutations that enhance their ability to disrupt cellular processes, evade immune surveillance, and promote malignant transformation [38].

6.3 Integration of viral DNA

In a subset of HPV-infected individuals, the viral genome may integrate into the host cell's genome, leading to dysregulation of cellular gene expression and genomic instability. Integration of high-risk HPV DNA into host chromosomal loci, particularly near cancer-associated genes, can contribute to the development of cervical intraepithelial neoplasia (CIN) and progression to invasive cervical cancer. Integration events often disrupt the E2 gene, resulting in overexpression of viral oncogenes E6 and E7, which play a central role in cervical carcinogenesis [39, 40].

6.4 DNA repair pathway defects

Impaired DNA repair mechanisms within the host cell may predispose individuals to HPV-induced genomic damage and increase the risk of cervical cancer development [41]. Germline mutations in genes encoding components of the DNA repair pathway, such as BRCA1, BRCA2, and mismatch repair genes (e.g., MLH1, MSH2), have been associated with an elevated risk of developing HPV-associated malignancies, including cervical cancer. These mutations may compromise the cell's ability to repair HPV-induced DNA lesions, leading to genomic instability and tumor progression [42].

6.5 Epigenetic alterations

Epigenetic modifications, including DNA methylation, histone modifications, and microRNA dysregulation, can modulate gene expression patterns and contribute to HPV-associated cervical carcinogenesis. Aberrant DNA methylation patterns, particularly in CpG islands within the promoter regions of tumor suppressor genes, may silence their expression and promote malignant transformation. Epigenetic alterations induced by HPV infection can disrupt cellular regulatory pathways, enhance viral oncogene activity, and facilitate tumor progression [43].

Understanding the interplay between host genetic factors, viral genetic variability, and epigenetic modifications is crucial for elucidating the molecular mechanisms underlying HPV infection and cervical cancer development. Integrating genetic and genomic information into cervical cancer prevention and treatment strategies may facilitate risk assessment, early detection, targeted therapy, and personalized interventions tailored to individual patients' genetic profiles. Additionally, ongoing research efforts focused on identifying novel genetic biomarkers and therapeutic targets hold promise for advancing precision medicine approaches in the management of HPV-associated cervical cancer [43, 44].

7. Screening methods for HPV and cervical cancer

Screening for HPV infection and cervical cancer is crucial for early detection, timely intervention, and prevention of cervical cancer-related morbidity and mortality. In China, various screening methods are utilized to detect HPV infection and cervical abnormalities. Below are some common screening methods for HPV and cervical cancer:

7.1 HPV testing

HPV testing involves detecting the presence of high-risk HPV DNA in cervical cells. This method is highly sensitive and specific for identifying women at risk of developing cervical cancer. In China, HPV testing is increasingly utilized as a primary screening tool, either alone or in combination with other screening methods. High-risk HPV types, such as HPV-16 and HPV-18, are particularly targeted due to their strong association with cervical cancer.

7.2 Pap smear (cytology)

Pap smear, also known as cervical cytology, involves collecting cervical cell samples and examining them under a microscope to detect cellular abnormalities indicative of precancerous or cancerous changes. Pap smear screening has been a cornerstone of cervical cancer prevention programs worldwide, including in China. However, its sensitivity for detecting cervical abnormalities is lower compared to HPV testing.

7.3 Visual inspection with acetic acid (VIA)

Visual inspection with acetic acid (VIA) is a low-cost, simple screening method that involves applying diluted acetic acid to the cervix and visually inspecting for acetowhite lesions indicative of cervical abnormalities. VIA is often used in resource-limited settings, including rural areas of China, where access to cytology and HPV testing may be limited. While VIA is less sensitive and specific than HPV testing, it can be a valuable screening tool in settings where more advanced methods are not feasible.

7.4 Visual inspection with Lugol's iodine (VILI)

Similar to VIA, visual inspection with Lugol's iodine (VILI) involves applying Lugol's iodine solution to the cervix and observing for staining patterns that may indicate the presence of cervical abnormalities. VILI is another low-cost and point-of-care screening method used in resource-limited settings. However, its sensitivity and specificity for detecting cervical abnormalities are lower compared to HPV testing and cytology.

7.5 Colposcopy and biopsy

Colposcopy involves using a specialized magnifying instrument (colposcope) to visually examine the cervix for suspicious lesions identified during screening tests, such as HPV testing or cytology. If abnormalities are detected, a biopsy may be performed to obtain tissue samples for histopathological examination. Colposcopy and biopsy are typically performed as follow-up procedures for women with abnormal screening results to confirm the presence of cervical intraepithelial neoplasia (CIN) or cervical cancer.

In China, cervical cancer screening programs often utilize a combination of these screening methods, tailored to the available resources, infrastructure, and population characteristics of each region. Integrating HPV testing with cytology (co-testing) or using HPV testing as a primary screening modality followed by triage with cytology or colposcopy for HPV-positive women has been shown to improve the sensitivity and effectiveness of cervical cancer screening programs. Additionally, efforts to enhance access to screening services, promote health education, and reduce barriers to care are essential for achieving widespread participation and maximizing the impact of cervical cancer prevention efforts in China.

8. Integrating screening and treatment

Integrating screening and treatment strategies is essential for maximizing the effectiveness of cervical cancer prevention programs in China. By combining screening methods with timely treatment interventions, healthcare providers can identify and manage cervical abnormalities early, reducing the risk of cervical cancer development and improving patient outcomes. Here are key components of integrating screening and treatment for cervical cancer prevention:

8.1 Screening

Utilizing a combination of screening methods, such as HPV testing, cytology (Pap smear), visual inspection techniques (VIA/VILI), and colposcopy, allows for comprehensive detection of cervical abnormalities. Screening programs should be tailored to the local epidemiological context, healthcare infrastructure, and population characteristics to optimize resource allocation and maximize coverage.

8.2 Diagnosis and triage

Positive screening results should prompt further diagnostic evaluation and triage to determine the severity of cervical abnormalities. Colposcopy-guided biopsy and histopathological examination are essential for confirming the presence and grade of cervical intraepithelial neoplasia (CIN) or cervical cancer. Triage algorithms, incorporating HPV genotyping, cytology, or other biomarkers, can help prioritize patients for additional evaluation and treatment.

8.3 Treatment

Timely treatment of cervical abnormalities is crucial for preventing the progression to invasive cervical cancer. Treatment modalities may include:

Excisional Procedures: Loop electrosurgical excision procedure (LEEP), cold knife conization, or laser ablation are commonly used to remove precancerous lesions (CIN) identified during colposcopy.

Cryotherapy: Cryotherapy involves freezing and destroying abnormal cervical tissue using a cryoprobe. It is a minimally invasive treatment option suitable for low-grade cervical lesions.

Hysterectomy: In cases of invasive cervical cancer or advanced-stage disease, surgical removal of the uterus (hysterectomy) may be necessary. This procedure may be complemented by adjuvant therapies, such as chemotherapy and radiation therapy.

8.4 Follow-up and surveillance

Patients treated for cervical abnormalities require regular follow-up and surveillance to monitor treatment outcomes and detect disease recurrence. Posttreatment follow-up may involve periodic Pap smears, HPV testing, colposcopy, or imaging studies, depending on the individual's risk profile and treatment history.

8.5 Health education and counseling

Integrating health education and counseling into cervical cancer prevention programs is essential for promoting awareness, encouraging screening participation, and facilitating informed decision-making regarding treatment options. Educational initiatives should address misconceptions about cervical cancer, promote the importance of screening and early detection, and provide support for patients undergoing treatment.

8.6 Multidisciplinary collaboration

Effective integration of screening and treatment requires collaboration among healthcare providers, including gynecologists, pathologists, oncologists, primary care physicians, nurses, and public health professionals. Multidisciplinary teams can ensure coordinated care delivery, optimize treatment decision-making, and streamline referral pathways for patients requiring specialized services.

By integrating screening and treatment strategies, China can enhance the effectiveness and efficiency of cervical cancer prevention programs, reduce the burden of cervical cancer, and improve the overall health outcomes of women across the country. Ongoing evaluation and adaptation of integrated approaches based on emerging evidence and local context are essential for achieving sustained impact and addressing evolving healthcare needs.

9. Challenges in HPV and cervical cancer prevention

HPV infection and cervical cancer pose several challenges, particularly in resource-limited settings, such as many regions in China. Addressing these challenges is essential for improving prevention efforts and reducing the burden of cervical cancer. Here are some key challenges in HPV and cervical cancer prevention:

9.1 Vaccine accessibility and coverage

Despite the availability of HPV vaccines, ensuring universal access and achieving high vaccination coverage rates remain significant challenges. In China, barriers to vaccine accessibility include cost, limited availability in rural areas, lack of awareness about vaccination benefits, and vaccine hesitancy driven by safety concerns or misinformation. Efforts to expand vaccine distribution, subsidize vaccine costs, and implement school-based vaccination programs can help improve coverage rates.

9.2 Health education and awareness

Limited awareness about HPV infection, cervical cancer, and preventive measures among the general population, healthcare providers, and policymakers poses a barrier to effective prevention efforts. Cultural taboos surrounding discussions of sexual health, stigma associated with HPV-related diseases, and misconceptions about vaccination efficacy and safety hinder health education initiatives. Comprehensive health education campaigns targeted outreach programs, and community engagement strategies are needed to raise awareness, dispel myths, and promote preventive behaviors.

9.3 Screening inequities

Disparities in access to cervical cancer screening services, particularly in rural and underserved areas of China, contribute to inequities in disease detection and treatment. Challenges, such as limited healthcare infrastructure, shortage of trained personnel, and logistical barriers, hinder screening uptake among high-risk populations. Implementing innovative screening approaches, such as mobile clinics, telemedicine, and self-sampling kits, can improve accessibility and coverage, especially in remote regions.

9.4 Limited diagnostic and treatment facilities

Inadequate diagnostic and treatment facilities for cervical cancer management pose a significant challenge in China, particularly in rural areas. Shortages of cytology laboratories, colposcopy equipment, and oncology centers limit timely diagnosis and treatment initiation, leading to delays in care and poorer outcomes for patients. Strengthening healthcare infrastructure, expanding capacity-building initiatives, and integrating cervical cancer services into primary care settings can help address these limitations.

9.5 Cervical cancer stigma and psychosocial support

The stigma surrounding cervical cancer diagnosis and treatment, fear of social ostracism, and psychological distress among affected individuals and their families impede prevention efforts and hinder access to care. Cultural beliefs, misconceptions about disease etiology, and fear of disclosure contribute to the perpetuation of stigma. Providing psychosocial support services, peer counseling, and community-based support groups can help mitigate stigma, empower patients, and improve their quality of life throughout the cancer journey.

9.6 Sustainability of prevention programs

The sustainability of HPV and cervical cancer prevention programs relies on long-term funding commitments, political support, and integration into existing healthcare systems. Limited financial resources, competing health priorities, and turnover of political leadership may undermine the continuity of prevention initiatives. Establishing sustainable financing mechanisms, fostering multisectoral collaborations, and advocating for policy continuity is essential for ensuring the long-term success of prevention efforts.

Addressing these challenges requires a multifaceted approach involving government agencies, healthcare providers, civil society organizations, and international partners. By prioritizing equity, promoting community engagement, and strengthening health systems, China can overcome barriers to HPV and cervical cancer prevention and advance toward achieving the goal of cervical cancer elimination.

10. Future directions and recommendations

As we look toward the future of HPV and cervical cancer prevention, several key directions and recommendations emerge to address ongoing challenges, enhance existing strategies, and advance toward the goal of eliminating cervical cancer. Here are some future directions and recommendations:

10.1 Strengthening vaccination efforts

- Expand HPV vaccination coverage through targeted immunization programs, particularly among adolescent girls and boys, with a focus on underserved populations and rural areas.

- Integrate HPV vaccination into routine immunization schedules and school-based vaccination programs to improve accessibility and uptake.

- Conduct outreach campaigns to increase awareness about the importance of vaccination, address vaccine hesitancy, and dispel myths and misinformation.

10.2 Enhancing screening programs

- Implement innovative and cost-effective screening approaches, such as HPV-based testing and self-sampling kits, to improve coverage and reach underserved populations.

- Integrate cervical cancer screening into primary healthcare services and community-based outreach programs to facilitate early detection and treatment initiation.

- Invest in strengthening laboratory infrastructure, training healthcare providers in cytology and colposcopy, and ensuring quality assurance mechanisms for screening services.

10.3 Promoting health education and awareness

Develop culturally tailored health education materials and campaigns to raise awareness about HPV infection, cervical cancer prevention, and the benefits of vaccination and screening.

Empower women and girls to take control of their sexual and reproductive health through comprehensive sex education programs and community engagement initiatives.

Foster partnerships with schools, community organizations, and media outlets to disseminate accurate information, challenge stigma, and promote preventive behaviors.

10.4 Integrating comprehensive care services

- Establish multidisciplinary cervical cancer care teams comprising gynecologists, oncologists, pathologists, nurses, and support staff to provide holistic care throughout the cancer continuum.

- Ensure access to timely diagnosis, treatment, and palliative care services for women diagnosed with cervical cancer, with a focus on reducing disparities in access to care.

- Strengthen psychosocial support services and survivorship programs to address the emotional, social, and practical needs of cervical cancer patients and their families.

10.5 Investing in research and innovation

- Support research initiatives to better understand the epidemiology, natural history, and molecular mechanisms of HPV infection and cervical carcinogenesis.

- Foster collaborations between academia, industry, and government agencies to develop novel prevention strategies, therapeutic interventions, and diagnostic tools.

- Harness advances in technology, such as artificial intelligence and genomic sequencing, to improve screening accuracy, risk stratification, and personalized treatment approaches.

10.6 Advocating for policy support and funding

- Advocate for policy reforms that prioritize cervical cancer prevention and control, including the allocation of sufficient resources, the enactment of legislation supporting vaccination mandates, and the integration of cervical cancer services into universal health coverage schemes.

- Mobilize political will and engage stakeholders at all levels to champion the cause of cervical cancer elimination, raise awareness among policymakers, and galvanize support for evidence-based interventions.

By pursuing these future directions and recommendations, China can accelerate progress toward eliminating HPV-related cervical cancer as a public health threat, reduce health disparities, and improve the well-being of women and communities nationwide. It requires concerted efforts, sustained commitment, and collaboration across sectors to achieve this ambitious goal.

11. Conclusion

The incidence of HPV infection and cervical cancer in China presents complex challenges yet also opportunities for effective intervention and control. Throughout this chapter, we have explored the epidemiological patterns, risk factors, prevention strategies, and challenges associated with HPV-related cervical cancer in China.

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Epidemiological patterns: The prevalence of HPV infection varies across different regions of China, with disparities influenced by factors such as age, socioeconomic status, and geographical location. Similarly, cervical cancer incidence rates exhibit variation, reflecting differences in healthcare access, screening practices, and vaccination coverage.

Risk factors: Behavioral, environmental, and genetic factors contribute to the risk of HPV infection and subsequent development of cervical cancer in Chinese women. Early onset of sexual activity, multiple sexual partners, smoking, and inadequate access to healthcare services are among the key risk factors identified.

Prevention strategies: Vaccination programs, screening initiatives, and health education campaigns play crucial roles in preventing and controlling HPV-related cervical cancer in China. Efforts to enhance vaccination coverage, improve screening methods, and promote awareness of cervical cancer risk factors are essential components of comprehensive prevention strategies.

Challenges and opportunities: Despite progress in HPV vaccination and cervical cancer screening, China faces challenges such as vaccine hesitancy, limited access to healthcare services in rural areas, and cultural stigma associated with reproductive health issues. Addressing these challenges requires multifaceted approaches, including targeted interventions, policy support, and community engagement.

Future directions: To further reduce the burden of HPV-related cervical cancer in China, future efforts should focus on expanding vaccination coverage, strengthening screening programs, addressing socioeconomic disparities, and fostering international collaboration. Additionally, ongoing research into HPV genotypes, transmission dynamics, and the effectiveness of prevention strategies will contribute to refining and optimizing public health interventions.

In conclusion, addressing the incidence of HPV and cervical cancer in China demands a comprehensive and coordinated approach that encompasses vaccination, screening, treatment, and health education. By leveraging existing infrastructure, harnessing technological advancements, and fostering partnerships across sectors, it is possible to mitigate the impact of HPV-related cervical cancer and improve women's health outcomes across China. With sustained commitment and collective action, we can strive toward the ultimate goal of eliminating cervical cancer as a public health threat in China and beyond.

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Chapter 3

HPV in Breast Carcinogenesis: Friend, Foe, or Fellow Traveler?

Usman Ayub Awan and Zeeshan Siddique

Abstract

Breast Cancer (BC) is a major public health problem and a leading cause of death and morbidity among women worldwide, with increasing incidence rates over the past decade. Several risk factors, such as reproductive history, lifestyle, and environmental exposure, have been associated with BC, but they only account for 20 to 50% of the cases. Viral infections, especially the Human papillomavirus (HPV), have been suggested as potential etiological agents of BC, but the causal link remains unclear. Herein, we review the prevalence of HPV in BC development and progression, focusing on the molecular mechanisms that HPV employs to infect and transform mammary epithelial cells. We also discuss the modes of transmission of HPV to the breast tissue, such as hematogenous or lymphatic spread, direct inoculation, or sexual contact, and the challenges and implications of HPV detection and prevention in BC. We highlight the possible interactions between HPV and other factors, such as genetic susceptibility and immune response, that may influence the outcome of HPV infection in BC. We provide some directions for future research and clinical practice in this field.

Keywords: human papillomavirus, breast cancer, viral oncology, molecular pathways, cancer

1. Introduction

BC is a significant public health issue and a primary cause of cancer-related mortality and morbidity among women [1, 2]. BC is one of the most prevalent malignancies in women, representing the sixth leading cause of cancer-related deaths and the primary source of cancer mortality among females. According to recent estimates, it has been projected that in 2020, there would be approximately 2.3 million new BC cases and around 685,000 related deaths worldwide. Over the past decade, there has been a steady increase in the incidence rate of BC [2–5]. Interestingly, various risk factors are linked to BC, some well-known, while others are still unknown. Several variables can influence the development of BC, including early onset of menstruation (before age 12), never giving birth, late menopause (beyond age 55), exposure to substantial levels of ionizing radiation, frequent consumption of alcohol, and a diet heavy in fat [6].

However, several viruses have been implicated as potential etiological agents of BC. However, it is noteworthy that these factors only account for a fraction, varying from 20 to 50% of BC cases [7–9]. Among these viruses, bovine leukemia virus, Epstein–Barr virus (EBV), mouse mammary tumor virus (MMTV), and Human Papillomavirus (HPV) are reported as associated with BC [10–14]. Understanding the role of these viruses in the development of BC is crucial for effective prevention and treatment. Here, we outline the various molecular pathways that explain how HPV increases the likelihood of BC.

