

Review Advances in Etiopathological Role and Control of HPV in Cervical Cancer Oncogenesis

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Abstract

The human papillomavirus (HPV) is a well-known oncovirus whose causal link in the occurrence and development of several cancers, such as cervical cancer (CC), has been well established. Indeed, numerous researches depicted the etiological role of HPV in CC pathogenesis in such a way as to develop efficient strategies, including early diagnoses and HPV vaccination, to mitigate HPV infection and CC occurrence. Despite the effectiveness of these strategies in preventing HPV infection, its persistence, and the progression to precancerous lesions and cancers, extensive work that could give a better understanding of other unknown factors favoring oncogenesis is much more needed. In this last decade, scarce or few but crucial and strategic studies have been carried out to improve and deepen our understanding of the etiopathological role of HPV in the progression towards the development of CC. In this review, we highlighted the recent findings on the pathological role of HPV in CC occurrence and the advances in novel adopted strategies to reduce HPV infection and prevent CC occurrence more effectively.

Keywords: HPV; cervical cancer; cervicovaginal microbiota; cervicovaginal metabolic profile; HPV diagnosis; HPV treatment

1. Introduction

Human papillomavirus (HPV) is a group of more than 200 small viruses characterized by an 8 kb non-enveloped circular double-stranded genomic DNA that infects human mucosal and cutaneous epithelia. Generally, it is estimated that more than 80% of the sexually active worldwide population will be infected with HPV [1]. As a sexually transmissible infection (STI), HPV transmission mainly occurs during the first sexual intercourse, regardless of race and gender. It is, therefore, a ubiquitous STI [1]. Fortunately, in around 75–90% of HPV-infected people, HPV is armless, transient, and resolves spontaneously without treatment within one or two years, with no lifestyle-threatening clinical symptoms (reviewed in [2]).

However, HPV infection persists in a few rates of the global population. Indeed, people with poor health conditions that weakened the immune system, including people with other infections such as Herpes simplex virus (HSV), Human immunodeficiency virus (HIV), Epstein-Barr virus (EBV), and other STIs (reviewed in [2]), congenital diseases (epidermodysplasia verruciformis, congenital lymphopenia) [3], organ transplantation, or under cancerrelated chemotherapy [2,4], are the most at risk for reinfection or infection persistence, leading to anogenital warts, precancerous lesions (PCLs), and subsequently to cancers in women and men. While HPV 6 and 11 are associated with benign infection and meaningless outcomes (cutaneous and genital warts), seventeen HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 58, 66, 68, 73, and 82) are noteworthy to be at high oncogenic risk (HR) and mainly found in women and men with PCLs and cancers [2].

Besides the vaginal, anal, penile, and upper aerodigestive tract cancers, cervical cancer (CC) is the most common and deadliest HPV-associated cancer caused by the persistence of high-risk HPV (HR-HPV) infections. In 2020, CC was responsible for 342,000 deaths [5], a slight increase compared with the death rate in 2018 (311,365) [6,7], and according to the latest available statistical data, HPV-associated CC would kill 443,000 women around the world by the year 2030 [2]. Most of these fatality cases oc-



cur and will continue to occur in low- and middle-income countries (reviewed in [2]). Despite the strategic means adopted so far (mandatory HPV vaccination in young girls from 9 years old and routine PCL screening) to reduce HPV infection and mitigate the occurrence of CC, the burden of CC cancer worldwide is still significant and stigmatizing. As the fourth most prevalent cancer, CC is ranked the fourth deadliest female cancer after breast cancer [6] and continues to cause significant fatalities [2]. This suggests that much effort should be put forward to deepen our understanding of the oncogenic role of HPV infection.

This past decade, few but crucial, strategic, and determinant studies have been carried out to improve and deepen our understanding of the etiopathological role of HPV in CC occurrence [2]. These studies mainly focused on the beneficial role of HPV infection in the presence of other pathological or congenital disorders or how HPV infection takes advantage of weakened immunity to induce PCLs and CC. This deepened and advanced understanding of the etiopathological role of HPV infection in PCLs and CC occurrence might lead to the adoption of enhanced strategies to control infection and mitigate the occurrence of CC. In this review, we highlighted the recent findings on the pathological role of HPV in CC occurrence and the advances in novel adopted strategies to reduce HPV infection and prevent CC occurrence.

