Molecular and Host Lifestyle Factors Associated with Persistent Human Papillomavirus Infection and Progression into Cervical Cancer: A Literature Review

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ABSTRACT
Background: Human papillomavirus (HPV) infection is the most common sexually transmitted infection (STI) worldwide, especially among low- and middle-income countries. The virus can establish persistent infection in the cervical epithelium, thereby increasing the risk of progression into cervical cancer. Since cervical cancer is one of the leading causes of cancer death among women worldwide, it is important to understand more about persistent HPV infection and potential therapeutic targets to suppress it. This study aims to summarize current insight into various molecular and host lifestyle factors that contribute to persistent HPV infection and ultimately cervical cancer.

Methods: This study adopts a literature review design by conducting a journal search through Google Scholar, PubMed, and ScienceDirect. The keywords used included “human papillomavirus”, “persistent infection”, “cervical cancer”, “immune evasion”, and “lifestyle”.

Results: Several diverse mechanisms are believed to facilitate persistent HPV infection, which can be classified under molecular and host lifestyle factors. Molecular factors include compartmentalization of HPV replication and gene expression as well as immune evasion, whereas host lifestyle factors include alcohol consumption, smoking, multiple sexual partners, STI coinfection, and certain contraceptive agents.

Conclusion: Persistent HPV infection acts as the intermediate phenotype before developing into cervical cancer. Understanding the molecular factors as well as host lifestyle factors underlying it can lead to more specific therapeutic options as well as better prevention and education programs. Future research is needed to better clarify the exact mechanisms underlying persistent infection.

INTRODUCTION
Human papillomavirus (HPV) is a small circular double-stranded DNA virus belonging to the Papillomaviridae family, and its infection is the most common sexually transmitted infection (STI) worldwide. Most sexually active individuals are exposed to it at least once during their lifetime, and the prevalence of HPV infection is highest among women in low- and middle-income countries [1]. Although the majority of HPV infections resolve spontaneously, infection can persist over long periods of time and cause cervical cancer in women [1,2]. Cervical cancer is now the fourth most common cancer among women worldwide, with approximately 604,000 new cases and 342,000 deaths occurring in 2020 [2,3]. Up to the present, more than 200 HPV genotypes have been documented, and around 40 of these target the genital mucosa. HPV types that infect stratified squamous epithelial cells in the genital tract can be divided into high-risk (HR) and low-risk (LR) types; HR types like HPV 16 and 18 are known to be responsible for most cervical cancers and precancerous cervical lesions, whereas LR types like HPV 6 and 11 also commonly infect the genital epithelial cells but are rarely found in cancerous lesions [1,4].
Other than HPV, known risk factors for cervical cancer include smoking, being sexually active from a young age, low socioeconomic status and education, multiple sexual partners, increased use of contraceptives, multiple childbirths, and coinfection with other STIs such as chlamydia and HIV [2,5-7]. Past evidence pointed out that some of these host lifestyle risk factors may potentiate persistent HPV infection, increasing its risk of progression into cervical cancer. For example, coinfection with HIV promotes the oncogenic potential of HPV, and antiretroviral treatment can deter the progression of HPV into cancerous lesions [1]. Moreover, HPV has molecular mechanisms related to its viral proteins and their interplay with our immune system which help facilitate persistent infection.

Hence, factors associated with persistent HPV infection and progression into cervical cancer are increasingly being studied. Understanding how each of these factors plays a role in facilitating persistent HPV infection can offer novel insight into potential therapeutic targets in order to suppress the progression into cervical cancer. This review aims to summarize the latest evidence regarding the association between molecular factors, host factors, and persistent HPV infection as well as the underlying pathological mechanisms.

METHODS

This study adopts a literature review design through searching, compiling, and analyzing findings from various primary studies and reviews that investigated the molecular mechanisms underlying persistent HPV infection as well as lifestyle risk factors contributing to it. Sources were cited from Google Scholar, PubMed, and ScienceDirect published between 2009-2022. The studies were selected by matching the keywords, identifying key information, and compiling the findings into a single comprehensive review to offer a new perspective and insight into persistent HPV infection and cervical cancer. The keywords that were used for searching included “human papillomavirus”, “persistent infection”, “cervical cancer”, “immune evasion”, and “lifestyle”.