2. HPV genome and breast carcinogenesis

The Human Papillomavirus (HPV) is a member of the Papillomaviridae family, which includes over 200 distinct genotypes, of which 40 are transmitted via the genital tract. Notably, 16 of these genotypes have been identified as potentially oncogenic, with a particular association with cervical cancer. High-risk HPV types include 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70. The oncogenic potential and transmission routes of these HPV genotypes are critical areas of study for developing effective prevention and intervention strategies [15, 16]. The genetic makeup of all HPV types includes a genome with eight open reading frames (ORF), transcribed from single-stranded DNA. The HPV genome comprises three central regions, which include early region (E), late region (L), and long control region (LCR). The LCR is 1 kb in length and lacks protein-coding regions. In the E area, there are six to seven ORFs, while in the L region, there are only two ORFs. HPV possesses conserved core genes, performs replication, and is involved in viral capsid development. However, there is a greater variation in E4, E5, E6, and E7 genes, which are involved in maturation, immune evasion, and the control of the cell cycle [17, 18], as shown in **Figure 1**.

Most HPV genomes, almost 86–100% within breast tissue, are incorporated with a low viral load between 0.00054 and 9.3 copies per cell [19]. HPV has a secondary role in tumors because of low viral titer. Different studies worldwide investigated a novel association between HPV and BC, while identification of HPV DNA (e.g., L1, L2, E1, E2, E6, and E7) in mammary carcinomas is highly inconsistent ranging between 0 and 86.2%, and it does not exhibit a correlation with the age of affected women [1, 28]. Therefore, molecular alterations in BC onset may be instigated via the "hit and run" mechanism. This conjecture implies that HPV plays a role in the initiation or facilitating of cancer development. However, in certain instances, due to the immune response vanishes from tumor cells before diagnosis [29]. The potential role of HPV as a modulator or contributor is still ambiguous and necessitates further research.

The carcinogenesis and chromosomal instability may be induced by HPV and host genome integration [30]. In BC tissues, the existence of E6 and E7 oncoproteins was confirmed, suggesting the potential involvement of HPV in BC progression. Specifically, E6/E7 mRNA was identified in 24 to 100% of HPV-positive BC specimens. Furthermore, novel E6/E7 fusion transcripts, namely E6^E7*I, E6^E7*II, were detected in breast tumors [19]. Substantial discrepancies were observed in HPV specimens, encompassing both HPV DNA and mRNA transcripts [31].

E6 degrades p53 through its interaction with E6-AP, a co-factor in the proteolytic pathway [30]. E6 exhibits functional and physical association with the telomerase complex [32]. E7 binds with pRb and retinoblastoma (pocket proteins), disrupts normal cell cycle mechanisms, and stimulates proliferation [19, 30]. The instability of the genome leads to normal cell transition to a malignant one. In an *in vitro* model, it is

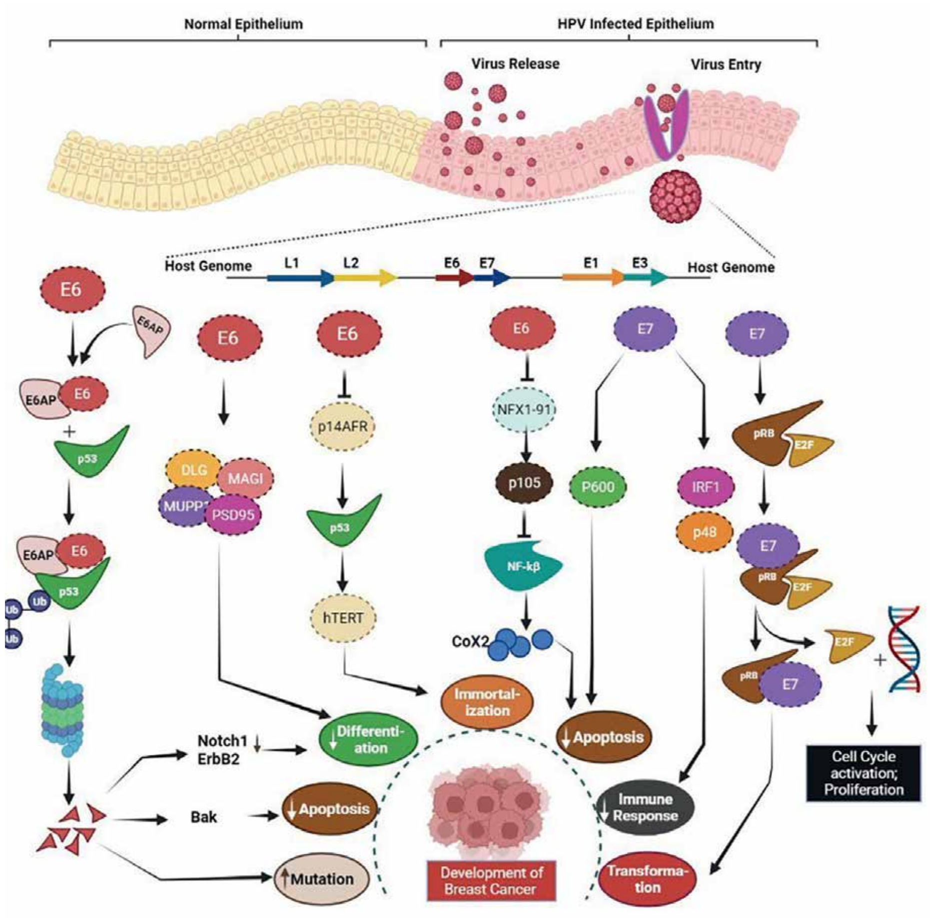

Figure 1.
This diagram illustrates the potential mechanisms underlying BC pathogenesis, focusing on the role of HPV oncogenic proteins E6 and E7. Specifically, the upregulation of E6 and E7 expression is implicated in initiating various pathways that contribute to BC progression [19–27].

demonstrated that the expression of E6 and E7 oncoproteins causes mammary epithelial cell transformation. The cell cycle disruption is associated with cyclin-dependent kinase inhibitor 2A (CDKN2A or p16INK4A), and CDKN2A is not a marker for HPV infection in BC [33]. E6 and E7 interact with BRCA 1 and BRCA 2, which are tumor-suppressor genes [34].

When HPV E6 and E7 proteins bind to mammary epithelial cells, multiple biological mechanisms come into play. Proteins such as these upregulate the pathways by inhibiting pRb, p53, NFX1, and BRCA1 [35, 36]. Additionally, E6 and E7 proteins may stabilize the HER2 receptor, upregulate BCL2, and block apoptosis, all while stimulating the proliferation of BC cells [37, 38]. A3B may have its expression altered by HPV infection, and reactive oxygen species generation may be increased. There was an increase in the prevalence of APOBEC-associated mutation signatures in East Asians in BC (31.2 vs. 9.0% in Europeans and 4.2% in West Africans) [39, 40]. APOBEC mutagenesis, a phenomenon characterized by specific mutations in cancer genomes, can be triggered by APOBEC3A and APOBEC3B proteins. The pattern of mutations observed in bladder and BC patients is influenced by various factors, which can be categorized into hereditary and environmental elements [41]. In addition, HPV-induced STAT3 activation is associated with the production of genes for pro-inflammatory cytokines in cervical and BCs [42].

The viruses' ability to cause cancer is related to several factors, including apoptosis evasion, deregulation of cellular processes, and persistent inflammation. BC cells proliferate more when there is ongoing inflammation, which is facilitated by cytokines such as interleukins (IL) and transforming growth factor beta (TGF-1) [43]. Moreover, HPV infection is connected to releasing IL-1, IL-6, IL-17, TGF-β, and TNF-α [44]. Furthermore, HPV synergizes with estrogen receptor (ER)-signaling pathways. Wu et al. demonstrated that, in conjunction with nuclear receptor coactivators, the HPV E2 protein increases the ERE-dependent transcriptional activity of Erα [45]. As a consequence, HPV-positive BC cells with strong estrogen signaling owing to ER gene amplification express the HPV oncogenes E6 and E7 more often, which accelerates the disease's growth and progression [46].

3. Mechanism of HPV-induced BC development

There is a paucity of research available on how HPV causes BC; however, one idea proposes that HPV invades the breast gland through macrophages and lymphatic cells using systemic flow and that virion can also spread to other specific locations [47]. An additional hypothesis posits that HPV may have the ability to infect breast tissue, thereby potentially linking the breast's exposure to external environmental factors and the development of BC. However, it is essential to note that this hypothesis requires further investigation and validation [48]. Sexual contact is one of the most common pathways for HPV invasion, and congenital HPV infection in children develops via placental or vaginal delivery [19, 49]. HPV enters breast cells via cell transport and endocytosis mechanism, and the alpha HPV genus is primarily involved in it [50]. The alpha-6 integrin protein is a receptor in cervix cells 51; however, in the breast, laminin-322 and alpha-6 integrin are involved in the morphogenesis of the mammary gland, and it is hypothesized that in case of a HPV infection, these proteins may promote tumor development [51, 52]. Integrin-mediated signaling pathways regulate neoplastic proliferation, programmed cell death, vascularization, and metastasis [53]. Upon cellular invasion, the viral DNA exists as an extrachromosomal element [54, 55]. At the same time, the mode of entry for LR-HPV is still unclear.

The potential role of extracellular vesicles (EVs), specifically exosomes, in disseminating HPV infection presents an intriguing hypothesis that may explain the increasing trend. This hypothesis is supported by the detection of blood-borne exosomes containing HPV genetic material in patients with HPV DNA-positive SCC of the middle rectum and BC, suggesting that EVs may play a role in HPV transmission and pathogenesis. However, further research is needed to confirm this hypothesis and elucidate the underlying mechanisms [56]. Notably, diverse cell types, tissues, and biofluids generate exosomes, which serve as cellular messengers encapsulating various biomolecules, including genetic material (DNA and RNA), proteins, and regulatory RNAs (non-coding RNAs) [57]. This exosome-mediated intercellular communication mechanism offers a plausible explanation for the non-contiguous spread of HPV infection, warranting further investigation [58]. Additionally, different studies have noted that oxidative stress could potentially affect the stromal compartment and the absorption of EVs [59]. By means of transmitting EVs positive for HPV from the primary site of infection to cells lacking the receptors of HPV, it is possible to induce the local proliferation of tumor cells. By means of in situ hybridization, the existence of HPV genome in stroma and epithelium was confirmed [60].

Both sexual and nonsexual ways can transmit the HPV. Most cases of genital HPV infection occur as a result of skin-to-skin or mucous membrane-to-muscle contact during oral or anal intercourse with an infected individual [61]. HPV can infect stratified epithelial cells once the virus enters the body by abrasions, lacerations, or epithelial surfaces. **Figure 2** shows the several pathways that HPV can use to spread from an infected area to the mammary tissues. Hematogenous or lymphatic transmission of HPV particles from other parts of the body—particularly the cervix, neck, or head—to the breasts is one way [63, 64]. Sexual contact with the nipples or cutaneous micro-injuries in the breast region is another way that HPV can directly inoculate the mammary glands [61, 65].

The specific mechanism through which HPV gains access to the breast tissue is still unknown. The HR-HPV genotype was consistent in BC samples and squamous intraepithelial lesions of the cervix from the same patients, according to Lawson et al. [66]. Furthermore, the presence of squamous intraepithelial lesions associated with

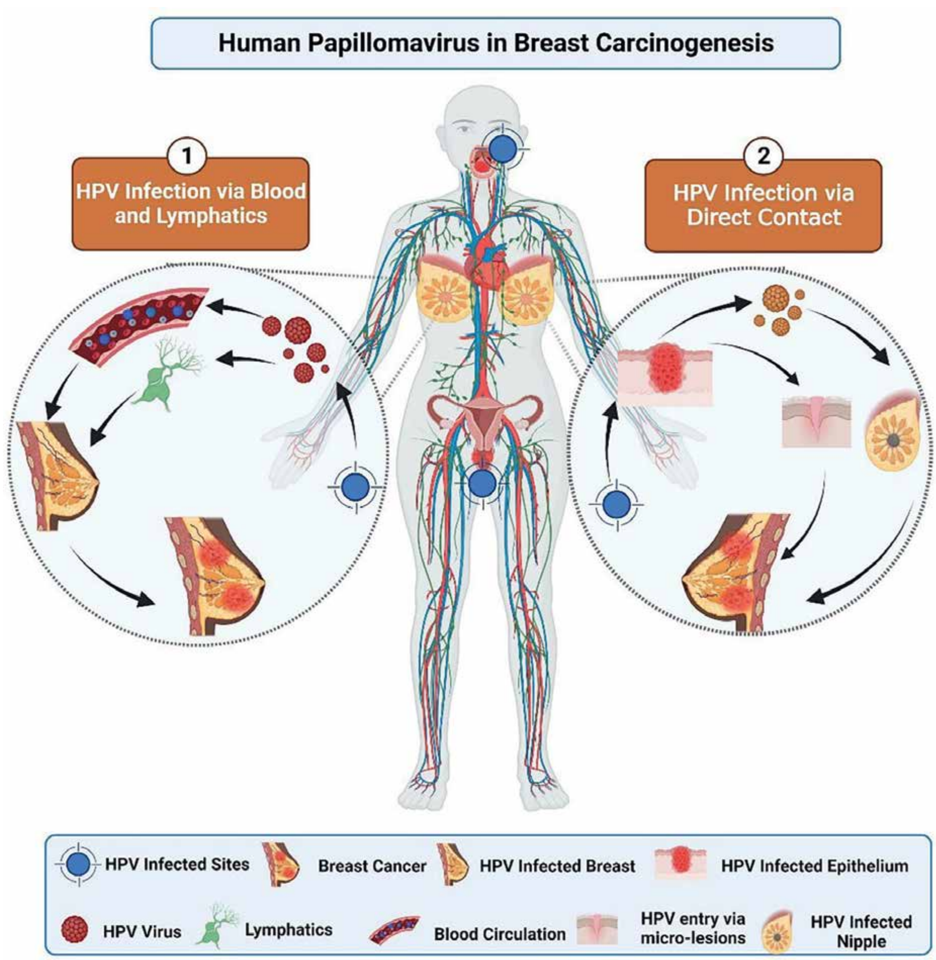

Figure 2.
Potential pathways for HPV-induced breast carcinogenesis [62].

HPV was confirmed in BC samples collected from women who had BC. The scientists postulated that in cases of HPV-positive cervical cancer, the virus may potentially spread to the breasts through the bloodstream. It was suggested by Bodaghi et al. and others that HPV could spread via circulation since HPV DNA was detected in monocytes [64]. Researchers could not uncover evidence that HPV could infect monocytes or that the virus could propagate via the blood. HPV can affect basal cells within a stratified epithelium; however, its susceptibility is limited to cells situated in the more specialized epithelial layers. Who knows how HPV particles make their way from infected areas to the breast or anywhere else in the body—it's just conjecture at this point. For virological reasons, explaining a circulation-based mechanism is challenging, as HPV multiplies in the squamous epithelium to evade immune detection. Circulating HPV DNA in the circulation may potentially be transferred to the breast, where it could be assimilated, according to one theory [67, 68].

De Carolis et al. emphasized the possible transfer of HPV genome from serum-derived extracellular vesicles (EVs) to TNBC [60]. The authors propose a potential role for HPV DNA from EVs in increasing the malignancy of BC, suggesting that HPV DNA may be transported from infected sites to the breast tissue via extracellular EVs. However, this hypothesis requires further investigation. One possibility is that certain sexual activities may cause minor skin injuries on the nipple or areola, providing a potential entry point for HPV to infect the breast tissue. HPV is most likely transmitted to the breast through direct contact with the skin or mucosal epithelium. Additionally, other potential factors, such as sexual transmission and EV transmission pathways, may also contribute to HPV infection of the breast tissue. Further research is needed to confirm these hypotheses and elucidate the underlying mechanisms [19, 46].

HPV has a particular affinity for infecting epithelial cells, which are responsible for lining different surfaces and cavities in the body. These may involve various parts of the body, such as the skin, respiratory tract, digestive tract, and reproductive organs. Epithelial differentiation is vital for the completion of the life cycle of HPV. It involves the specialization of cells in both structure and function [46, 69]. During the early stages of infection, HPV virions specifically attack the basal epithelial cells that have been exposed because of injury.

The virus can infiltrate these cells and integrate itself as a small episome in the nucleus, enclosed in a vesicle that contains the L2 protein. This process signifies the initiation of the HPV infection and lays the foundation for the subsequent replication and its gene expression. This process is unique to HPV and is not observed with other types of viruses [70]. After entering the host cell nucleus, the HPV genome goes through an initial amplification phase where it rapidly replicates multiple copies of itself within each cell. This process signifies the initiation of the HPV infection within the host cell and is crucial for the subsequent expression of viral genes [71].

4. Prevalence of HPV in BC

The global prevalence of HPV in BC ranges between 1.6% and 86.2% [7, 72, 73], even though other researchers found no HPV in these types of tumors [65, 74]. The incidence of HPV infection in BC did not appear to vary by region [19]. Comparing research is difficult, though, because of the variety of materials, which include fresh frozen tissue and PCR techniques with various primers used for HPV detection. Furthermore, it's not impossible that contamination with earlier PCR products affected some of the preanalytical and analytical data. There has been reported geographic HPV transmission despite these restrictions. For instance, 32.42 and 12.91% of BC patients in Europe and Asia, respectively, were found to be HPV positive [75]. Moreover, 42.9% of BC population from North America and Australia had HPV, compared to 15.1% from South and Central America [46]. According to research that started with information from The Cancer Genome Atlas (TCGA) database and Australian BC specimens, 2.3% of BCs carried HR-HPVs. Next-Generation Sequencing (NGS) was then used to evaluate the data. Interestingly, benign breast specimens that tested positive for HR-HPV were linked to BCs that tested positive for the virus in the same subjects, according to the researchers. Furthermore, physiological activity of HR-HPV has been observed in British Columbia [76]. Bae and Kim [77] found that among those with positive HPV, the probability of having BC was increased by 4.02-fold (95% CI: 2.42–6.68). Similarly, Choi et al. found a link (OR = 5.43, 95% CI: 3.24–9.12) between BC and HPV infection [78].

Additionally, similar research by Ren et al. found a statistically significant correlation between BC development and HPV infection (OR = 6.22, 95% CI: 4.25–9.12) [79]. Notably, normal tissues did not show any evidence of HPV, but BC samples did [80]. In contrast to BC, the incidence of HPV infection in normal breast samples was significantly lower. Moreover, a higher prevalence of HPV infection was observed in BC compared to benign breast lesions, including fibroadenomas, fibrocystic changes, mastitis [46, 80], intraductal papilloma's [81], and breast adenosis [65]. Furthermore, samples from fibroadenomas and neighboring normal breasts showed higher rates of HPV infection compared to samples from British Columbia (64.8%) [46]. Koilocytes, which are indicative of HPV-positive cells, were identified in BC cells using PCR [82]. Most HR-HPV types have been found in BC samples [19]. However, LR-HPV subtypes are reported in certain cases [83]. HPV16 is the most prevalent genotype in BC patients, accounting for 87.5% of cases, with HPV18 present in 12.5% of cases. Moreover, HPV16 was identified in 77.37% of BCs that tested positive for HPV, followed by HPV33 (13.64%) and HPV31 (9.09%) [84]. However, other papers state that HPV33 [85], HPV39 [86], or HPV51 [87] are widely frequent HPV subtypes in breast cancer. HPV DNA has been observed to be preferentially present in high-grade BC (II/III) [88].