2. Unbalanced Cervicovaginal Microbiota and Metabolome: A Risk for HPV-Associated Cancers

2.1 Cervicovaginal Microbiota (CVM) in HPV Infection Persistence

It is noteworthy that HR-HPV infection may persist in certain individuals known as "people at risk". These include people with sexually-transmissible diseases (AIDS-HIV, Chlamydia, and Gonorrhea), organ-transplant receivers, and the elderly; in short, people with an innate or acquired immune deficiency [2]. However, besides these people qualified as people at risk, it has been newly discovered that healthy women with a disrupted cervicovaginal microbiota (CVM) have a high risk for developing cervical intra-epithelial neoplasia grade 1 or 2 (CIN1/2) and invasive CC. Indeed, these studies described that the unbalanced bacterial population in the cervicovaginal area, caused by an increase or decrease of certain bacterial populations, is a major factor for HR-HPV acquisition and persistence of the infection towards high-grade lesions and cancer development [2,8,9].

A healthy CVM, which consists of a balanced variable population of bacteria, is dominated by *Lactobacillus spp.* [9,10] and plays a crucial role in vaginal area protection against STIs and diseases [2,8,9]. Its disruption has been associated with several vaginal infections and diseases [8,9]. Bacterial vaginosis (BV), for instance, which is a vaginal pathological condition, is characterized by a de-

creased amount of vaginal Lactobacillus spp. and an abundance of polymicrobial anaerobic bacteria, including Gardnerella vaginalis, Prevotella spp., Mobiluncus spp., Ureaplasma urealyticum, and Mycoplasma hominis [9]. Also, BV has been found to be a strong predictor of Neisseria gonorrhoeae and Chlamydia trachomatis infections [11], which are among the risk factors for HPV acquisition and infection persistence, the occurrence of CIN1/2, and malignancies, leading to the hypothesis that changes in CVM homeostasis have a crucial implication in the occurrence of cancerous lesions (CINs) [12]. Other studies have evidenced the strong causal link between genital immune mediators and the decrease in Lactobacillus spp. in cervical carcinogenesis [13,14]. Several studies further confirmed this hypothesis (reviewed in [2]). More precisely, in their study, Usyk et al. [15] demonstrated that Lactobacillus spp. depletion in vaginal microbiota along with the abundance of strictly anaerobic bacteria, including Gardnerella vaginalis, Prevotella spp., Mobiluncus spp., Atopobium vaginae, and Mycoplasma hominis, is associated with a high risk of persistence of HR-HPV infections an occurrence of CIN2/3. Moreover, Norenhag et al. [10] confirmed that a CVM dominated by other bacterial populations than Lactobacillus spp. was associated with up to five times higher risk of contracting any HPVs and up to three times higher for HR-HPVs, which may induce invasive CC. Taken together, this demonstrates that-the abundance of-strictly anaerobic bacteria, but especially Gardnerella spp., are associated with the persistence of HR-HPVs infection and occurrence of malignancies, making "abnormal Gardnerella spp. increase" a point of control for monitoring HPV infection persistence (reviewed in [2]).

Controversially, while BV (or imbalanced CVM) has been since no long ago taught to be among the risk factors for HR-HPV infection and related carcinogenesis, Lebeau et al. [16] claimed in their recent study that it is instead the HR-HPV infection that leads to CVM alteration, making their causal relationship again questionable. Given that a healthy CVM consists of a low microbial diversity largely dominated (>90%) by a few *Lactobacillus spp.* (mainly L. crispatus, L. jensenii and L. iners) [9,10,16–18], the authors showed that, in the course of HPV infection, HR-HPV oncoproteins (E6 but mainly E7) down-regulate expression of the host innate peptides that are required for survival and sustainability of Lactobacillus spp., and antimicrobial activity on Gardnerella vaginalis in healthy vaginal mucosa [16]. Thus, inhibition of these host peptides, including defensin family (H β D1-4, HD5, HD6) and antimicrobial defensin-like family (SLPI, S100A7, Elafin, and LL-37) peptides, through the interaction of HPV E7 with NEMO, CK1, and β -TrCP of both NF- κ B and Wnt/ β -catenin signaling pathways, leads to BV (or dysbiosis), characterized by changes of the normally dominant lactic acid bacteria as described above [16-18] by a high diversity of bacterial population predominated by Gardnerella vaginalis and

other anaerobic bacteria including *Atopobium vaginae*, *Prevotella spp.*, *Mobiluncus spp.*, *Sneathia spp.*, *Ureaplasma urealyticum*, and *Mycoplasma hominis* [9,16].