RESULTS AND DISCUSSION

Persistent HPV infection and its progression into cervical cancer

Patients with persistent HR HPV infection are at risk of developing cervical cancer. Persistent HR HPV may gradually induce the development of precancerous cervical lesions that ultimately can become cervical cancer, and the course of this disease may take up to 5-15 years. The slow course of this disease precisely underscores the need for routine HPV screening before precancerous lesions arise [8,9]. Because of its established role in the incidence of cervical cancer, some studies have regarded persistent HPV as an intermediate phenotype. Once HR HPV infection becomes persistent, it is unclear whether or not it will inevitably progress into precancerous lesions or it can still be cleared or suppressed by the immune system. It was found that HPV viral genomes are present in as many as 95% of cervical cancer tissue biopsies [10]. Among the many HR HPV types already identified, HPV 16 and 18 are believed to be responsible for approximately 70% of all cervical cancers [11]. Gravitt et al. [12] recently proposed an expanded view of the natural history of HPV, which implied that the course of HPV infection is not linear across a woman’s lifespan. When a woman tests positive for HPV, this positive result can be interpreted as either a new infection or reinfection from current sexual activity, or a reactivation of latent infection from past sexual activity. On the other hand, when HPV is no longer detected, it can be interpreted as either complete clearance of the virus by the immune system or a decline in viral load below the test limit for detection [12].

It is believed that HPV infection requires wounding of the epithelium to expose the basement membrane and the basal layer. This then allows viral entry which leads to HPV infection of the basal keratinocytes. Considering that the cervix undergoes repetitive mechanical trauma in sexually active women, this makes sense that the cervix is prone to HPV infection. More specifically, cervical cancers associated with HPV infection predominantly arise in the transformation zone, the site that becomes a border between the squamous epithelium of the ectocervix and the columnar epithelium of the endocervix. The transformation zone may be more vulnerable as its basal cells are more accessible to HPV due to fewer overlying layers compared to in other locations [4,13].

Basal cells are targeted by the virus as they are the only actively dividing cells in the epithelium. HPV takes advantage of these cells’ mitotic activity and eventually establishes the basal layer as an ideal reservoir for viral replication at low levels. This stage of the infectious cycle is also known as maintenance replication [4,10,13]. The ability of HPV to organize its life cycle and gene expression in accordance with the epithelial cells is a crucial factor that contributes to the persistence of HPV infection.

Molecular mechanisms underlying persistent high-risk HPV infection

Compartmentalization of HPV replication and gene expression

HPV employs various molecular mechanisms to establish persistent infection in the cervix. As mentioned before, the process begins with maintenance replication, in which the virus maintains its genome copy number in the host cells by synchronizing its replication with the basal cells’ mitosis. HPV E6 and E7 genes, which
are oncoproteins, first act to disrupt the normal cell cycle regulation in order to enable long-term constant replication of HPV episomes in undifferentiated basal cells and also exponential viral replication in differentiated cells at a later stage. E6 and E7 proteins inhibit the tumor-suppressing function of p53 and retinoblastoma protein, respectively, and these combined events override cell cycle checkpoints that are necessary for deciding whether cell replication should progress or not. Then, HPV E1 and E2 proteins help initiate viral DNA replication. HPV utilizes a tethering mechanism in which the viral genome attaches itself to host mitotic chromosomes throughout all stages of mitosis including cytokinesis. These extrachromosomal HPV copies are then partitioned into the newly formed daughter cells along with the host DNA. The tethering mechanism, also colloquially referred to as hitchhiking, is facilitated by the HPV E2 protein as it binds to particular regions in the HPV episome through its C-terminal DNA-binding domain as well as interacts with host chromosomal proteins through its N-terminal domain. The E2 protein does not work alone; several tethering co-factors including bromodomain-containing protein 4 (BRD4), TopBP1, and ChlR1 have previously been proven to interact with E2 to establish viral tethering to host chromosomes [4,10,13]. Moreover, E2 also contributes to the activation and repression of the HPV gene promoter region itself. It helps keep the gene expression of E6 and E7 at bay, thus explaining the barely detectable levels of E6 and E7 in the undifferentiated basal cells. When there is a loss of E2 expression, oncogenic progression may ensue as there is an upregulation of E6 and E7 gene expression. Not only that, but genomic instability also arises and ultimately interferes with the HPV infection cycle [10]. Thus, it can be inferred that the HPV viral proteins help establish persistent infection by not only cooperating with one another but also regulating one another’s expression and function.