Furthermore, grade II BC included the majority of HPV16 and 58, although grade III cancers had a higher rate of HPV18 infection [89]. According to the molecular classification (reviewed in [19]), all subtypes of BC, such as Luminal A, Luminal B, HER2-enriched, and triple-negative BC (TNBC), have HPV infection. However, HPV was shown to be more common in TNBC and HER2-enriched malignancies than in Luminal A and B types [90]. BC's more aggressive biological activity is linked to HER2-enriched cancers and TNBC. Furthermore, compared to HER2-positive tumors, hormone receptor-positive BCs had greater HPV infection rates [81]. Furthermore, HPV was specifically present in HER2-negative Luminal B tumors; however, no statistically significant difference was seen between these cancers and other molecular subtypes, such as the Luminal B/HER2+ phenotype [83]. Surprisingly, HPV DNA was linked to increased lymph node invasion and greater rates of proliferation in Luminal A and Luminal B tumors, respectively. These results suggest a link between HPV infection and more severe types of BC illness. Nevertheless, no correlation with a specific molecular subtype was discovered [84].

There has been a long-standing debate over the precise etiology of BC that is associated with HPV. However, recent studies have shown that there is a significant connection between HPV infection and the development of BC. In spite of this, there are still a great deal of problems and difficulties that need to be solved in this area of study [62]. For detecting HPV, the majority of studies used either conventional or nested PCR using commercial primers for the L1 gene (capsid protein). They presented plausible reasons for false positive and false negative findings, as well as limiting variables linked to the diagnostic procedures utilized in those investigations, such as viral load and DNA/RNA quality [62]. This was done after the positive results were obtained. Primers were also used for the E6 and E7 genes, as well as sequencing processes. Only 13–15 of the 200 subtypes of HPV are high-risk, which are linked to a variety of malignancies [91]. The thorough meta-analysis conducted by Awan and colleagues revealed that the pooled prevalence of HPV infection among females diagnosed with BC was 25.6% (95% confidence interval (CI) = 0.24–0.33, I2 = 97%, $\tau2$ = 0.0364, p = 0). New risk factors for the development of BC must be identified, as this result serves to highlight [62].

Following the stratification of the control group and the control breast tissues, Awan et al. discovered significant variability between the control and breast tissues [62]. The pooled frequency of HPV in BC tissues was found to be 26.2%, with overall odds of 5.55 (95% CI = 3.67–8.41, I2 = 38%, $\tau2$ = 1.4878, p-value less than 0.01). The fact that the I2 value across subgroups was less than 50% and suggested that there was an acceptable amount of heterogeneity brought to light the significance of control selection. There have been reports of similar findings from other meta-analyses, which indicate that there is a substantial connection between HPV and BC. As an example, a meta-analysis of 37 case-control studies, which included 1728 controls and 3607 cases of BC, found overall odds of 6.22 (95% CI = 4.25 to 9.12, p = 0.0002) [79]. The odds were estimated to be 5.9 (95% CI = 3.26–10.67) in another study that included nine case-control studies [92]. Bae et al. found odds of 4.02 (95% CI: 2.42–6.68) [93] in a meta-analysis that included 22 different investigations. A meta-analysis of 10 case–control studies, which included 447 cases of BC and 275 controls, revealed that the presence of HPV positive was associated with an elevated risk of BC (OR = 3.63, 95% CI = 1.42–9.27) [75]. On the other hand, the consistency of several techniques of HPV detection, which suggest a much greater incidence of HPVs in BC than in control tissues [94], lends confidence to the hypothesis that there is a connection between the control group and BC.

The study and meta-analysis that Awan and colleagues conducted included 74 publications that were published throughout the last three decades [62]. In addition, the meta-analyses carried out in Europe, North America, and Australia by Simoes et al. [92] have provided an additional piece of evidence that supports their conclusions. In Iran, the prevalence of HPV infection among BC patients is notably higher compared to European women. This could be attributed to various factors, including differences in sexual behavior, cultural practices, or the prevalence of high-risk HPV strains in the region. Conversely, the prevalence is lower among women from North America and Australia, indicating that these regions might have more effective screening programs, higher HPV vaccination rates, or different patterns of HPV type distribution.

The report of up to 86% HPV infection rate in British Columbia is particularly striking. Such a high prevalence rate could point toward a lack of adequate screening or vaccination programs, or it could reflect a population with a higher risk of exposure to HPV. It's important to note that this figure may not represent the general population but rather a specific subgroup within British Columbia that was studied. These findings underscore the importance of considering demographic and geographical factors when analyzing the prevalence of HPV infections. They also highlight the need for tailored public health strategies that address the unique challenges and risk factors present in different regions to effectively combat HPV-related diseases [48, 92]. The conclusion that can be derived from the numbers that have been published in the past is that about one in every four women have been diagnosed with HPV-positive BC [73, 75, 95].

The prevalence of HPV strains that were discovered in various groups was shown to be significantly different from one another [19]. HPV infections, which include HPV 16, 18, and 33, have been found in British Columbia [96]. HPV infections are frequently associated with genital atypical lesions and malignancies. Notably, HPV 11, 16, 18, and 33 were the most common types found in European women with BC, while HR or LR subtypes HPV 52, 59, and 83 were more prevalent in African women with BC [96, 97]. The HR subtypes of HPV, including HPV 16, 18, 31, 33, 35, 52, and 58, were investigated by Awan and colleagues [62]. The findings of the study revealed a significant association between all HPV types and an increased risk of BC ($p < 0.05$), supporting the current literature and suggesting that HPV infection may play a role in BC development and progression. Specifically, the frequency of HR HPVs in BC tissue was six times higher compared to normal and benign breast tissue controls. HPV-16 was the most frequently detected type in BC tissues, with an overall frequency of 9.7% (95% CI = 3.15–11.73, I2 = 0%, $\tau 2 = 0.5766$, $p < 0.01$). Additionally, HPV-18 was the second most prevalent type found in BC tissues, with a frequency of 6.6% (95% CI = 1.95–4.04, I2 = 0%, $\tau 2 = 0.2734$, $p < 0.01$). These findings suggest a potential role of HPV infection in BC pathogenesis and warrant further investigation [62].

The review and meta-analysis conducted by Awan and colleagues included 3156 publications that were published throughout the last three decades [62]. In order to establish the prevalence of HPV in BC tissues, they conducted a thorough analysis of 1223 investigations and examined 74 publications. However, they excluded 1130 studies since they were deemed insufficient. Meta-analyses carried out in Europe, North America, and Australia by Simoes et al. [92] have provided an additional piece of evidence that supports their conclusions. Interestingly, the prevalence of HPV infection was higher among patients with BC in Iran than among women from Europe, although it was lower among women from North America and Australia. It has been reported that up to 86% of people in British Columbia are infected with HPV, which suggests that demographic variables and geographical variances may be responsible for the discrepancies that exist across nations [48, 92]. The conclusion that can be derived from the numbers that have been published in the past is that about one in every four women diagnosed with BC are infected with HPV [75].

The HPV prevalence strains that were discovered in various groups were shown to be significantly different from one another [19]. HPV infections, which include HPV 16, 18, and 33, have been found in British Columbia [96]. These infections are often cited as etiological agents for cancer. It is interesting to note that HPV 11, 16, 18, and 33 were the most prevalent kinds among European women who had BC. On the other hand, African women were more likely to have HPV 52, 59, and 83, which were either HR or LR subtypes [96, 97]. The HR subtypes of HPV, including HPV 16, 18, 31, 33, 35, 52, and 58, were investigated by Awan and colleagues [62]. The results of their investigation indicated that all these HPVs were linked to an elevated chance of getting BC ($p < 0.05$). Their research lends credence to the current body of research and hints that the presence of HPV infection may have a role, either as a cause or a contributor, in the development and progression of BC. When compared to the frequency of HR HPVs in normal and benign breast tissue controls, the frequency of HR HPVs in BC tissue is six times greater [98]. Furthermore, the presence of HPV-16 in BC tissues was reported in 21 studies, resulting in an overall frequency of 9.7% (95% CI = 3.15–11.73, I2 = 0%, $\tau 2$ = 0.5766, $p < 0.01$). In addition, the second most prevalent form of HPV detected in BC tissues was HPV-18, which had a frequency of 6.6% (95% CI = 1.95–4.04, I2 = 0%, $\tau 2$ = 0.2734, $p < 0.01$) [62].

Awan et al. carried out a comprehensive study with the purpose of determining the prevalence and distribution of different HPV subtypes in breast cancer tissues from a variety of geographical locations. According to the findings of the study, the HPV-18 strain was considerably more widespread in Australia, but the HPV-16 strain was the most prevalent kind around the globe, including in Asia, America, Europe, and Africa. There were 45 articles that were examined, and 25 of them were from Asia. This indicates that the overall prevalence was 22.7%. The study also discovered that the prevalence of HPV in patients with breast cancer varied depending on the geographic location of the patients. Europe had the highest rate (39.1%), followed by Africa (31.8%), Australia (30.5%), and the United States (33%). In addition, this study indicates that the incidence of HPV in patients with breast cancer varies from area to region. These findings suggest that HPV prevalence in BC patients differs across regions, which could have significant implications for tailoring prevention and treatment strategies for BC caused by cervical cancer [62, 99].

Several factors could explain the regional disparity in HPV infection and its link to BC. These factors encompass sexual behavior, hygiene practices, vaccination history, screening routines, and socioeconomic standing [4, 5]. However, the high rate of BC in Asia attributed to HPV (22.7%) is because a considerable portion of its female population has multiple sexual partners, less condom usage, and restricted HPV vaccination access, healthcare disparities [5, 100] and cervical cancer screening [101]. Europe, conversely, exhibits a lower rate of HPV in BC cases (13.4%). This is because of the advanced health systems, increased knowledge on preventing HPV, and wider availability of the HPV vaccine [73, 95]. Additionally, the distribution of HPV subtypes and their carcinogenic potential can vary geographically. Specifically, HPV 16 and 18 are more prevalent and potentially oncogenic subtypes in BC tissues compared to other subtypes, although their distribution is not uniform worldwide. These variations in HPV subtypes could influence the risk of BC development across regions. Moreover, differences in detection methods, sample selection, and data quality and availability can impact HPV prevalence estimation in BC across countries and regions. However, the interpretation of findings may be complicated by factors such as the scope of research, accuracy of tests, and comprehensiveness of samples [62, 73].

Because the causal connection between HPV and BC cannot be definitively proven, there are still unanswered questions about the potential underlying mechanism. To prove that the virus is involved in the pathophysiological BC development, it is essential to address this issue, as the detection of HPV alone is not enough to establish this connection.

5. Conclusions

BC is a multifaceted and intricate disease that impacts a significant number of women globally. While the role of viral infections, particularly HPV, in BC etiology and progression remains contentious and unclear, mounting evidence suggests that HPV may be involved in BC development and progression. HPV may infect and transform mammary epithelial cells through various molecular pathways, and its interaction with other factors, such as genetic susceptibility, hormonal status, and

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immune response, may influence BC outcomes. Consequently, HPV detection and prevention in BC is a critical and complex task that necessitates further investigation and clinical application. Gaining a deeper understanding of HPV's role in BC may offer novel insights into the pathogenesis, diagnosis, and treatment of this disease.

Conflict of interest

There is nothing to declare.

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Chapter 4

New Evidences about Unusual Behavior of HeLa Cells in Stress Environment Concerning Immortality Status

Natalya Rekoslavskaya, Anna Chemezova and Alexei Tchemezov

Abstract

To produce an anticancer vaccine, we harnessed a plant viral expression system utilizing transgenic tomato fruit containing the genes HPV16 E2, E6, and E7. Notably, antibodies from the serum of mice orally vaccinated with HPV16 E2 spurred the formation of biofilms in HeLa cells, resembling dendrimer structures initially colored with sublethal trypan blue (TB) dye, which later faded. These biofilms emerged *ex vivo* upon the introduction of HPV16 E2, L-amino acid oxidase, D-amino acid oxidase, HPV16 E6, and E7, in conjunction with a CRISPR/CAS cassette. Subsequently, HeLa cells adopted a symplast-like structure devoid of cell demarcation, composed of fused membranes encircling the cytoplasm. In a separate experiment, mice spleens, rich in immune cells and red blood cells, were inoculated with HeLa cells. Following isolation, splenocytes underwent Elispot analysis after exposure to HPV16 E2, L-amino acid oxidase, and D-amino acid oxidase as activators. Significantly elevated levels of interferon, T-cell receptors, CD4/CD8 T lymphocytes, and apoptotic enzymes (granzyme B, perforin, and granulysin) were detected. Furthermore, splenocytes derived from HeLa-treated spleens exhibited the ability to induce regression of mice lung tumors *ex vivo*. These findings suggest that splenocytes, when exposed to HeLa cells, may undergo a form of training or education, facilitating the development of a microenvironment.

Keywords: biofilms of HeLa cells, HPV16 E2, L-amino acid oxidase, D-amino acid oxidase, antibodies to HPV16 E2, regression of mice lung tumors, “education” of splenocytes

1. Introduction

1.1 Characteristics of papillomaviruses

The term “papillomavirus” originated from the Latin for nipple (*papillo*) and tumor (*ome*). Papillomaviruses (PVs) are nonenveloped DNA viruses that induce exophytic lesions of the skin and mucosal surfaces. PVs have been around for millions of years; fossil remains of ancient organisms have been found to contain PV DNA.

About 450 genotypes of PVs have been documented, with 12 types characterized as carcinogenic [1]. PVs have been found in all living vertebrates and invertebrates, including birds, turtles, snakes, shellfish, and so on.

The first animal PV was described in 1933 by Richard Shope who investigated papillomata in “warty” wild cottontail rabbits, also known as “jackalopes” [2]. These animals were infected with PVs and formed benign tumors on the head as horns and on the body together with keratin plaques. But if this benign tumor was minced and infected to health cottontail rabbit, there were formed carcinogenic tumors then on the body.

In 1975, Harald zur Hausen published the hypothesis that human papillomavirus (HPV) played a specific inductive role in the etiology of cervical cancer. For this work, he was awarded a Nobel Prize in Physiology and Medicine in 2008 [3].

Each HPV genome contains six structural genes within the “early” region: E1, which encodes helicase; E2, known as a suppressor of the expression of oncogenes E6 and E7; and E4 and E5, with functions that are not fully understood. Additionally, there are two genes in the “late” region, which encode the major L1 coat protein and the minor L2 coat protein.

HPV types 16 and 18 are responsible for approximately 70% of cervical cancers and precancerous cervical lesions. It is noteworthy that up to 50 genomic copies of HPV18, which have undergone recombination with cellular sequences, can be identified in HeLa cells. These cells, derived from the cervical carcinoma tissue of a 31-year-old patient, were the first human cells to be successfully cultured in vitro. HeLa cells have since become widely used as a laboratory cell line [4].

Comparing the behavior of immortal HeLa cells with that of non-immortal bacteria surviving in biofilms could offer valuable insights into cellular immortality and microbial survival strategies.

1.2 The conditions of biofilm formation in connection with HeLa cells

Biofilms are complex communities in which resident bacteria exist within a self-derived extracellular matrix. Every bacterial species, or consortium thereof, forms its own unique multicellular community [5]. Architectural and behavioral investigations of biofilms of different bacteria have led to the conclusion that biofilm formation on both living and inanimate surfaces serves as an adaptation for survival, allowing bacteria to overcome environmental threats such as antimicrobial agents and host defense mechanisms. However, as biofilms mature, they encounter challenges such as nutrient limitation and accumulation of waste products. As such, biofilm-associated bacteria may return to a planktonic state, which offers advantages in nutrient availability [6]. To facilitate the study of the association between biofilm formation and the virulence determinants of pathogenicity, different ex vivo models have been developed. These models offer more strictly controlled experimental conditions compared to in vivo models, allowing for intensive research. Ex vivo models are also useful for assessing therapeutic pathways for combating biofilm-related infections [5].

Nevertheless, biofilms have had a global impact on modern medical and microbial literature, but their study continues to raise more questions. Despite extensive research, there is lack of evidence regarding the formation of biofilms by HeLa cells.

In a search spanning 5 years, only a few instances were found suggesting the involvement of HeLa cells in biofilm formation with various bacteria species. Examples include enteropathogenic and uropathogenic *Escherichia coli* [7], *Providencia stuartii* [8], *Candida tropicalis* [9], *Aliarcobacter butzleri* [10], mastitopathogenic *E. coli* [11], and *Lactobacilli* [12]. However, these instances typically involve bacteria settling on certain areas of HeLa cell surfaces without forming cohesive structures or consortia. It appears that HeLa cells remain largely unaffected by these bacterial interactions.

The limited evidence suggests that HeLa cells may possess resistance to invasion by other bacteria. This could imply that the cell walls and membranes of HeLa cells are highly stable and able to withstand the presence of other organisms.

Nevertheless, certain natural products can destroy HeLa cells. For example, extracts from medicinal cactus plant *Morinda citrifolia* [13] were found to induce aging and loss of adhesion of HeLa cells, leading to their detachment and as single cells. This suggests that HeLa cells possess an integral surface membrane that can be affected by certain compounds. Similarly, the antimicrobial peptide brevedin, derived from the skin of lake frogs, has been shown to permeate bacterial membranes via endocytosis and inhibit the proliferation of HeLa cells [14]. Furthermore, a cytotoxic extract from the anticancer medicinal plant *Vassobia breviflora* [15] has been found to block the growth of HeLa cells at a concentration of 0.03 mg/ml. This extract also exhibits antibiofilm activity against other bacterial species.

It was of interest to investigate the potential anti-HeLa cell activities of well-known oncolytics such as HPV16 E2, L-amino acid oxidase (LAAO), and D-amino acid oxidase (DAAO). The literature has documented the apoptotic activity [16–25] of HPV16 E2, its antiproliferative properties [24], and anticancer action [26, 27] alongside the well-documented activities of LAAO [27–29] and DAAO [6, 27, 29].

Therefore, the goal of this study is to investigate the effects of superoncosupressor "early" protein HPV16 E2, LAAO, DAAO, and other "early" proteins like HPV16 E6 and HPV16 E7 on HeLa cells ex vivo. The study aims to elucidate whether these proteins could potentially "educate mice" splenocytes to recognize or learn to target lung tumor cells induced by HeLa cells, ultimately driving them into regression. This approach holds promise for developing novel therapeutic strategies against HeLa cell-derived tumors by harnessing the immune system's ability to recognize and eliminate cancerous cells.