Overall, it should be retained that while unbalanced CVM (where *Gardnerella spp.* is high and *Lactobacillus spp.* is low) is now a well-characterized risk factor for HPV acquisition and infection persistence, HR-HPV itself participates in CVM alteration through molecular interactions, which weaken the bacterial cervicovaginal protection and enhance the occurrence of CC. Hence, an unbalanced CVM is a microbial marker that should alert clinicians to precancerous lesion risk and should be considered with high priority for treatment.

2.2 HPV Infection and Severity Induce Typical Cervicovaginal Metabolic Profiles (CVMPs)

Additionally, to the negative effect of HPV infection on CVM (and/or vis-versa) leading to HPV-associated disorders, the cervicovaginal metabolic profile (CVMP) might constitute another point of control for monitoring HPV infection risk and persistence of HR-HPV infection, but preferably for monitoring cervicovaginal cancers, because as metabolic diseases, cancers target and modify metabolome composition at the local environment [19,20]. Studies in women with CC showed unique and typical CVMPs defined by a specific composition from amino acids to nucleic acids in CC tissues, which was completely absent from cancer-free/healthy women [21]. In the context of HPV-related CC, this typical CVM change is indirectly induced by HR-HPV infection through inhibition of host innate peptide [16] and cytokine [22] production that subsequently alters bacterial dynamics and antimicrobial activity in the cervicovaginal microenvironment and causes BV as described hereinbefore. For instance, changes from a healthy Lactobacillus spp.-dominated CVM towards an increased Gardnerella spp.-dominated anaerobic and microaerophilic poly-bacterial communities are strongly associated with increased amino acid catabolites and polyamines [23,24]. More precisely, in a study profiling the cervicovaginal metabolism in HPV-infected women, with PCLs, CIN1/2/3, and CC, it was reported that for each woman and each specific grade of HPVinfection severity, the CVMP was typical and specifically different with a huge variation in vaginal pH and genital inflammation (Fig. 1) [25]. The CVMP in HPVpositive women with PCLs and CIN1/2/3 showed a decrease of amino acids metabolites and depletion of dipeptides characterized by HPV-positive women with CIN3. However, in HPV-positive women diagnosed with CC, the CVMP profile is characterized by increased xenobiotics and lipids from multiple macromolecule sources [25]. Hence, any increase of xenobiotics (mainly antiinflammatory and analgesic drugs) and lipids (mainly 3hydroxybutyrate, eicosenoate, and oleate/vaccinate) in an HPV-infected woman should be a suspicion of cancer development [25,26], because many others studies evidenced that high level of 3-hydroxybutyrate, eicosenoate, and oleate/vaccinate augment are associated with tumor development and growth [27,28], specifically CC [26,29].

3. Immunological Insight of Congenital Disorder in HPV Infection Persistence

Immunodeficiency is one of the main causes of HR-HPV infection persistence, leading to invasive CCs. Two main types of immunodeficiency are known: acquired and congenital immunodeficiency. Acquired or secondary immunodeficiency (AID or SID), mainly due to HIV infection or immunosuppressive treatments, is the most attributable risk factor for HR-HPV infection, infection persistence, and cancer occurrence [2]. Congenital or primary immunodeficiency (CID or PID) is due to inborn errors of immunity caused by mutations in genes involved in immune host defense and immune regulation [30]. The causal link of CID with HPV infection susceptibility was speculative. It was previously discovered that patients without AID but with severe HPV infection leading to malignancies were suffering from CID. Interestingly, many studies recently focused on the etiological role of CID in HPV susceptibility and reported that CID is involved in the persistence of HR-HPV infection in individuals without AID. This supports that CID, like AID, is a risk factor for HPV acquisition, persistence, and HPV-associated disease development [31-35].