During this maintenance phase in the undifferentiated, proliferating compartment of the epithelium, the number of viral copies remains relatively constant, and viral gene expression remains minimal. When the infected basal keratinocyte moves up the differentiating compartment, however, there is a significant upregulation of viral DNA replication and gene expression. Under normal conditions, differentiating cells tend to downregulate the cellular replication process. However, as mentioned before, E6 and especially E7 force the differentiating keratinocytes to remain actively dividing [4,14]. During this phase, the number of viral copies can dramatically increase from 50-200 copies to thousands of copies per cell [15]. Following HPV genome upregulation, HPV L1 and L2 viral capsid proteins are produced to facilitate the arrangement and assembly of virions. The expression of L1 and L2 is restricted to the most differentiated epithelial layer as they are highly immunogenic. The virions are then released along with the desquamation of the fully differentiated keratinocytes without triggering the immune system as there is no virus-induced cell lysis and any inflammation [16]. In other words, by adjusting its life cycle to the host cell proliferation and differentiation cycle, the virus becomes invisible to the circulating immune cells [4,13]. This compartmentalization of HPV replication and gene expression according to specific epithelial layers is a crucial strategy that enables immune evasion and subsequently persistent infection. Inevitably, persistent HPV infection may allow gene mutations and cellular genetic changes to accumulate and become cancerous lesions after a long period of time. Ironically, cervical cancer cells lose their ability to properly differentiate and so prevent the productive amplification of the HPV genome and viral proteins [4,17].

**Suppression of innate and adaptive immune responses**

Compartmentalization of HPV replication and gene expression discussed above enables a passive immune evasion strategy, in which the immunogenic L1 and L2 capsid proteins are only expressed in the late phase of infection and shed off quickly from the outermost epithelial layer. This outermost layer has a low density of antigen presenting cells (APCs). Moreover, HPV makes use of a more aggressive immune evasion strategy through the binding of E6 and E7 oncoproteins to various cellular proteins that regulate the immune system [4,13,18]. Normal keratinocytes form an important part of the innate immune system by expressing pathogen recognition receptors (PRRs), one of which is the group of toll-like receptors (TLRs). Upon the presence of pathogens or cell injury, TLRs of the keratinocytes will be activated and lead to the production of type 1 interferons (i.e., IFN-α, IFN-β) and inflammatory cytokines (i.e., IL-1, IL-6, TNF-α, TGF-β) [13,19]. However, in HPV-infected keratinocytes, E6 and E7 directly bind to and suppress numerous regulators of the interferon response pathway, notably IRF-3 and IRF-1 [20]. Both IRF-3 and IRF-1 are important transcription factors that mediate expression of the IFN-α and IFN-β genes. In addition to a decrease in the production of inflammatory cytokines, HPV-infected keratinocytes also lead to upregulation of anti-inflammatory cytokines such as IL-10 [21]. Furthermore, HPV has also been shown to dampen the activity of natural killer (NK) cells which are another important part of the innate immune response [20].

Altogether, the impaired innate immune responses result in the failure to create a pro-inflammatory microenvironment, which leads to the diminished capacity of the adaptive immune system to detect infected cells [4,13,18]. Langerhans cells and dendritic cells act as important APCs that can infiltrate infected tissues and trigger pathogen clearance via cell-mediated immune mechanisms preceded by cross-presentation...
of antigens to effector T cells [18]. In HPV-infected tissues, however, infiltration of APCs is reduced through downregulation of the chemokine CCL20 and cell adhesion molecule E-cadherin [22,23]. The maturation and migratory capacity of APCs have also been shown to be suppressed in HPV persistent infection [18].