2. Methodology

The genetic constructs and synthetic capabilities of the plant virus expression system, based on tomato fruit, to synthesize HPV16 E2, E6, and E7 were described previously [29].

HeLa cells were purchased from Biolot (Saint Petersburg, Russia Federation) and cultured in Corning flasks using DMEM supplemented with 10% bovine fetal serum. Before experiments, HeLa cells were kept at −62°C in DMEM + bovine fetal serum and 30% glycerol for cryoconservation. The viability of HeLa cells was detected with trypan blue dye coloring, which stains dead cells dark blue.

Mice were obtained from the vivarium of the Irkutsk Anti-Plague Institute of Siberia and the Far East (Irkutsk, Russia).

For Elispot analyses, splenocytes (T lymphocytes) were isolated from mouse spleens. The spleen tissues were minced into tiny pieces using a needle from a sterile syringe in 1 ml of DMEM. The splenocyte fractions (T lymphocytes) were purified by centrifugation at 700 g in a refrigerated centrifuge for 7 minutes at 4°C. The fractions were then examined under a microscope for purity.

In Elispot analyses to study the activation of the immune system, antibodies from Abcam (UK) were utilized as activators (effectors). These antibodies included:

- Murine IFNγ ELISPOT KIT [AB64029]
- Rabbit monoclonal [EPR1108] to interferon gamma (AB133566)
- Anti-T-cell receptor antibody (JOVI.1) (AB5465) mouse monoclonal
- Rabbit monoclonal anti-CD4 antibody [EPR19514] (AB183685)
- Rabbit monoclonal anti-CD8 alpha (SP16) antibody [EPR21769] (AB217344)

Rabbit monoclonal antibodies for enzymes of apoptosis:

- Rabbit polyclonal anti-granzyme B (AB53097)
- Rat monoclonal anti-perforin antibody [CB5.4] (AB16074)
- Anti-human granulysin (AB213787) monoclonal

The second antibodies used were goat immunoglobulins to mice conjugated with alkaline phosphatase, and substrates BCIP(NCIP)/NBT (5-bromo-4-chloro-3-indolylphosphate/nitrotetrazolium blue) from Sigma (USA).

L-amino acid oxidase (LAAO) from *Crotalus adamanteus* and D-amino acid oxidase (DAAO) from porcine kidney were sourced from Sigma (USA).

To induce tumor formation, fresh isolated mouse lungs were placed in flasks with a suspension of HeLa cells and inoculated for 5 days. Similarly, fresh isolated mouse spleens were placed in another set of flasks with a suspension of HeLa cells and inoculated for 5 days.

Experiments were repeated 2–5 times with replicates to ensure robustness and reliability of results.

3. Results and discussion

3.1 The action of HPV16 E2, vaccine material of tomato transgenic with "early" genes HPV16 E2, E6 and E7, therapeutic proteins LAAO and DAAO on the cultivation of cancer HeLa cells in vitro

Before conducting the experiments, the suspension of HeLa cells was kept in a low-temperature freezer in DMEM medium supplemented with 30% glycerol or DMSO. During seeding, 0.5–1 ml of unfrozen suspension of HeLa cells was placed in 5 ml of DMEM medium with the addition of 10% of fetal cow serum and antibiotics penicillin 500 units and streptomycin 50 mg/l. After 30 minutes of seeding, a monolayer of HeLa cells was formed on the bottom of the Corning flasks, which was evidence of the high viability of HeLa cells. On the second day, the bottom of the Corning flasks was covered with a monolayer of HeLa cells and all subsequent experiments were conducted using this material.

Figure 1 shows HeLa cells grown during 2 days as a monolayer on the bottom of Corning flasks. At a magnification of 400 times, intact HeLa cells could be observed, characterized by thick cell walls and large nuclei containing 1–3 nucleoli.

As shown in **Figure 1(2)**, the dye NBT stained HeLa cells with functional mitochondria, resulting in a purple-blue coloration. Conversely, the indicator of dead cells with damaged membranes, trypan blue (TB) (**Figure 1(3)**), did not stain the original HeLa cells.

Figure 1(2) illustrates the outcome of the action of HPV16 E2 on a two-day suspension of HeLa cells obtained from the monolayer on the bottom of corning flasks. The cells were suspended in DMEM medium supplemented with antibiotics.

Immediately after adding 30 μl of HeLa cell suspension to the supernatant of the homogenate of transgenic tomato (containing HPV16 E2) and 20 μl of 0.4% trypan blue (TB), staining of the HeLa cells was observed, accompanied by flotation due to disruption of adhesion to the glass slide (**Figure 2(1)**). After 30 minutes, these free-floating HeLa cells aggregated into flakes, eventually forming tightly dyed lumps (**Figure 2(2)**). Subsequent changes in the condition of the stained HeLa cells with TB resulted in the compaction of these floating cells (**Figure 2(3)**).

It was discovered that the blood serum of mice vaccinated with the vaccine material derived from transgenic tomato fruit containing the HPV16 E2 gene had a similar effect as the transgenic protein HPV16 E2 itself. This serum facilitated the penetration

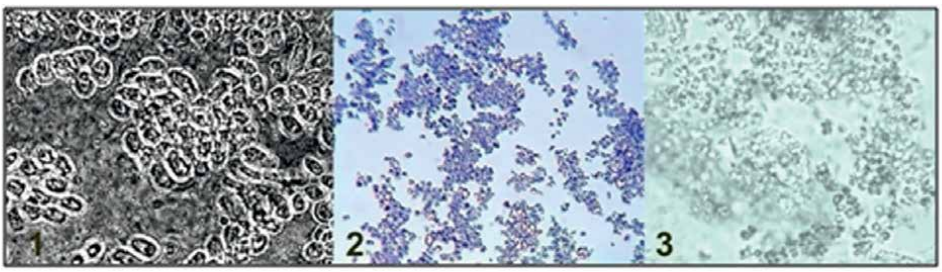

Figure 1.
1: Native suspension of HeLa cells taken from the monolayer on the bottom of the corning flask after 2 days of growing on the DMEM medium without staining. Light microscope, multiplication ×400, video camera Levenhuk C310 NG (Levenhuk Ltd., USA). The preparate was placed in Cedarwood oil (PanReac AppliChem, Darmstadt, Germany); 2: HeLa cells, stained with vital dye nitrotetrazolium blue (NBT) ×100; 3: HeLa cells in the presence of sublethal dye, the indicator of dead cells with damaged permeable membrane trypan blue (TB), ×300.

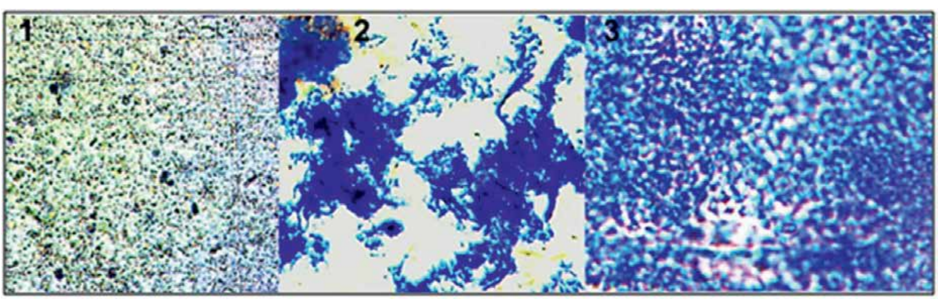

Figure 2.
The action of HPV16 E2 on HeLa cells in the presence of TB. 1: 5 minutes after the addition of HPV16 E2 and TB; 2: 30 minutes after the addition of HPV16 E2 and TB; 3: 60 minutes after the addition of HPV16 E2 and TB. Light microscope, video camera Levenhuk C310 NG; ×300.

of trypan blue (TB) into HeLa cells with damaged membranes, resulting in the aggregation of HeLa cells into tightly dyed lumps (**Figure 3(1)** and **(2)**).

However, during the exposure period, the structured conglomerates of stained cells began to undergo changes, gradually forming various dendritic forms in some areas and losing their color (**Figure 3(3–6)**), despite the entire slide being loaded with TB. Notably, no such transformation was observed after the action of blood serum from non-vaccinated native mice on HeLa cells (data not presented).

After the addition of LAAO (30 μl of a 10 mg/ml solution), tightly stained flakes of HeLa cells formed in the presence of TB within the first 30 minutes (**Figure 4(1)**). Subsequently, after 2 hours (**Figures 4(2)**) and 3 hours (**Figure 4(3)**), there was a

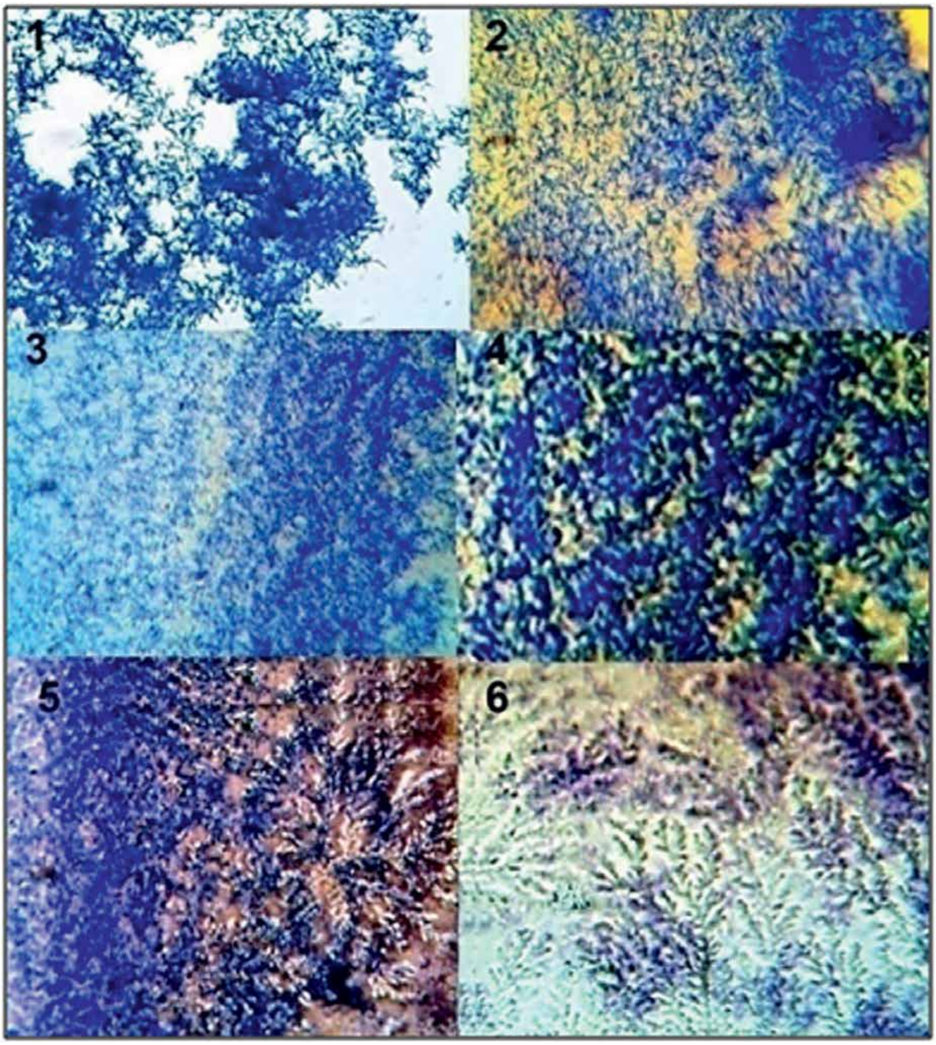

Figure 3.
The dynamics of the action on the HeLa cells of the blood serum of mice vaccinated per os by vaccine material of tomato fruit, transgenic with HPV16 E2. 1: after 5 minutes; 2: after 20 minutes; 3: after 2 hours; 4: after 6 hours; 5: after 16 hours; 6: 20 hours after the addition of mice blood serum (20 μl) and TB (20 μl). Light microscope, video camera Levenhuk, ×100.

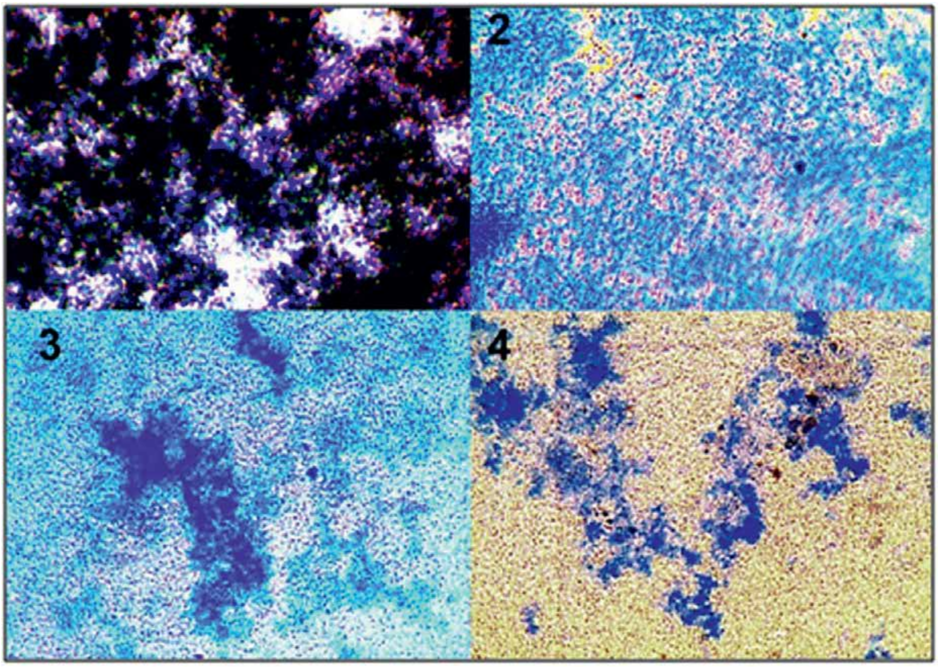

Figure 4.
The dynamics of the action of LAAO on the suspension of HeLa cells; 1: after 30 minutes; 2: after 2 hours; 3: after 3 hours, 4: after 4 hours.

reduction in color intensity and the compactization of HeLa cells into more organized structures. By 4 hours, more than half of the HeLa cells had become colorless and densely compacted in a monolayer, with approximately 30% of the remaining cells remaining unpainted with TB (**Figure 4(4)**).

The metamorphosis of HeLa cells following the addition of 10 μl of a 10 mg/ml solution of LAAO from the North American rattlesnake, Crotalus adamanteus, along with 30 μl of TB, is presented in **Figure 5**. Within the first 5–30 minutes, stained blue conglomerates of HeLa cells formed (**Figure 5**, upper row). Subsequently, these clusters began to exhibit dichotomic branching and appeared as colorless branches during the subsequent hours of exposure to the snake venom solution with TB (**Figure 5**, middle row). After 5 hours, all HeLa cells were observed in the form of a continuous symplast, resembling bilayer membranes in three dimensions with branching without subdivision into individual cells (**Figure 5**, lower row).

From **Figure 5** (final lower row), it is apparent that single HeLa cells were not observable under the light microscope even at a magnification of 1000 times. This suggests the possibility of membrane fusion of all cells into a uniform, continuous symplast without cytokinesis under the action of LAAO. It can be speculated that these structures serve functions of protection and sustainable self-preservation for HeLa cells. The dimensions of this symplast can be estimated based on the dimensions of the Goryaev chamber, where the distance between two vertical lines (**Figure 5**, middle row, photo in the center) amounted to 1/1600 mm^2.

The addition of DAAO (from porcine kidney, Sigma, USA) to HeLa cells resulted in the formation of unusual structures (**Figure 6**). Upon the addition of TB together with DAAO, there was a loss of adhesion, and individual cells floated

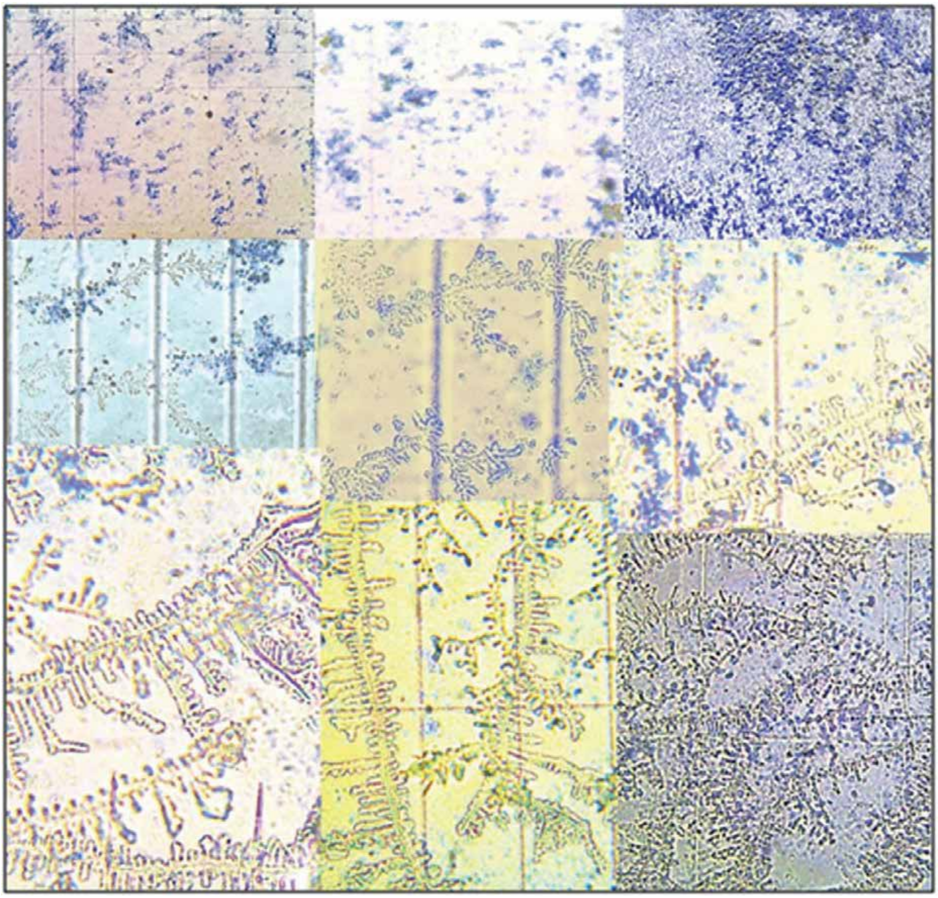

Figure 5.
The dynamics of the gradual formation of branching continuous symplast of HeLa cells during the interval of 5 minutes to 5 hours in the presence of 30 μl of the solution 10 mg/ml of LAAO from snake venom of north American rattle-rattler Crotalus adamanteus after staining with TB.

in the suspension of HeLa cells with DMEM. Subsequently, the HeLa cells began to conglomerate, and after 5 hours of exposure, the cellular structures transformed into a tightly compacted single grainy layer (**Figure 6**).