To understand the involvement of CID in susceptibility to HPV acquisition and the persistence of the infection towards CC, findings from immunological studies have contributed quite a lot. It was reported that in either CID or AID, or both cases of patients, the cellular immune response was too low, with a low count of CD4⁺ and CD8⁺ T-cells, which is the main characteristic of lymphopenia states. Also, it is known that HIV specifically targets CD4⁺ T-cells and kills them. The immunological profile in CID and AID patients infected with HR-HPV, but not low-risk HPV, is comparable and characterized by a decreased T-cell response. These results suggested that T-cells, especially CD4⁺ T-cells, play a central role in the clearance of HPV and prevention of cancer occurrence [31-35]. A study by Nicholls et al. [36] reported that infiltration of CD4⁺, CD8⁺ T-cells, and dendritic cells in dogs infected with canine oral papillomavirus induces regression of papillomavirus-associated cancerous lesions, with CD4⁺ T-cells providing the most efficient healing effect. This study corroborates and confirms previous studies conducted by Iwatsuki et al. [37] and Coleman et al. [38], reporting that T-cells infiltration (more CD4⁺ and low CD8⁺ T-cells) are responsible for HPV6 and 11- associated warts regression.

Taken together, this highlights the protective role of CD8⁺ and preferentially CD4⁺ T-cells in the clearance of HPV infection, especially HR-HPVs, and their importance



Fig. 1. Effect of human papillomavirus (HPV) on cervicovaginal microbiota and metabolome profile and implication in carcinogenesis. The background color circled on the female's body indicates the health state of the cervicovaginal microbiota: healthy and balanced cytomegalovirus (CMV) (colorless), slightly unbalanced cervicovaginal microbiota (CVM) (yellow), moderately unbalanced CVM (pink), severely unbalanced CVM (red). The color of the arrow bar (yellow [acid] to blue [basic]) indicates the vaginal pH. CIN1/2/3: cervical intra-epithelial neoplasia grade 1, 2 or 3.

in preventing CC development. In other words, lymphocyte depletion caused by CID (as well as AID) constitutes a risk factor for HPV infection, persistence, and CC development.

4. Molecular Implication of HPV/HIV Co-Infection in CC Occurrence

The HPV/HIV co-infections are associated with an increased risk of HR-HPV persistence, acquisition of opportunistic infections, and development of genital malignancies [39]. The effect of HPV and HIV co-infection has been established, and co-infected HPV/HIV women are known to have reduced HPV clearance rates and an increased risk for persistent HR-HPV infection compared with HIV- negative women [40]. By promoting HPV-related carcinogenesis [39,41], HIV positivity remained strongly associated with HR-HPV infection and HPV-associated cancer occurrence [40].

HIV-infected people have a suppressed immunity resulting in a high prevalence of HIV/AIDS-associated malignancies such as HPV-associated cancers, as described in paragraph 2. Besides the immunological point of view, other molecular mechanisms in which HPV and HIV are engaged have been suggested. However, the molecular mechanism behind these phenomena remains largely unclear [42].

Besides the lack of or the low titer of CD8⁺/CD4⁺ T-cell response due to HIV infection, host molecular genetic variations and HPV/HIV co-infection have an impact and play a crucial role in CC occurrence and progression [43,44]. Some studies reported that people with a certain predisposition to human leukocyte antigen (HLA) molecules might be susceptible to HPV infection and persistence. Indeed, the persistence of oncogenic HPV in some women with specific HLA II alleles is strongly associated with the development of CC. For instance, the HLA II DRB1*13:01, DQB1*03:01, DQB1*03:19, and DQB1*06:02 alleles can modulate cervical carcinogenesis in HPV/HIV-1 co-infected women [45].

CC has also been associated with DNA genomic instability and DNA repair mechanisms. In HIV/AIDS, other factors than immunodeficiency, such as oncogenic DNA viruses and the HIV-1 protein Tat, have been associated with malignancies, as they significantly increase the incidence of carcinoma associated with DNA repair pathway dysregulation. It has been found that Tat-induced DNA repair deficiencies may play a significant role in the development of HIV-associated malignancies [46]. Similarly, based on this observation, Barillari et al. [47] could demonstrate that the extracellular Tat protein plays a significant role in the development of HPV-associated cancers, including CC. The prolonged interaction of viral envelope gp120 and transactivator proteins Tat with cervical induces an epithelial-mesenchymal transition mediated by SMAD2 phosphorylation and activation of transcription factors Slug, Snail, Twist1, and ZEB1. This interaction of gp120 and Tat proteins reduces E-cadherin expression and activates vimentin and N-cadherin expression [48]. The HIV Tat protein is incorporated into HIV-1 virions as a potent transcriptional transactivator of virus gene expression, which is essential for virus infectivity, replication, and transmission [49]. The HIV Tat protein transactivates the HPV long control region, increasing the expression of the oncogenes E6 and E7. One of the molecular interactions between HIV and HPV has been recently proposed [50]. HIV-1 Tat protein up-regulates HPV E6 and E7 gene expressions, which are mainly involved in cervical carcinogenesis and, therefore, the development of tumor cells.

Furthermore, the HIV-1 Tat protein induces cell cycle proliferation through the expression of cell cycle regulator genes [51], meaning that Tat promotes metastasis of cervical carcinoma during carcinogenesis mechanisms. Precisely, Tat protein is taken up by human uterine cervical carcinoma cells, and this affects (up-regulates) the expression of HPV oncoproteins (E6 or E7) and down-regulate cellular proteins (p16 and/or p53), which are key to cervical carcinoma development or progression. It has been demonstrated that HIV-1 Tat protein is taken up by human uterine cervical carcinoma cells and directly followed by an increase in the expression of the E6 protein of HPV, which is associated with the high incidence and clinical aggressiveness of uterine cervical carcinoma observed in HPV/HIV co-infected women [47].

5. New Regulations for Screening and Treatment of Cervical Pre-Cancer Lesions and CC Prevention

As previously reviewed [2], the two main strategies to fight against HPV-associated precancerous lesions and CC occurrence are vaccination and early diagnosis/screening, followed by appropriate treatment. However, because vaccines are prophylactics and only limited to preventing from getting infected by HPVs, preventing the persistence of HPV infection may slightly or not be overcome by HPV vaccines and not can it guarantee cancer occurrence, as the infection is already present. To a lesser extent, vaccines can prevent new infections after a primary transient infection, autoinfection, or sur-infection and limit the risk of developing CC. There is, unfortunately, no available direct treatment to eradicate HPV infection to date. Thus, for over-



all better prevention, HPV vaccines should be affordable, evenly, and equally available to all populations, specifically to people and areas of high prevalence, and implemented at an early age [52]. Yet, in developing countries, where the HPV-associated burden is tremendous (accounting for more than 80% of the global CC burden [5,6,53]), accessibility to prophylactic treatments is limited because of cost, cold chain transportation challenges, and poor health care.

The millennium objective targeted by the WHO is "to vaccinate 90% of eligible girls from a very young age against HPV, to screen 70% of eligible women at least twice in their lifetimes, and to effectively treat 90% of those with a positive screening test or a cervical lesion, including palliative care when needed", in order to accelerate the elimination of CC as a public health problem, by 2030 ([54], https://www.who.int/publications/i/ite m/9789240014107). Therefore, reinforcing the currently implemented vaccination programs or designing alternative HPV vaccines with cost-effective development strategies for affordable products to control HPV infection and prevent CC occurrence are needed. Besides, diagnosing HPV infection and screening for cervical precancerous lesions are recommended to prevent CC occurrence.

5.1 Molecular Basis for the Development of Next-Generation HPV Prophylactics

Cervical cancer is a preventable disease as it lies on infection by HPV. The currently available HPV prophylaxis, including Cevarix, Gardasil, and Gardasil-9, are made from one of the main HPV proteins involved in the first steps of the infection, the major capsid L1 protein, obtained in the form of virus-like particles [55,56]. Targeting this protein blocks further HPV interactions with host cells to initiate the early steps and prevents infection progression, which explains the proven efficacy of these three vaccines [52,57] and long-term preventive immunity [58–60]. However, these vaccines have limitations, including limited protection against some but not all HR-HPVs.