It was observed that during the action of the "early" protein HPV16 E7 in the presence of TB, HeLa cells lost adhesion and became suspended within the first few minutes of addition but remained unstained (**Figure 7**).

The phenomenon of flotation and disruption of monolayer growth is typically considered an indication of aging in HeLa cells. Some of these cells showed slight staining with TB after 3 hours of exposure. Slow and weak staining was observed with HPV16 E6 in the presence of TB (data not presented).

Since oncoproteins HPV16 E6 and E7 do not possess the supersuppressor-oncolytic activity observed with HPV16 E2, these proteins may not activate the instability of HeLa cell membranes. Hence, staining occurred slowly (**Figure 7**).

CAS was used to excise oncogenes E6 and E7 from HeLa cells. Endonuclease CAS targeted these oncogenes using a guide RNA with a template derived from RdRP. This decision was made based on the assumption that multiple genomes of related papillomavirus HPV18 were present in HeLa cells (**Figure 8**).

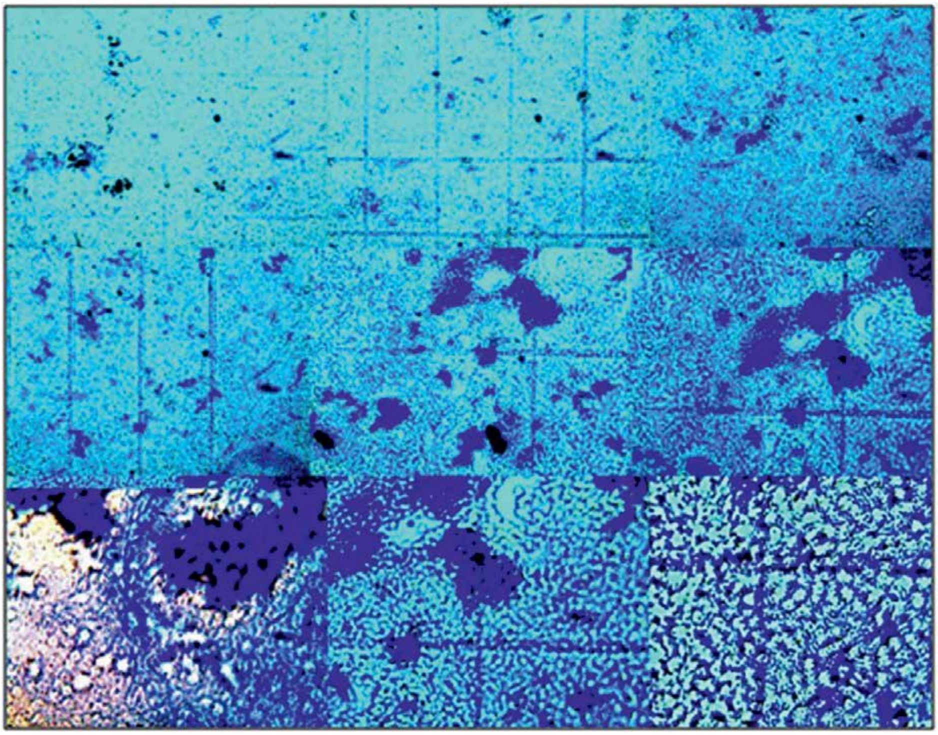

Figure 6.
The sequential change of structures of compactization of HeLa cells in the presence of DAAO and TB for the period from 5 minutes to 5 hours of exposure (the viewing direction offered from the left to the right). The area of the small square of the Goryaev chamber was 1/400 mm^2.

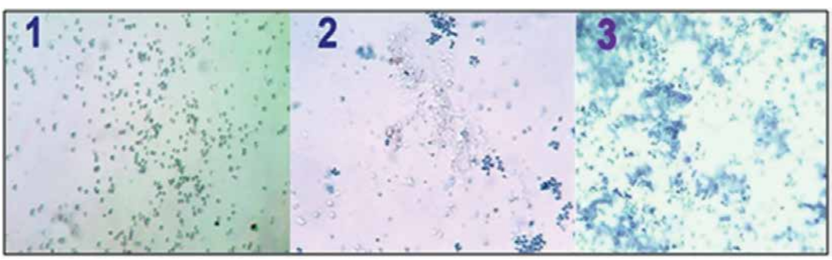

Figure 7.
The monitoring of the action of the "early" protein HPV16 E7 on HeLa cells in the presence of TB. 1: after 10 minutes, 2: after 1 hour, 3: after 3 hours after the addition of HPV16 E7.

3.2 Unusual "alien" structures found in the abdomens of mice infected with Hela cells sensitive to HPV16 E2

In addition to the unusual behaviors observed in HeLa cells under stress conditions, unique structures were discovered in the abdomens of mice (**Figure 9**). Male mice were initially infected with HeLa cells by injecting 100 μl of the cells into the lateral thigh muscle. Subsequently, after 1 month, they were vaccinated orally three times with an interval of 1 month using vaccine material transgenic with HPV16 E2.

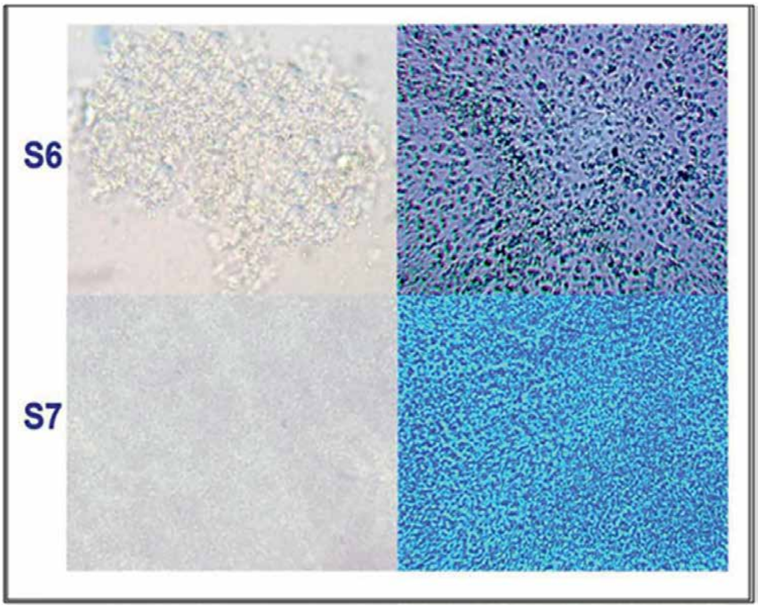

Figure 8.
The action of HPV16 E6 and E7 in combination with the cassette of module system CRISPR/CAS (sigma, USA) after 5 minutes (left vertical row) and after 3 hours (right vertical row) in the presence of TB. The indication was S6 for HPV16 E6 + CRISPR/CAS and S7 for HPV16 E7 + CRISPR/CAS.

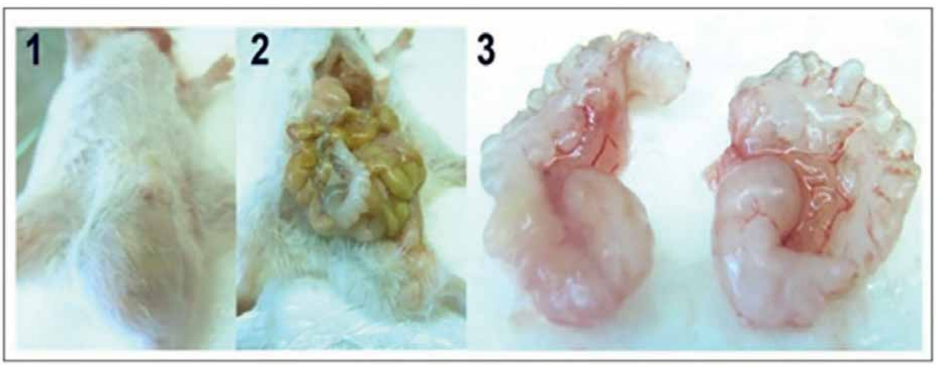

Figure 9.
The finding of "alien residents" of the unusual structures in mice abdomen infected with HeLa cells and then vaccinated per os with HPV16 E2. 1: after 1 month after injection of HeLa cells, hypertrophy of testis occurred; 2: the detection of hypertrophy of guts and the structure of the "alien resident" in the opened overgrown guts; 3: the view of isolated "alien residents" from opened guts of male mice, infected with HeLa cells and after vaccinating with HPV16 E2.

"Alien residents" manifested as coral-like structures that were fragile and lacked any discernible internal structural elements. Occasionally, one or two of these "aliens" were observed in the mouse gut, unattached to the abdomen. The traces of blood seen in **Figure 9** were from the wound incurred during abdominal dissection, indicating that these structures likely developed autonomously.

Another peculiar finding frequently observed in the abdomens of infected mice were spheres of white or pearl color, measuring 1.5–1.8 cm in diameter. These spheres rapidly shrunk in size upon exposure to air or DMEM medium, and fully dissolved upon addition of supernatant from buffer homogenates of tomato fruit containing HPV16 E2 (**Figure 10**).

Figure 10 depicts the pearl-colored spheres observed upon opening the abdomens of mice infected with HeLa cells. No connecting elements were identified on the inner surface of the abdominal cavity, nor were there any blood vessels or traces of blood present. It is presumed that the development of these spheres occurred autonomously.

Following investigations conducted with HeLa cells in vitro, it was deemed necessary to examine the effects of HPV16 E2, LAAO, and DAAO on mice lung tumors ex vivo using a model of T lymphocytes isolated from spleens infected with HeLa cells.

3.3 The "education" of "naive" mice splenocytes after incubation in suspension of HeLa cells on tumor growths in mice to force tumors into regression

Non-effector (inactive and unloaded with antigen) "naive" macrophages, T lymphocytes, and splenocytes are powerful factories of protein biosynthesis, particularly in terms of synthesizing receptors on the surfaces of lymphocytes. It is estimated that approximately 100,000 different types of receptors may be present on the surfaces of lymphocytes. Therefore, it can be assumed that mice T lymphocytes and splenocytes

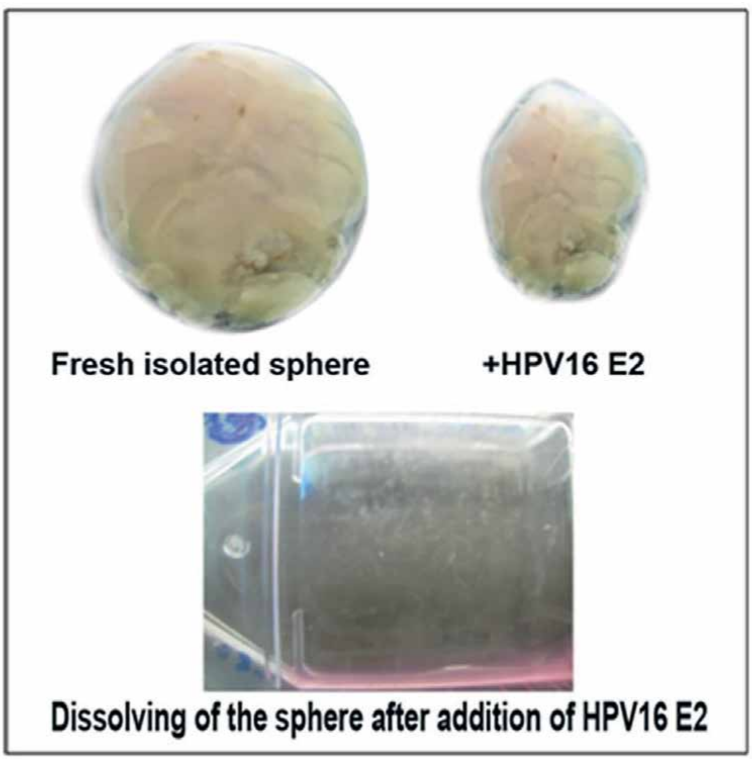

Figure 10.
The view of the sphere isolated from mice abdomen infected with HeLa cells. Upper left—fresh isolated sphere, upper right—beginning to subside sphere after digging of few drops of the supernatant of homogenate of tomato fruit transgenic with HPV16 E2. Bottom—remaining traces after dissolving spheres in the cultivated flasks with DMEM and HPV16 E2 to the next day. The seeding of the monolayer of HeLa cells can be seen in the flask (bottom).

can be trained to serve as effectors bearing receptors capable of recognizing tumor antigens. The number of T lymphocytes in an adult organism may reach as much as 10^{16} cells.

The training, or "education," of T lymphocytes typically occurs in their native habitat, such as the bone marrow or thymus. Antigens of host proteins are presented to naive T cells to eliminate those lymphocytes that might potentially react with host antigens. Those lymphocytes that respond after recognition are eliminated.

The aim of the experiment was to train splenocytes, predominantly naive ones, isolated from the spleen of native mice by exposing them to a suspension of HeLa cells for 5 days. After 5 days, the fractions of splenocytes trained with HeLa cells were placed in cultivated 24-well plates with lung tumor growths caused by HeLa cells during their two-day incubation with HeLa cells.

Five mice (3 females and 2 males, aged 7 months, with masses of 35–40 g) were used in the experiment. Lungs and spleens were immediately placed into a two-day suspension of HeLa cells (taken from a low-temperature freezer) in cultivating Corning flasks (25 ml) with DMEM medium containing 500 units of penicillin and 50 mg/l streptomycin. Membrane nitrocellulose disks with a diameter of 14 mm were placed into wells of 5 plates with 24 wells each, with each plate corresponding to a different experimental variant: (1) DMEM only, (2) HPV16 E2 as the activator, (3) LAAO as the activator, (4) DAAO as the activator, and (5) HPV16 E2 as the activator (replication of variant 2). Each well contained 300 μl DMEM, 30 μl of splenocyte fraction, lungs or their tumor fragments, and 300 μl of each activator. The plates were covered with lids, packed into plastic films, and left for 5 days at room temperature.

After 5 days, the disks in the wells were washed three times with TNT buffer, and relevant antibodies (IgG) targeting interferon, CD4/CD8 T lymphocytes, T cell receptors, and apoptotic enzymes (granzyme B, perforin, and granulysin) were added. Incubation lasted for 1 day, followed by washing the plates three times with TNT buffer. Secondary antibodies to mice IgG conjugated with alkaline phosphatase were then added. After another day of incubation, the plates were washed with TNT buffer, dried in the air, and the substrate BCIP (or NCIP)/NBT was used to develop spots of colonies of immunopositive T cells.

The incubation of isolated lungs resulted in extensive tumor growths, as depicted in **Figure 11** (left and center). These growths appeared as cellular tumor neoplasms of light color in the central part and partly on the periphery of the inoculated lungs.

However, keeping the spleens in the suspension of HeLa cells did not lead to any visible changes in the surface, color, volume, or structure of the spleens, as shown in **Figure 11** (right).

It appears that the "trained" splenocytes induced the regression of tumor lungs independently of the presence of activators such as oncolytics HPV16 E2, LAAO, and DAAO, as shown in **Figure 12**.

3.4 Elispot analyses of immunogenicity of "educated" splenocytes

Despite the well-known oncolytic and antiproliferative activity of LAAO and DAAO [27–29], it was decided to investigate their effects on the immunogenicity of components of the T-cell unit in mice. This included studying their action on peripheral mononuclear blood cells (PMBCs), splenocytes, and lungs inoculated with HeLa cells, as illustrated in **Figure 13**.

The results shown in **Figure 13** demonstrate that LAAO and DAAO significantly increased the synthesis of interferon and apoptotic enzymes (granzyme B, perforin,

Figure 11.
The holding of isolated mice lungs with the purpose of the tumor induction (left and center) in suspension of HeLa cells and "training" of mice spleen loaded into HeLa suspension for 5 days (right).

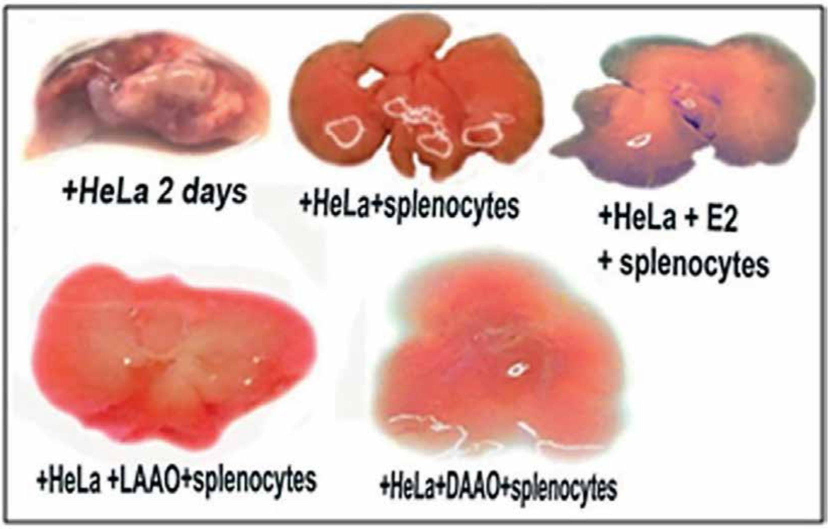

Figure 12.
The view of isolated mice lungs inoculated in suspension of HeLa cells. From the left to the right, upper row: mice lungs inoculated only with HeLa cells; in the center: mice lungs inoculated with HeLa cells at first, then processed with "trained" splenocytes; upper right: mice lungs after inoculation with HeLa cells holding for 5 days with "trained" splenocytes in the presence of the activator HPV16 E2; lower left: lungs were keeping for 5 days with "trained" splenocytes in the presence of the activator LAAO; lower right: lungs after inoculation with HeLa cells were holding for 5 days with "trained" splenocytes in the presence of the activator DAAO.

and granulysin) in both PMBCs and splenocytes. The counts of inducible immunopositive cells were notably higher than the control groups, showing an increase of up to two times or more.

Furthermore, the induction of components of the T-cell response in mice lungs, which were incubated with HeLa cells for 5 days, yielded intriguing results. Mice lungs incubated with HeLa cells for 2 days developed tumor growths, specifically small cell sarcomas. T lymphocytes isolated from these tumor-bearing lungs were subjected to Elispot analysis using antibodies against interferon and apoptotic enzymes (granzyme B, perforin, and granulysin), as depicted in **Figure 14**.

The results of Elispot analyses (**Figure 14**) clearly demonstrate the activation of the antitumor defense mediated by T lymphocytes in the lungs of mice bearing tumors treated with HeLa cells. This activation is evidenced by a significant increase

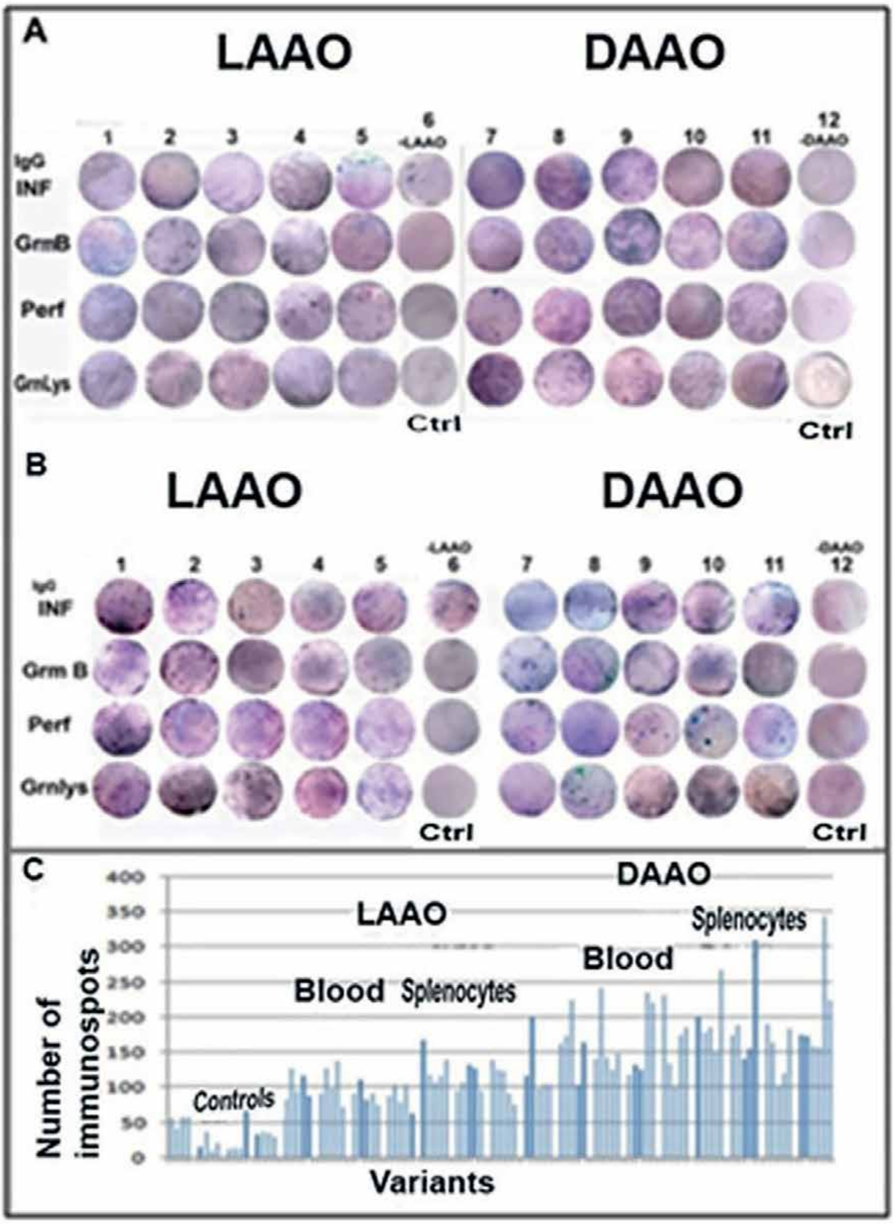

Figure 13.
The action of LAAO and DAAO on the induction T-cell response in peripheral mononuclear blood cells (PMBCs) (A) and in splenocytes (B). A: Elispot with PMBC, B: Elispot with splenocytes, C: The graph of counted amounts of immunospots synthesized, the generation of which was induced by the presence of LAAO and DAAO. The order of values in columns (C) corresponded to positions of immunospots. INF—antibodies to interferon, Grm B—antibodies to granzyme B, perf—antibodies to perforin, Grnlys—antibodies to granulysin. Ctrl—controls. Numbers 1–5 are the number of mice used.

in the synthesis of interferon and apoptotic enzymes, including granzyme B, perforin, and granulysin. Numerous colonies of immunospots generated by T lymphocytes, well stained and dense, were observed.

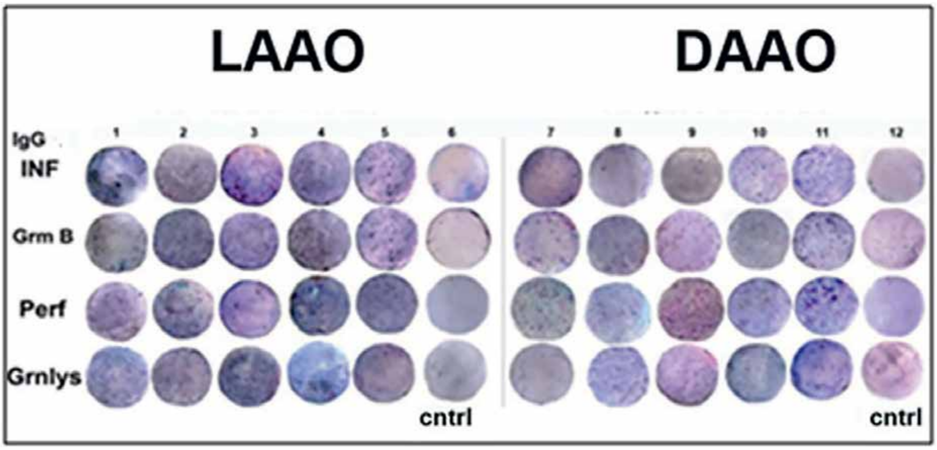

Figure 14.
The action of LAAO and DAAO on the generation of colonies of immunospots of T lymphocytes isolated from mice lungs inoculated in the suspension of HeLa cells for 5 days. Cntrl meant variants without LAAO (left) and without DAAO (right). IgG to INF, GrmB, perf and Grnlys implied antibodies to interferon, granzyme B, perforin and granulysin. Numbers meant numbers of mice used.

It was hypothesized that T lymphocytes from the lungs of mice infected with HeLa cells were capable of being "trained" by internal antitumor mechanisms induced during the infection. Based on the results of **Figures 12** and **14**, it could be assumed that splenocytes and T lymphocytes were also "trained" by similar internal antitumor mechanisms, likely components of the microenvironment.

To test this assumption, experiments were conducted in which spleens and lungs of mice were infected with HeLa cells for 5 days, resulting in tumors on the lung surfaces while leaving the spleens visibly unchanged. Subsequently, splenocytes were isolated from the treated spleens and placed into wells containing tumor-bearing mice lungs (or fragments thereof), with nitrocellulose membrane disks. Additionally, activators such as HPV16 E2, LAAO, and DAAO were added. Controls included wells with only nitrocellulose membrane disks, supplemented with DMEM with antibiotics and 20 μl of isolated splenocyte fraction.

Intact splenocytes from untreated spleens, isolated from mice infected with HeLa cells injected into the lateral thigh muscle for 2 months, showed a weak immune response. Only a few T lymphocytes reacted with the corresponding antibodies to T cell receptor (TCR) and apoptotic enzymes (granzyme B, granulysin, and perforin). Activated colonies of T lymphocytes, numbering between 200 and 500 immunospots on nitrocellulose membrane disks, were found.

Therefore, it was decided to investigate the effects of oncolytics (HPV16 E2, LAAO, and DAAO) on isolated splenocytes from spleens inoculated with a suspension of HeLa cells for 5 days. Five mice, comprising 3 females and 2 males, aged 7 months and weighing between 30 and 40 g, were selected for analysis.

Based on the characteristics of immunospots on nitrocellulose membranes and the results of counting their numbers (**Figure 15**), it can be inferred that a significant and robust immune response occurred in spleens inoculated with HeLa cells, indicating a strong impact in response to the presence of HeLa cells. The number of immunospots increased by a factor of 10 compared to the number detected on membrane nitrocellulose disks visible in **Figure 16**. Furthermore, an additional increase in the number

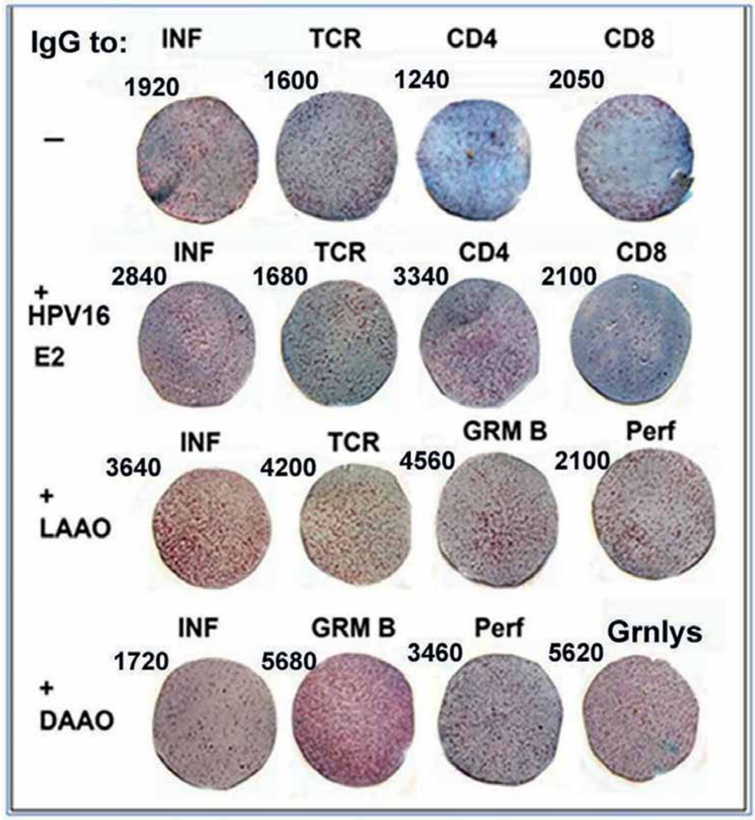

Figure 15.
The view of characteristic membrane nitrocellulose disks with immunospots of colored colonies of "trained" induced splenocytes (T lymphocytes) from isolated mice spleens inoculated with HeLa cells in vitro for 5 days in corning flasks. IgG to inf, TCR, CD4/CD8, GRM B, perf and Grnlys were used in Elispot. Numbers correspond to the counted amounts of immunospots on characteristic membrane nitrocellulose disks.

of immunospots was observed in the presence of activators such as HPV16 E2, LAAO, and DAAO. Particularly noteworthy was the pronounced impact of LAAO and DAAO, with the number of immunospots increasing by 20–30 times compared to those represented on the membrane nitrocellulose disks in **Figure 16**.

These findings are consistent with the observed regression of lung tumors in mice in the presence of HPV16 E2, LAAO, and DAAO (**Figure 12**), demonstrating a correlation between the immune response observed in the spleens and the therapeutic effects on tumor regression induced by these agents.

4. Conclusion

The phenomenon of biofilm formation or symplast of the internal cellular content of HeLa cells surrounded by a continuous sustained membrane Impermeable to sublethal dye TB is of great interest. It has greater implications for general biology because the formation of symplast represents an example of the "intelligent" behavior of HeLa cells, which are highly pathogenic and immortalized. Understanding this

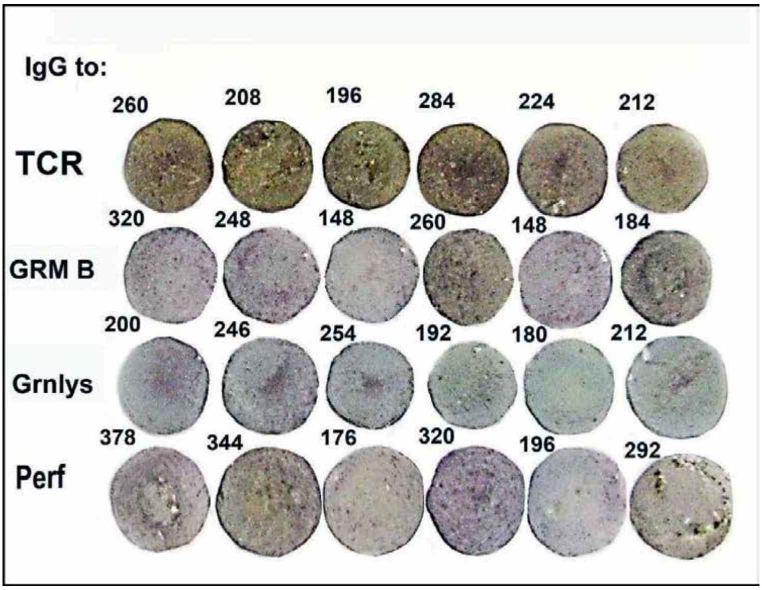

Figure 16.
Elispot with intact mice splenocytes isolated from mice after 2 months of the injection of the suspension of HeLa cells into lateral thigh muscle. Antibodies to T-cell receptor and to apoptotic enzymes: granzyme B, perforin and granulysin—were used in analysis.

behavior is not only relevant to oncology and vaccinology but also sheds light on alternative modes of existence for "alien" organisms.

Our experiments revealed that HeLa cells were capable of forming sustaining "avoiding" symplast structures under stress conditions, yet could easily transition from this symplast state to continue proliferation as a monolayer. Additionally, our unpublished data showed instances where plant cell vacuoles fused into a single extensive pool without demarcation, indicating fusion of tonoplasts and the formation of a single large vacuole. Polyethylene glycol, another membrane-acting agent, induced rearrangement of lipid bilayers and membrane fusion, suggesting potential antibacterial properties of membranotropic compounds.

Based on this data, it might be assumed that many bacteria were able to use HeLa cells as nutrient-sustained and safe bioplatforms for the organization of their own biofilms, essentially parasitizing on HeLa cells. This is facilitated by the relative security and stability of HeLa cell membranes and cell walls. However, HeLa cells may also associate with bacteria capable of forming special adhesive structures such as fimbriae [12], polymerized F-actin [15], special needles, filaments, and hollow tubes [16], as well as adhesive crepe and rough filaments [13].

Regarding the appearance of "alien" structures in the stomach of mice infected with HeLa cells and later vaccinated with HPV16 E2, this finding is entirely novel. It is plausible that these structures originate from HeLa cells organizing into protective structures in the hostile abdominal environment. No similar occurrences have been reported in invertebrates, insects, or helminths. However, it is noteworthy that these structures were sensitive to the addition of HPV16 E2.

The discovery of the induction of immunogenicity in vitro in HeLa cells caused by "trained" splenocytes isolated from spleens inoculated with cancerous HeLa cells is of significant interest. This effect, found for the first time, has no analogues in the world literature. Regarding the oncolytic action of LAAO and DAAO, most literature focuses on their redox properties attributing to them the ability to damage cancer cells by increasing oxidation. However, in this context, the induction of immunogenicity caused by LAAO and DAAO should be considered.

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Chapter 5

Human Papilloma Virus Vaccines

Princy Louis Palatty, Dhanya Sacheendran and Mamatha Jayachandran

Abstract

We intend to delve into the history of evolution and development of human papilloma virus vaccine (HPV Vaccine). The related ethical misconduct as a learning exercise shall be explored and the lessons learnt will spur better research. The present use oh HPV vaccine, tolerability, efficacy, and safety will be highlighted and explained. The factors associated with the perception leading to vaccine hesitancy would also be discussed. We shall also look at the types of HPV vaccines available, and the regimens implemented. The present challenges in HPV vaccines and the potential strategies to overcome them shall be dealt with. Venturing into vaccines is very essential.

Keywords: HPV vaccine, consent, ethical misconduct, effectiveness, safety

1. Introduction

It has been said that communities, countries, and ultimately the world are only as strong as the health of their women. Cervical cancer is a bane to women in their reproductive age. Addressing this dynamic issue will keep societies intact. Globally, cervical cancer is the fourth most common cancer in women, with nearly 6,04,000 new cases being reported in 2020. Almost 90% of Cervical cancer related deaths occur in low- and middle-income countries. Human papillomavirus (HPV) is the most common sexually transmitted infection globally [1–3]. Though majority of HPV infections are asymptomatic with spontaneous resolution, persistent infections pose the risk of developing into anogenital warts, precancerous lesions, and cervical, anogenital, or oropharyngeal cancers in both women and men. Over 200 identified HPV types exist, each with its own distinct tropism – a preference for specific tissues. Low-risk HPV strains like 6 and 11, often acquired through skin-to-skin contact, are the culprits behind familiar warts, whereas the high-risk HPV strains, like 16 and 18, promote uncontrolled proliferation, paving way for the development of various cancers [1–3].

The history and discovery of the human papillomavirus (HPV) cradles a fascinating journey through scientific theories, scientific investigations, virology, and medical breakthroughs. Despite being a radical breakthrough in preventing cancer, the evolution of HPV vaccines brought home some disregarded ethical issues. HPV, the human papilloma virus is a diverse group of DNA viruses, that was first identified and studied in the mid-20th century, paving way for understanding its implications in various medical conditions, particularly in the realm of oncology. Head start research began in the 1930s and 1940s with focus on causative agents behind genital warts and cervical cancer [1, 2].

The identification and isolation of HPV strains happened gradually over years. History was etched in 1976, with isolation of the first HPV types, HPV-1 and HPV-2, from plantar warts by Dr. F.M. Jablonska-Kaszewska and colleagues. It wasn't until the use of techniques like DNA hybridization during the 1970s and early 1980s that identification of specific types of HPV associated with cervical cancer was made possible. Dr. Zur Hausen and his team in the early 1980s, identified HPV types 16 and 18 as the prominent culprits behind cervical cancer. Dr. Zur Hausen's groundbreaking discoveries led to a paradigm shift in understanding the role of HPV in cervical cancer and thus initiating a cascade of research in this arena. He was later awarded the Nobel Prize in Physiology or Medicine in 2008.

Following these discoveries, the development of HPV vaccines became a significant stride in preventive medicine. The first HPV vaccine, Gardasil, targeting the most prevalent cancer-causing HPV types was introduced in 2006 [3, 4]. Subsequent advancements led to the development of more comprehensive vaccines with broader coverage against multiple high-risk HPV strains. Vaccination offers robust protection against HPV-associated cancers and precancerous lesions. Research into HPV's implications in various cancers has propelled medical understanding and kickstarting of public health initiatives. Ongoing efforts tend to focus on improving vaccination accessibility, enhancing screening methods, and expanding knowledge of HPV-associated diseases, emphasizing its role in diverse malignancies.

For bringing this into action, the World Health Organization (WHO) evolved strategies for cervical cancer elimination. Achieving a global target of 90% vaccination coverage, providing 70% twice-lifetime screening, and enabling 90% treatment of preinvasive lesions and invasive cancer by 2030 was devised. Global statistics on HPV disease prevalence and the extent of vaccination coverage provided will help in achieving this global target by 2030.

2. Epidemiology

Global statistics reveal that this preventable disease had claimed the lives of over 310,000 women in 2018 [5]. Around 90% of the deaths reported occurred in the low-income and middle-income countries (LMICs). This same year saw approximately 570,000 new cases of cervical cancer being reported. Five major genera of HPV have been identified as alpha, beta, gamma, mu, and nu. The most common identifies variant is gamma with 99 known HPV types. A recent study has further identified 69 additional gamma variants. (VIDE **Figure 1**).