Screening the molecular and intracellular trafficking of the HPV infection steps provides molecular insights for developing alternative drugs against HPV infections and CC (reviewed in [61]). Specifically, three main molecular target sites in the early steps of HPV infection, including (i) the heparan sulfate binding site, (ii) the HPV cellular internalization site, and (iii) the intracellular trafficking molecules, are promising in preventing HPV entry into cells and replication [61]. Of interest, targeting these therapeutic sites could allow the development of broad-spectrum prophylactics against all HPV types and, to a lesser extent, other viruses, as the antiviral mechanism lies on inhibiting the viral attachment to the cell surface or preventing the interactions between viral particles and intracellular components, by either binding to viral particles or interacting with intra- or extracellular receptors [61].

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Screening tests	Screening principle/method	Ref.
Pap smear	Visual detection of epithelial cells (cytological) abnormalities/metastasis and dyspla-	[74,75]
	sia in a cervix smear sample directly onto a microscope slide	
Liquid-based cytology (LBC)	Visual detection and semi-automated quantitation of epithelial cells (cytological)	[72]
	abnormalities/meta-, and dysplasia in a cervix smear sample, pre-treated in physi-	
	ologic liquid	
Dual staining cytology to identify p16 and Ki-67	Automated staining and semi-quantitative evaluation of slides/samples from the	[85]
	cervico-epithelial cells	
Visual inspection with acetic acid or with Lugol's	Visual (necked eye) detection of cervical epithelial cells with acetic acid or with	[54]
iodine (VIA/VILI)	Lugol's iodine (VIA/VILI)	
Automated visual evaluation of digital image	Intelligence artificial or deep learning-based cytological image evaluation	[83]
Nucleic acid amplification tests (NAAT)	Detection of high-risk HPV (HR-HPV) DNA or mRNA through nucleic acid ampli-	[84]
	fication (PCR)	
Tests under evaluation (future tests) HPV-DNA methylation	PCR-based quantitative site-specific CpG methylation using next-generation se-	[82]
	quencing (NGS) approach	
Protein biomarkers	Detection of HR-HPV antibodies or oncoproteins	[54]
	Screening tests Pap smear Liquid-based cytology (LBC) Dual staining cytology to identify p16 and Ki-67 Visual inspection with acetic acid or with Lugol's iodine (VIA/VILI) Automated visual evaluation of digital image Nucleic acid amplification tests (NAAT) HPV-DNA methylation Protein biomarkers	Screening testsScreening principle/methodPap smearVisual detection of epithelial cells (cytological) abnormalities/metastasis and dyspla- sia in a cervix smear sample directly onto a microscope slideLiquid-based cytology (LBC)Visual detection and semi-automated quantitation of epithelial cells (cytological) abnormalities/meta-, and dysplasia in a cervix smear sample, pre-treated in physi- ologic liquidDual staining cytology to identify p16 and Ki-67Automated staining and semi-quantitative evaluation of slides/samples from the cervico-epithelial cellsVisual inspection with acetic acid or with Lugol's iodine (VIA/VILI)Visual (necked eye) detection of cervical epithelial cells with acetic acid or with Lugol's iodine (VIA/VILI)Automated visual evaluation of digital image Nucleic acid amplification tests (NAAT)Intelligence artificial or deep learning-based cytological image evaluation Detection of high-risk HPV (HR-HPV) DNA or mRNA through nucleic acid ampli- fication (PCR)HPV-DNA methylationPCR-based quantitative site-specific CpG methylation using next-generation se- quencing (NGS) approach Detection of HR-HPV antibodies or oncoproteins

Table 1. Diagnostic tests for HPV infection and precancerous lesion screening.

Specifically, heparan sulfate proteoglycans (HSPGs) are required cell receptors for the initial attachment of HPV and many other viruses, including coronaviruses [62,63], herpes simplex viruses 1 and 2 (HSV-1/2), Dengue virus (DENV), echoviruses 5 and 6, enterovirus, human cytomegalovirus (HCMV), HIV, polyomaviruses [64], and hepatitis viruses [64,65]. Thus, potential inhibitors that target the HSPG-depending early attachment might be promising against broad-spectrum viral infections [61,64]. Interestingly, several molecules mimicking or interacting with HSPGs, including heparin, dendrimers, and carrageenan, have proven to be effective against HPV, HSV-1/2, HCMV, SARS-CoV-2, and DENV [61,62,64]. Hence, deeper *in vivo* studies to assess the protective efficacy and safety of these molecules are highly encouraged.