An alpha subgroup of human papillomavirus has been attributed to be a high-risk group (HR HPV). They are implicated in the progression of HPV infections to anogenital cancer and a subset of head and neck cancer. Though the beta and gamma genera are present on the skin surface in the general population, the etiological role of beta papillomaviruses (beta HPVs) in non-melanoma skin cancer (NMSC), is gaining importance. Studies have revealed that the oncoproteins E6 and E7 alter host immune response pathways, promote cellular transformation, and establish a persistent HPV infection thereby progressing to cervical cancer. E6 and E7 oncoproteins need to be expressed continuously for mucosal HR HPV type infection maintenance. But this is not required in case of cutaneous HPV types progressing to skin cancers. Beta HPV type act as facilitators of skin carcinogenesis especially NMSC by amassing UV radiation induced DNA breaks and mutations [6, 7].

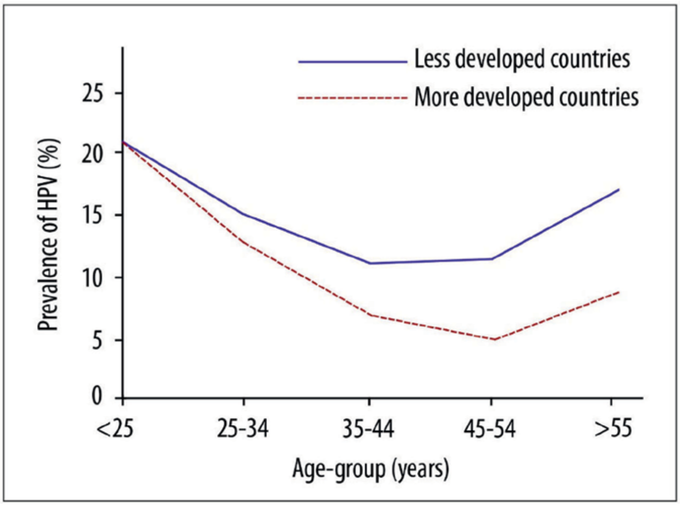

Figure 1.
Global HPV prevalence.

3. Etiopathogenesis

Various factors influence the pathogenesis of HPV. Viral genotype, host susceptibility, immune response, and environmental factors contribute to the clinical manifestations associated with HPV infection. Anybody can contract HPV, but certain factors increase the likelihood like – multiple sexual partners, early age of sexual debut, weakened immune system as in patients with HIV/AIDS.

HPV is a diverse family of non-enveloped, double-stranded DNA viruses, with various genotypes exhibiting exquisite tropism for specific epithelial tissues. Low-risk strains, commonly HPV6 and 11 have a predilection for skin keratinocytes, leading to common and plantar warts. High-risk genotypes like HPV16 and 18, exhibit a predilection for mucosal epithelia, of anogenital and oropharyngeal regions, thus leading to development of various cancers associated with HPV [1] (VIDE **Table 1**).

An icosahedral capsid contains the HPV genome and is divided into three regions – the early (E) region, late (L) region and the Long Control Region (LCR). The early (E) region has different types of precursor proteins E1, E2, E4, E5, E6, and E7, which are

S. No	Diseases	Associated HPV types
1	Cutaneous warts	1, 2, 3, 4, 10, 27,57
2	Anogenital warts	6, 11, 53
3	Mucosal cancers	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, 82
4	Non-Melanoma Skin Cancers	1, 5, 8, 9, 17, 20, 23, 38
5	Bowen disease	16, 18, 31, 32, 34
6	Epidermodysplasia verruciform	5, 8, 9, 12, 14, 15, 17, 19–25, 36–38, 46, 47, 49, 50

Table 1.
Diseases & HPV types.

essential in the early stages of infection and DNA replication. Among these, E6 and E7 have the major role in regulating the pathogenicity of the virus. The major and minor viral capsid structure is coded by the late (L) region proteins with two parts, L1 and L2. Viral replication and transcription are controlled by a non-coding region, the Long Control Region (LCR).

Proteins and specific functions

E1 Regulate viral DNA replication
E2 Regulatory factors of viral transcription
E4 Promote virus maturation and release
E5 Regulate growth factor signaling pathway
E6 Promotes the degradation of P53 and increases resistance to apoptosis
E7 Promotes retinoblastoma protein (pRb) degradation, affects the cell cycle and stimulates cell proliferation
L1 Major capsid protein is important for virus assembly and stability
L2 Secondary capsid protein is important for virus infection

Persistent HPV infections can lead to precancerous squamous intraepithelial lesions (SIL) or cervical intraepithelial lesions (CIN). SIL is further graded as LSIL / HSIL and CIN is further graded as CIN 1, CIN 2, and CIN 3. (VIDE **Figures 2** and **3**).

Microabrasions and hair follicles serve as entry points for the viral particles to invade the basal keratinocytes. HR HPV can directly invade the squamocolumnar

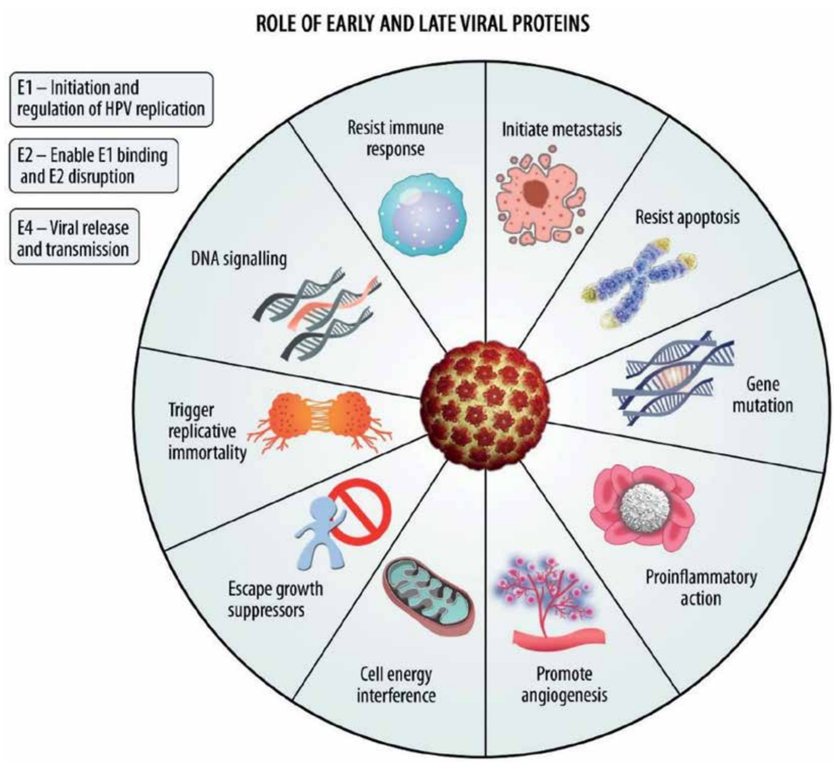

Figure 2.
*Role of early and late viral proteins. *E5 induces immune evasion thus triggering increased cell proliferation. *L1 and L2 late capsid proteins promote replication of viral life cycle through progeny virions in host nuclei cells.*

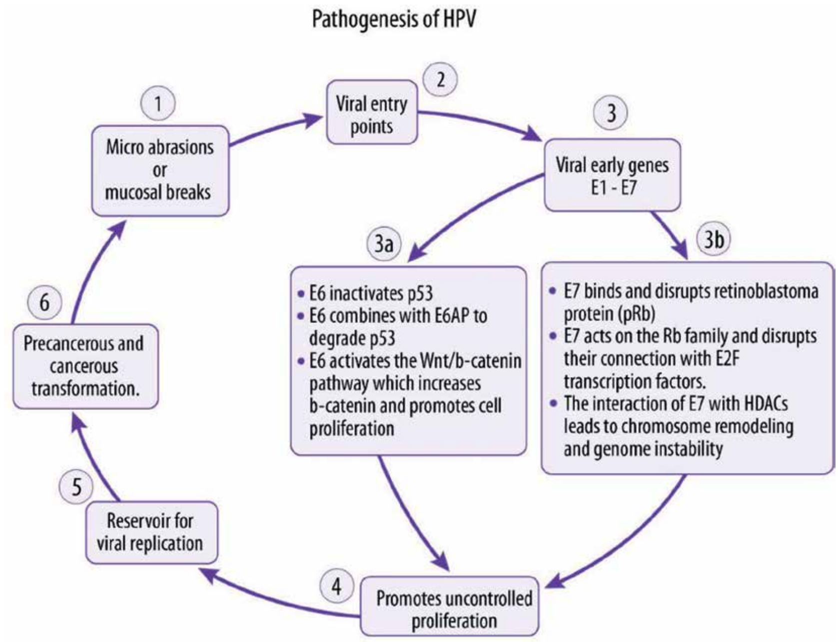

Figure 3.
Pathogenesis of HPV.

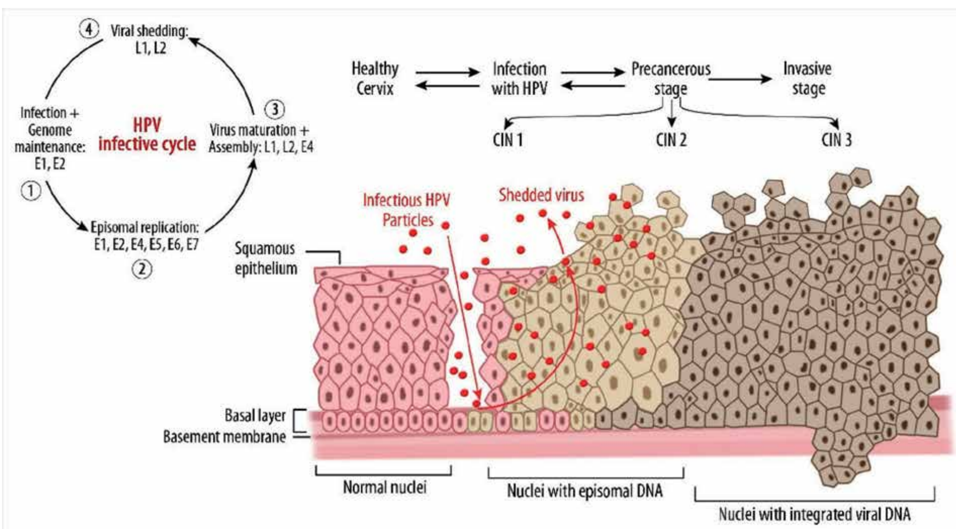

Figure 4.
Infective cycle of HPV.

junction of the cervix making it a prime target site for infection. Another similar direct breach of the epithelial lining is found to occur in the tonsillar crypts. E6 and E7 oncoproteins defy the cellular aging process and boost impromptu cell growth [5–7]. HPV mediated cellular transformation requires high expression of oncoproteins E6 and E7. This enhanced expression is favored by the loss of E2 repressive functions (VIDE **Figure 4**).

The pathogenesis of Human Papilloma Virus can lead to serious health consequences. Through vaccination, early detection, and targeted treatment, we can significantly reduce the disease burden of HPV-associated illnesses. Unraveling the mysteries of HPV pathogenesis equips us to break the cycle of infection and create a future free from its shadow.

4. History of vaccine development

The notoriety of HPV to cause malignancies has led to aggressive multi-modal therapies. This includes primary surgery coupled with radiation and chemotherapy. The consequences of these treatment methods are highly toxic with most patients experiencing adverse effects like xerostomia, dysphagia, and in the long run become dependent on gastrostomy tubes thus markedly compromising the quality of life [8, 9]. This led to the search for better and alternative methods of treatment. Experimental therapy for patients with HPV mediated malignancies led to the development of vaccines to combat HPV infections. It led to the development of vaccines which can be grouped as prophylactic vaccines and therapeutic vaccines. Vaccination curbs HPV infections proving beneficial for the vaccinated person as well their future sex partners by preventing the spread of transmission along with other barrier methods of protection (VIDE **Table 2**).

4.1 Clinical trials

The timeline of medical research highlights the remarkable story of Henrietta Lacks, the Afro-American woman who succumbed to cervical cancer in 1951. Derived from her name, the HeLa cell line was born from her cervical samples, and these were the first established in-vitro immortal cancer cell line. This initiated advancements in medical research which included the development of the human papilloma virus (HPV) vaccine. Most screening programs for cervical cancers utilize the detection of HPV as a primary screening tool thus complying with the World Health Organization (WHO) recommendations.

All HPV vaccines were subjected to clinical trials before being introduced into the market. It was of paramount importance to determine the efficacy, effectiveness, and safety of these vaccines. After determining the appropriate HPV vaccine endpoints at a WHO convention in 2003, a consensus was arrived at that ethical and time constraints could not allow for cervical cancer to be an appropriate trial endpoint, given

Vaccine	Country	Institution/Manufacturing company
Gardasil	United States of America	Merck and Co
Cervarix	United Kingdom	GlaxoSmithKline (GSK)
Gardasil 9	United States of America	Merck and Co
Cecoline	China	Xiamen University and Xiamen Innovax Biotech Co Ltd
Walrinvax	China	Walvax Biotechnology Co Ltd
Cervavac	India	Serum Institute of India Pvt. Ltd. (SIIPL)

Table 2.
Vaccine development.

that participants under follow-up during the trial period would receive treatment of any cervical precancerous lesions detected. To determine vaccine efficacy at reducing HPV infections and precancerous cervical lesions, Cervical Intraepithelial Neoplasia (CIN) Grade 2 and above were included as study endpoints. Following licensure, many HPV vaccine trials were conducted [4, 8].

4.1.1 Clinical trials in young women

These were multinational Phase 3 efficacy trials that included thousands of women in the age group 15–26 years. It was conducted by the respective manufacturing companies.

Gardasil (The Quadrivalent HPV vaccine) – The efficacy of Gardasil was assessed by the FUTURE I and II trials. Around 12,000 women aged 15–26 years were randomized into two arms. One arm of the participants received three doses of Gardasil while the other arm received a placebo. The results were published in 2007. After a follow up period of 3 years, the vaccine efficacy against HPV 16 and HPV 18 associated high grade cervical disease was 98% in women without prior exposure while it was 44% in women who were previously exposed. The FUTURE II trial subset of European participants were followed up for 14 years which revealed a vaccine efficacy of 100% as no cases of HPV 16 or 18 associated high grade cervical dysplasia was noted. A double-blind randomized study among Japanese women aged 18–26 years, showed high efficacy against vaccine type high grade cervical dysplasia [4, 8].

Cervarix (The Bivalent HPV vaccine) – The efficacy of Cervarix was first reported by the PATRICIA trial. Here, 18,000 women aged 15–25 years were randomized to receive three doses of Cervarix or the Hepatitis A vaccine. The 3 years follow up period revealed a 92.9% vaccine efficacy against HPV-16 or HPV-18-associated high-grade CIN. The efficacy against vaccine-type CIN3+ was as high as 100% in the HPV-naïve cohort. The second largest prelicensure trial that explored the efficacy and safety of Cervarix was the Costa Rican Vaccine Trial (CVT). A vaccine efficacy of 89% against HPV 16 or HPV 18 associated CIN2+ was reported after a 4 year follow up period, while an efficacy of 100% was achieved after an 11 year follow up. A vaccine efficacy of 100% in around 6000 HPV naive Chinese women aged 18–25 years was also reported with Cervarix [4, 8].

Gardasil 9 (The Nonavalent HPV vaccine) – This was a multinational double-blind trial. Over 14,000 women aged 16–25 years were recruited for the study, but here, the control group received the quadrivalent Gardasil vaccine. All the HPV subtypes not covered by Gardasil were addressed in the Gardasil 9 HPV vaccine (HPV 31, HPV 33, HPV 45, HPV 52, and HPV 58). An efficacy of 97.1% was reported. The incidence of abnormalities associated with HPV 6, HPV 11, HPV 16, and HPV 18 (i.e., HPV subtypes covered by the quadrivalent vaccine) was comparable between both groups of participants. Furthermore, the immunogenicity of the nonavalent vaccine with respect to HPV-6, HPV-11, HPV-16 and HPV-18 was comparable to that of the quadrivalent vaccine, with antibody response persisting for up to 5 years. The Gardasil 9 vaccine could potentially confer better protection against cervical cancers by providing broader coverage against all vaccine HPV subtypes [4, 8].

Cecolin (The Bivalent HPV Vaccine) – This multicentric trial in China trial analyzed the efficacy of this bivalent vaccine in over 7000 female participants aged 18–45 years from 2012 to 2013. The control group here received the Hepatitis E vaccine. The participants were age stratified into two groups allowing a subgroup analysis. Vaccine efficacy against high-grade genital disease and persistent infection associated with HPV-16 or HPV-18 were 100% and 97.3%, respectively. A phase 3 clinical trial of Cecolin vaccine is underway in Ghana and Bangladesh with the findings expected to be released this year (NCT04508309) [4, 8].

Cervavac (The Recombinant Quadrivalent vaccine from India) – Cervavac is the first HPV vaccine developed in India from the Serum Institute of India (SIIPL). A randomized, active-controlled phase 2/3 trial incorporating 12 tertiary care hospitals in India was conducted between 2018 and 2021. Of the 2341 individuals (both women and men) who were screened, 2307 were found to be eligible and were enrolled in the study. 1107 belonged in the 9–14 years age group and 1200 were in the cohort of 15–26 years. A non-inferior immune response was observed with the SIIPL quadrivalent HPV vaccine in comparison to the comparator Gardasil HPV vaccine. It was concluded that this new recombinant vaccine could help meet the demand for HPV vaccines globally [9].

Walrinvax (Recombinant Bivalent HPV Vaccine) – Targets HPV 16 and 18. The safety of this vaccine was assessed in 4 clinical trials conducted in China. A total of 7371 females belonging from 9 through 30 age group were participants in the trial.

4.1.2 Clinical trials in older women

HPV vaccine efficacy is known to have reduced efficacy with a decline in age in women with prior HPV infection. Gardasil and Cervarix have both undergone trials in such group of women. The FUTURE III trial evaluated the efficacy Gardasil in over 3800 women aged 24–45. Vaccine efficacy against vaccine type CIN1+ was 88.7% in HPV-naïve women and 30.9% in all women. Another double-blinded trial of the quadrivalent vaccine in Chinese women aged 20–45 found a high vaccine efficacy of 94%. The VIVIANE trial evaluated the bivalent Cervarix vaccine's efficacy in women aged >25. Vaccine efficacy against combined endpoint of vaccine-type 6-month persistent infection and CIN1+ was 90.5% in the per protocol group and 86.5% in the total vaccinated cohort. Estimated vaccine efficacy against vaccine-type CIN2+ was high but insignificant due to low numbers. Though studies have validated the use of HPV vaccines in older women, the effectiveness of the vaccine in this group is lesser than in adolescents [4, 8].