Likewise, during infection, HPVs use similar downstream intracellular trafficking with other viruses, which may constitute therapeutic targets against a broad range of viruses [61]. Several reports describe the promising use of molecules to prevent infection by HPVs and the occurrence of CC. Zhang *et al.* [66] designed a short peptide that can bind to retromer (an intracellular virus interacting protein that binds to HPV L2 protein [67]) and potentially compete with HPV16 as an antiviral strategy. They demonstrated that the synthetic peptide can enter cells, sequester retromer by occupying the HPV-16 L2 retromer-binding site and inhibit HPV infection [66]. Such a drug could prevent the occurrence of CC.

Moreover, endoplasmic reticulum (ER) chaperones have been identified as common proteins involved and required in intracellular viral trafficking, from entry and viral replication to the assembly and release of virions. To develop broad-spectrum antiviral therapeutics, several studies have proposed potential inhibitors of ER chaperones that can be used against HPV, HIV, hepatitis viruses, SARS-CoV-2, Zika virus, DENV, Ebola virus, HCMV, and many others [68]. ER chaperone inhibitors, including AR-12 (OSU-03012), EGCG (polyphenol), PU-WS13, Migitol, Indonlizidine derivatives, Juniferdin, PACMA 31, 16F16, Isoquecetin, Bacitracin, Origamicin, LOC14, and Tizoxanide, have proven to block the progression of viral infection [68]. Similarly, myosin motors are proteins that bind to actin filaments to generate force, mobility, and shape for cell structure and dynamics [69]. They play a central role in intracellular trafficking, exo- and endocytosis, and transcription. Thus, viruses, including HPVs, use or take advantage of these proteins of the cytoskeleton, which are necessary for HPV infection, specifically for viral internalization, packaging, and release [70,71]. Therefore, the specific interactions between HPVs and myosin motors serve as a basis for developing antiviral drugs that could compromise such interactions. Overall, these studies might help develop broad-spectrum antiviral prophylactics that target common intracellular virus trafficking.

5.2 Diagnostic Approaches for CC Screening and Next-Generation Tests

For effective CC prevention, sexually active women are recommended to undergo cervicovaginal screening to identify those who are at risk of cervical pre-cancer (CIN1, 2, or 3). Currently, several diagnostic tests are available on the market. The well-known of these precancerous lesion screening methods include the Papanicolaou test, also known as the Pap smear or smear test, a cervicovaginal cytological test designed to detect abnormal cervical cells [72–75]. Although wildly considered an effective method for detecting and preventing CC, the Pap smear test sensitivity is low (ranging from 30% to 87%) [72,73,76-78]. Hence, it was highly recommended that the Pap smear must be performed yearly for a better follow-up [73]. Besides, it was also proposed that the Pap smear may be replaced by liquid-based cytology (LBC) (which is slightly more sensitive than the Pap smear [72]) or associated with colposcopy-directed biopsy [73] to reduce the rate of undiagnosed cervical dysplasia. Therefore, it has been finally adopted that colposcopy-directed biopsy should be done in cases of macroscopic cervical architectural changes, abnormal findings, or positivity of the Pap smear cytology result [54,73,79]. Appropriate treatment is then determined after a biopsy of suspicious lesions for histological diagnosis. This approach has been proven effective in developed countries where cytology-based CC screening programs are well-implemented and strictly followed, yielding a five-time reduce fatalities from CC [80]. This observation is unfortunately not the case in developing countries [81], where access to health care and availability of standard care facilities are still major public health concerns. This implies that governments from low- and middle-income countries should make available financial resources to set up or strengthen screening programs, to reduce the CC-related death rate.

In the last decade, researchers have developed improved diagnostic/screening tests in order to reach the WHO's goal by 2030. These screening tests include visual inspection with acetic acid (VIA) and molecular tests, specifically HR-HPV DNA detection-based tests by PCR [54], which are more suitable to use in any laboratory setting (Table 1, Ref. [54,72,74,75,82-85]). More interestingly, it was recently reported that newer cervicovaginal screening tests had been made available for diagnosing HR-HPV infection and precancerous lesions, among which molecular tests, specifically directed to the detection of HPV mRNA, HPV-E6/E7, and HPV-DNA methylation. Besides that, more conclusive screening tests performed on cytological samples (p16/Ki-67 dual staining) and highly technology-based advanced visual inspection screening tests based on artificial intelligence or machine learning platforms (automated visual evaluation of digital images) have been launched (Table 1) [54,82-86].