4.1.3 HPV vaccine with previous known infection

HPV based cervical screening has gained prominence in many countries and this helps to detect the early if women have had a previous HPV infection. Therefore, it is important to determine the efficacy of HPV vaccine in this group. Most of the HPV vaccine clinical trials recruited individuals who were HPV DNA or HPV antibody seropositive in the totally vaccinated cohort group. The findings in this trial support the use of the vaccine in HPV-DNA-negative women, irrespective of their serostatus [4, 8].

4.1.4 HPV vaccine in HIV infection

A few studies that have investigated the safety and efficacy of HPV vaccines among HIV patients have shown seroconversion rates of 100% post vaccination with no adverse outcomes. But further investigative and HPV vaccine efficacy data are deficient in this population and needs further assessment [4, 8].

4.1.5 HPV vaccinations in men

HPV is known to have a causal relationship with anogenital and oropharyngeal diseases. 90% of anal cancers, 70% of oropharyngeal cancers and 48% of penile cancers have been associated with HPV. Hence, in males, HPV vaccines have the potential to reduce HPV associated lesions and infections. A prelicensure randomized controlled trial with Gardasil in 4000 men aged 16–26 years found it to have an efficacy of 90.4% against vaccine type anogenital lesions and 85.6% against persistent vaccine type HPV infections. The results of the trial showed vaccine efficacy of 89.9% in vaccine type genital warts and 90.8% in external genital lesions, in the HPV naïve group. The vaccine efficacy against vaccine-type external genital lesions was 66.7%, in the intention-to-treat group [8]. However, the Gardasil 9 vaccine did not undergo prelicensure clinical trials in men. Instead, immunogenicity studies were done which showed that the Gardasil 9 vaccine elicited immune responses like Gardasil vaccine against the HPV subtypes 6,11,16, and 18. Hence Gardasil 9 has been approved for use in men [8].

4.1.6 Cross-protection against nonvaccine HPV subtypes

The FUTURE I and II trials of Gardasil analyzed the cross-protection against ten other HPV subtypes like 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59. These showed some degree of cross-protection against HPV infection and low-grade CIN, which was most marked against HPV-31, but not against high-grade CIN. A high vaccine efficacy was noted against infection and CIN2+ associated with HPV 31, HPV 33 (both subtypes closely related to HPV 16), HPV 45 (closely related to HPV 18) and HPV 51. Assessment of Cervarix through the PATRICIA and Costa Rica trials also revealed the effects of the bivalent vaccine against nonvaccine-type persistent infection and CIN2+. In women who were HPV naïve at the baseline, the vaccine was demonstrated to have the highest efficacy. Cross-protective effects lasting at least 11 years were indicated in the long-term follow up data from Cervarix [8].

4.2 Ethical misconduct

The history of HPV vaccine trials in India brought home some bitter truths in the ethical conduct of clinical trials. The past has been a witness to various atrocities and unethical research conducted against certain sections of society. The Tuskegee syphilis trial is the most prominent among them that violated the rights of participants. It led to the establishment of various guidelines for the conduct of ethical research. Despite stringent guidelines being enforced, ethical misconduct continued. In India, the Parliamentary Standing Committee on Health and Family Welfare recommended legal action against a prominent Non-Government Organization (NGO) that was accused of violating ethical standards and national law during a study that was conducted to assess the possibility of initiating a nationwide cervical cancer vaccination program in India. A public interest litigation into the irregularities of the HPV vaccine trial in India, laid bare the opportunities for misconduct. Addressed as the field test of two HPV vaccines, seven vaccinated children died. The trial was then halted by the Indian government in March 2010. The trial failed to follow proper procedures, adequately monitor related events, and obtain informed consent from all participants as many of them were illiterate. It argued that the study should have fallen under the clinical trials legislation despite being projected as a demonstration project. The inquiry that concluded in 2011, reported that the deaths were unrelated to the vaccination and no ethical norms were violated [10]. For the first time ever, the judiciary put a clamp closure on all clinical trials occurring in the country at that moment. This unprecedented move brought forth streamlined clinical trials guided by mitigatory guidelines. The famous "Gatekeeper Permission" strategy tool over the routine "Consent from authorities" mode. Audio visual consent process gained prominence since then. Regrettably the lucrative clinical trial hub shifted from South Asia to East Asia.

5. Vaccines currently in use

The Human Papilloma Virus vaccine was first developed by Professors Ian Frazer and Jian Zhou at the University of Queensland in Australia. They synthesized "virus-like particles" (VLPs), containing proteins like those from the outer layer of the HPV, in 1990. After 7 years, the first human trials for Gardasil, the first HPV vaccine was completed. This was followed by approval for Cervarix – a bivalent vaccine and Gardasil 9 – a nonavalent vaccine. Only HPV prophylactic vaccines have received approval for use. Considering the role of early proteins E6 and E7 in the pathogenesis of cancerous and precancerous lesions associated with HPV, therapeutic vaccines are under clinical research and development. There are various molecules under phase II and phase III trials. (VIDE **Figure 5**).

5.1 Types of vaccines

HPV vaccines were developed with the chief aim to address prophylactic and therapeutic needs. Prophylactic vaccines were developed to be used in those patients who are expected to be exposed to the virus but have not yet contracted the infection, while therapeutic vaccines were needed to avert further development of the disease or the need for other invasive therapies. The evolution of preventive vaccines began with the discovery of VLPs and their enhanced immune capabilities.

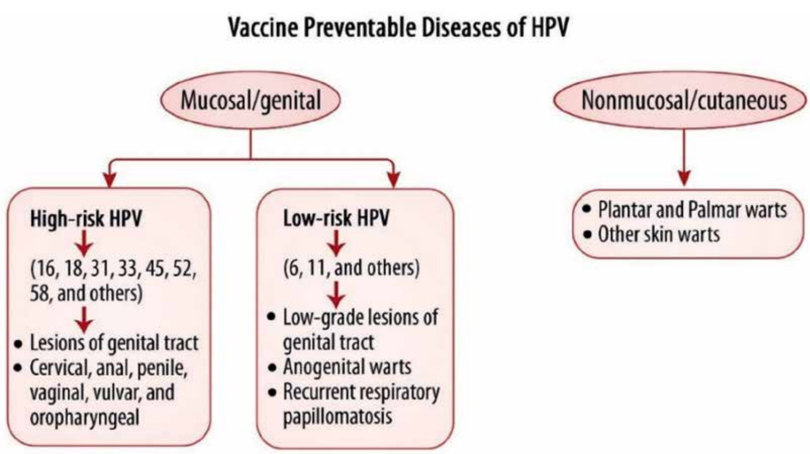

Figure 5.
Vaccine preventable diseases of HPV.

The licensed HPV vaccines in use were developed based on a virus-like particle (VLP) of the major papillomavirus capsid protein L1. The VLPs are merely proteins, with no viral genome. This gives them the edge of being non-infectious and non-oncogenic, making them safer than HPV-attenuated vaccines. The virus-like particles can be produced in bacteria, yeast, or insect cells. Cervarix comprises HPV16 and 18 VLPs, monophosphoryl lipid A (MPL), and aluminum hydroxide as an adjuvant. Monophosphoryl lipid A is a toll/like receptor 4 (TLR4) agonist which has potency to induce high levels of antibodies [2, 8, 11]. Gardasil contains VLPs against HPV6, 11, 16, and 18, while Gardasil 9 contains VLPs against HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58. Cervarix – a bivalent vaccine that prevents HPV16 and 18. Cervavac is a recombinant quadrivalent vaccine (HPV 6, 11, 16, 18) developed by the Serum Institute of India, Pune [10] (VIDE **Table 3**).

5.2 Walrinvax

It is a recombinant bivalent HPV vaccine developed by Shanghai Zerun Biotechnology, a subsidiary of Walvax Biotechnology. It targets the more virulent HPV 16 and 18 and was licensed by the WHO in 2022. The adjuvant used is aluminum phosphate and the expression system is yeast [4]. The most common adverse reactions were vaccine related like erythema, swelling and induration at the injection site. The systemic adverse reactions included headache, myalgia, fatigue, nausea, vomiting, and diarrhea. Rare instances of hypoesthesia and paresthesia were also reported (<0.1%).

5.3 Safety and efficacy of prophylactic vaccines

HPV vaccine demonstrates a high degree of immunogenicity, with nearly 98% of the vaccine recipients eliciting evidence of developing antibody response. Previous

Vaccine	Date of approval	Description and features
Gardasil*	June 8, 2006	• Quadrivalent HPV vaccine targeting HPV 6, 11, 16 and 18 • Expression system: Yeast • Adjuvant: 225 μg aluminum hydroxyphosphate sulfate • Cervical cancer Protection rate: 70%–75%
Cervarix*	October 16, 2009	• Bivalent HPV vaccine targeting HPV 16 and 18 • Expression system: Baculovirus -Insect Cell • Adjuvant: 50 μg MPL absorbed on 500 μg aluminum hydroxide (ASO_4) • Cervical cancer Protection rate: 70%
Gardasil 9	December 10, 2014	• Novanavalent HPV vaccine targeting HPV 6, 11, 16, 18, 31, 33, 45, 52, 58. • Expression system: Yeast • Adjuvant: 500 μg aluminum hydroxy-phosphate sulfate • Cervical cancer Protection rate: 90%

**The use of Gardasil and Cervarix has ceased in the United States after the advent of Gardasil 9. However, India continues its use of Gardasil, Gardasil 9 and the newly developed indigenous quadrivalent Cervavac vaccine.*

Table 3.
Prophylactic vaccines – features.

Vaccine	Adverse effects
Cervarix	Injection site reactions like pain and swelling. Headache, fatigue Fever, nausea and vomiting, diarrhea, dizziness, myalgia
Gardasil	Injection site reactions like pain, swelling and erythema. Headache
Gardasil 9	Injection site reactions like pain, swelling and erythema. Headache Muscle and joint pain

Table 4.
Adverse effects of prophylactic HPV vaccines.

infection with one HPV type did nor lower the efficacy of the prophylactic vaccine against other HPV types [12] (VIDE **Table 4**).

As a precaution the HPV vaccines are deferred until symptom improvement in moderate or severe acute illnesses. The quadrivalent and nonavalent HPV vaccines are produced using the yeast medium. This could pose a potential threat to individuals with a history of immediate hypersensitivity to yeast. These vaccines are contraindicated in such individuals. The bivalent Cervarix vaccine was contraindicated in individuals with latex allergy as the prefilled Cervarix syringes contained latex in the cap tip [2, 8, 11].

Cervavac also exhibits similar adverse effects like other HPV vaccines. Hypersensitivity including reactions to the yeast component of the vaccine is a contraindication. It should be administered with extreme caution in patients with history of thrombocytopenia or any coagulation disorder as the intramuscular administration of this vaccine may cause bleeding [10, 12].

5.4 Dosing schedule and regimens of prophylactic vaccines

Prophylactic HPV vaccines are initiated from minimum 9 years of age in both genders and is not licensed for adults over 45 years of age. The vaccines are administered either as a two dose or three dose regimens. The two-dose regimen is recommended for persons in the age group 9–14 years. The three-dose regimen is for any individual who is immunocompromised or falls in the age group 15–45 years [2–4, 8] (VIDE **Table 5**).

Cervavac has been approved for use in girls and women from 9 through 26 years of age [10].

Each dose is 0.5-mL administered intramuscularly.

Age	Regimen	Schedule
9–14 years	2-dose	0, 6 to 12 months
	3-dose	0, 2, 6 months
15–45 years	3-dose	0, 2, 6 months

Table 5.
Prophylactic vaccines – Dosing schedule.

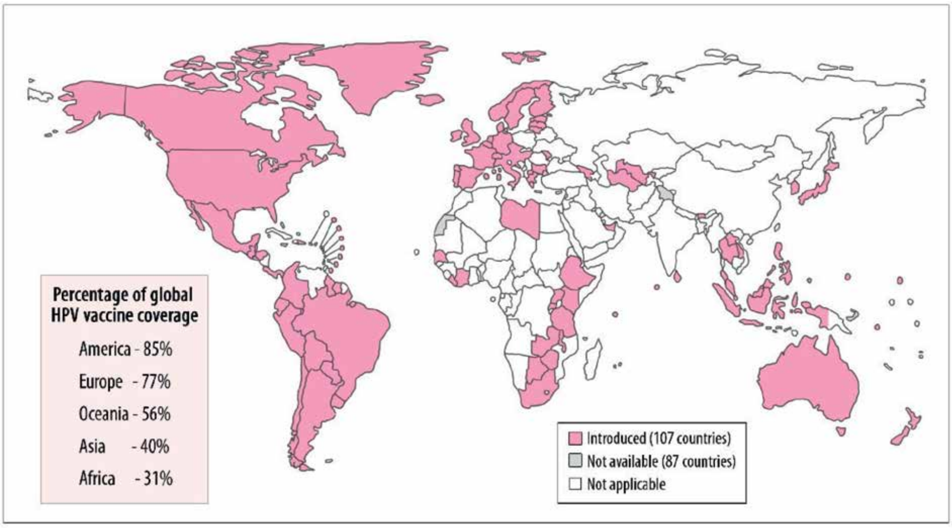

Figure 6.
Global HPV vaccine coverage as of 2020.

5.5 Alternative single dose schedule

In view of the decline in the first dose of HPV vaccination coverage from 25–15% between 2019 and 2021, the WHO's independent expert advisory group, SAGE (Strategic Advisory Group of Experts) came up with an alternative single dose regimen (VIDE **Figure 6**). It was suggested as an off-label option to be used in girls and boys aged 9–20 years. Compared to the routine dosing schedule, this single dose schedule is deemed to offer better compliance and improved coverage. Single dose schedule is not recommended in immunocompromised individuals [4].

One dose efficacy with Cervavac has not yet been established [10].

5.6 Considerations in special populations

HPV vaccines are not advocated for use during pregnancy. However, this does not call for any immediate intervention in case a dose has been administered during pregnancy. Vaccination should be delayed until after delivery if not initiated before pregnancy. HPV can be given to breastfeeding women aged 26 years and younger and not previously vaccinated. Presence of immunosuppression is not a contraindication for HPV vaccination. In children with history of sexual assault the earliest administration of HPV vaccine is recommended. Healthcare professionals should promote shared clinical decision making in women aged 27–45 years, if previously unvaccinated [2, 8].

5.7 Prophylactic HPV vaccines: Current updates

The prophylactic vaccines that have been licensed for use so far are either bivalent, quadrivalent, or nonavalent. To encompass more HPV strains, further research was aimed to target a single HPV variant. Efforts were also directed to target two, eight, and eleven variants of HPV. Most of these newer prophylactic vaccines are in their phase 2 or phase 3 clinical trials [2].

6. Vaccines in the pipeline

6.1 Therapeutic HPV vaccines

Currently, therapeutic vaccines for HPV are still in the different phases of clinical trials. Most of them are still in phase 1 and phase 2 trials. Therapeutic vaccines target the early proteins E6 and E7 which play an important role in the pathogenesis of the HPV [13].

Therapeutic HPV vaccines have categorized as nuclei acid vaccines, protein and polypeptide vaccines, dendritic cell vaccines, and recombinant vector vaccines (VIDE **Table 6**).

The therapeutic HPV vaccines discussed above are in phases 1 and 2 of clinical trials. There are few limitations and drawbacks that have hampered the progress of therapeutic HPV vaccines. In the case of live vector-based vaccines, there is a possibility that the body's immune response to the vector is stronger than the immune response to the antigen. Although protein and peptide vaccines are deemed to be safe and stable, their poor immunogenicity might hinder their efficacy. Though DNA vaccines project an effective antigen specific immunotherapy, insufficient immunogenicity remains a disadvantage. mRNA vaccine development in general, are slow because of their poor stability and low delivery efficiency [13, 14]. To combat this, liposomal preparations of RNA lipid complexes with antigen HPV 16, E7 was introduced. mRNA vaccine research has shown a bright future with the advent of mRNA covid vaccines. Failure to extract good quality dendritic cells for vaccine development, whole cell

S. No	Type of vaccine	Platform	Antigen
1.	Live vector-based vaccines	Bacterial vector-based vaccine	HPV16 E7
			HPV16/18 E6/E7
		Viral vector-based vaccine	HPV16 E6/E7
			HPV16/18 E6/E7
2.	Peptide and Protein-based vaccines	Synthetic long peptides and Specific epitope (short) peptides	HPV16 E7
			HPV16 L2/E6/E7
			HPV16/18 E7
			HPV16 E6
3.	Nucleic acid-based vaccines	DNA vaccines	HPV16/18 E6/E7
			HPV16 E7
			HPV16 L2/E6/E7
		mRNA vaccines (liposome-based vaccine)	HPV16 E6/E7
			HPV16 E7
4.	Whole-cell vaccines	Dendritic cells	HPV16/18 E6/E7

Table 6.
Carriers and antigens of therapeutic HPV vaccines.

vaccine development has faced a crisis. Hence newer adjuvants, potential antigen targets are further being investigated [2].

7. Conclusion

The HPV vaccination program has markedly transformed the health status of women globally. It has helped in decreasing HPV infections and associated cancers. The hesitancy of individuals to opt for HPV vaccinations remains a hurdle in achieving the WHO goal of global cervical cancer elimination by 2030. The impact of HPV vaccination in males has been overlooked and one of the prime reasons cited is the lack of physician recommendation on it [14]. A systematic review published from Portugal in June 2023, has thrown light on the agonizing truth that only 4% of men worldwide were fully vaccinated against HPV as of 2019. Literatures imply evidence of HPV vaccine efficacy in men up to 26 years of age. Keeping increased vaccination coverage as a public health priority, the FASTER Strategy protocol was put forth to bridge the disconnect between HPV screening and vaccination and hasten reduction in HPV related infections and cervical cancer mortality [15]. Considering the role of early proteins other than E6 and E7 in the development of HPV related cancers, further exploration to encompass other antigens will widen the horizon to successfully generate more effective therapeutic vaccines.

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Human papillomavirus (HPV) is a global health concern that impacts millions, contributing to conditions ranging from benign warts to life-threatening cancers. Despite the availability of vaccines and advancements in treatment, HPV continues to pose significant public health challenges worldwide. *Confronting HPV - Insights and Solutions* offers an in-depth exploration of the virus, providing the latest research and expert perspectives on its epidemiology, treatment, and prevention strategies. This comprehensive resource tackles critical questions surrounding HPV, addressing both its biological complexity and the social impact of the disease. From understanding the spread of the virus and its role in various cancers to examining the effectiveness of vaccination programs, this book equips healthcare professionals, researchers, and public health advocates with the knowledge needed to fight HPV more effectively. In addition to discussing clinical and preventive approaches, *Confronting HPV - Insights and Solutions* highlights the importance of its vaccine. With actionable insights and forward-thinking solutions, this book serves as an essential guide for anyone working to reduce the burden of HPV on individuals and communities alike.

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