Fig. 2. The seven algorithms considered for screening and treatment of cervical pre-cancer lesions and cervical cancer (CC) prevention. HPV, human papillomavirus; VIA, visual inspection with acetic acid; Cyt., cytology; +, positive; -, negative; Susp, suspicion of positive cytology test.

5.3 Approaches to Cervical Cancer Screening and Treatment

There are two regulation approaches for screening and treatment of precancerous lesions and preventing CC occurrence, categorized into seven possible combinations or algorithms, which include (i) the screen-and-treat approach, for which the treatment is only based on a positive primary screening test (2 algorithms), and (ii) the screen-triage-and-treat approach for which the adequate treatment is based on and follows a positive primary test followed by a positive secondary test [54] (Fig. 2).

Specifically, in the screen-and-treat approach, women positive for HR-HPV and precancerous lesions after the primary screening test are directly eligible for the appropriate treatment. Secondary screening is not needed in this approach to start the treatment. Precancerous cell ablation and excision are the main treatments for women to prevent CC development. Once the primary screening is positive, the appropriate treatment is done on either the same visit of the screening test (single-visit approach) or another day (multiple-visit approach) [2,54].

In a screen-triage-and-treat approach, women who are positive for HR-HPV and precancerous lesions after the primary screening test undergo a secondary screening test. If this later is confirmed positive, the appropriate treatment is determined by prime colposcopy with biopsy and histopathological examination. However, if the secondary screening test is negative, only an appropriate follow-up evaluation is required [2,54].

6. Conclusions

Cervical cancer is the fourth deadliest cancer in women, and HPV, the leading viral cause of CC, is the most common transmissible virus in the world. Persistence of the infection mainly occurs in case of infection with HR-HPVs, and this constitutes the primary risk factor for leading CC. Besides, host factors, notably immunodeficiency due to poor health conditions, including viral infections [2], congenital diseases [3], organ transplantation, and cancerrelated chemotherapy [2,4], constitute the second risk for infection persistence, responsible for PCLs, and CC.

Recent studies on the etiopathological role of HPV infection in CC occurrence demonstrated that the persistence of HR-HPV infection might also be more prominent in case of disruption of CVM, characterized by the abundance of Gardnerella spp. and scarcity of Lactobacillus spp. In fact, HPV infection promotes its own persistence by inducing CVM disruption through molecular inhibition of the host innate peptides required for the survival and sustainability of Lactobacillus spp. and antimicrobial activity on Gardnerella vaginalis in the healthy vaginal mucosa. Moreover, the HPV-associated disrupted cervicovaginal microenvironment is characterized by a typical metabolome profile specific for a given HPV infection severity or cervicovaginal inflammation. Furthermore, HIV-HPV co-infection and primary lymphopenia constitute other crucial factors for the persistence of HR-HPV infection and the occurrence of CC.

Given the causal role of HR-HPV infection in the occurrence of CC, the fighting strategies lie in diagnosing and treating precancerous intraepithelial lesions based on new regulations. This includes that every woman from 20 years old should undergo a cervicovaginal screening to identify those who are at risk of cervical pre-cancer and should be received an adequate treatment determined by one of the seven screening-treatment algorithms adopted Fig. 2. To end, while diagnosing HPV and cervical cytology have reduced incidence of CC, government and international decision-makers should provide resource lines to implement HPV vaccination programs rather than only screening programs around the world, but especially in low-and middle-income countries, where the HPV associated burden is blatant [2]. Furthermore, given the limitations of the current HPV vaccines, the development of broad-spectrum antiviral drugs and vaccines is highly encouraged.

Author Contributions

AJKK conceptualized and conceived the main idea, extracted the data, wrote the original draft, and formatted the manuscript for submission. SZA, GAB and FANB helped in designing the work, contributed in the main idea, extensively reviewed and edited the final version of the manuscript before submission. TJ and AAZ conceptualized the main idea and provided resources. All authors contributed to editorial changes in the manuscript. All the authors read and approved the final version of the manuscript for publication.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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