By evading both innate and adaptive immune systems, HPV can establish an immunosuppressive environment where infection persists and subsequently promotes tumorigenesis.

**Host lifestyle factors contributing to persistent HPV infection**

In addition to oncogenic virus types and genetic susceptibility, several host lifestyle factors increase not only the risk of HPV acquisition but also the persistence of HR HPV infection. These factors include alcohol consumption, smoking, use of contraceptive agents, coinfection with other STIs, multiple sexual partners, and possibly many others. Up to the present, there have been conflicting findings about whether one factor truly increases the risk of persistent HPV infection. This can be partly explained by limitations in conducting the research, in which most of the existing evidence is almost exclusively derived from longitudinal epidemiological studies such as cohort studies. This type of study is costly and time-consuming, which can explain why there is still a lack of concrete evidence. Another limitation may be due to the nature of the relationship itself, in which persistent HPV infection stems from multifactorial factors rather than influenced by only one or two causative factors.

A cohort study among 9230 Korean women by Oh et al. [24] is the first study to demonstrate the relationship between alcohol consumption and persistent HPV infection. Compared to non-drinkers, alcohol drinkers had a higher risk of still testing positive for HPV after 1 and 2 years of follow-up. In particular, women who usually drank 3 or more glasses of beer had a 3-fold increased risk of 2-year HR HPV persistence compared to non-drinkers. There may also be a linear positive relationship between drinking alcohol and HPV persistence, in which women with a drinking habit of more than 5 years had higher risks of 1-year and 2-year HR HPV persistence than women with a drinking habit of less than 5 years. Alcohol interferes with folate absorption in the colon, and folate deficiency has been found to increase the risk of cervical cancer through DNA hypomethylation and faulty DNA repair [25]. Moreover, alcohol promotes the formation of reactive oxygen species by activating cytochrome P450 2E1, which contributes to carcinogenesis [26]. Although poor folate intake has been established as a risk factor for the development of cervical cancer, the benefits of folic acid supplementation in hindering its development have so far been unclear [27].

In addition to drinking alcohol, smoking has also been suggested to correlate with persistent HPV infection. Schmeink et al. [28] discovered that the risk of persistent HPV infection was doubled in smokers compared to non-smokers. Utami et al. [29] also found that current smokers had higher HR HPV (HPV 16 and 18) DNA load compared to former smokers. However, there are other studies that did not find any significant association between smoking and the risk of persistent infection [30-32]. It is believed that smoking possesses direct carcinogenic and immunosuppressive effects in the cervix microenvironment by altering the number and activity of lymphocytes, APCs like Langerhans cells, and natural killer cells [29,33,34]. It is possible that the observed association between smoking and HPV persistence may be underestimated due to limitations in the study designs. As many prospective cohort studies rely on questionnaires to gather information regarding smoking behavior among women with HPV, response bias can arise as it can be hard to objectively determine certain factors such as including secondhand smoke, smoking frequency, and number of cigarettes smoked. Moreover, in several countries where smoking as well as alcohol consumption among women are perceived as culturally or socially inappropriate, it is also possible that the participants may not be entirely honest regarding their behavior [35].

Regarding sexual behavior, having multiple sexual partners is a possible risk factor for persistent HPV infection, particularly among women with 6-10 lifetime sexual partners. However, evidence for this association is still limited and ambivalent [28,31]. Multiple sexual partners also increase the risk of contracting other STIs, and coinfection of HPV with other STIs such as HIV, chlamydia, herpes, and gonorrhea may increase its persistence. This may be caused by impaired local immune response in the cervix, thus rendering it incapable of clearing HPV infection [2].

Contraceptive agents may also modify the risk of persistent HPV infection among women. Earlier studies already revealed that the use of oral contraceptives is associated with a higher risk of developing cervical cancer, and this risk increases proportionately with the duration of use [36-38]. However, the exact pathological mechanisms underlying this remain unclear. Hormonal contraceptive agents, which contain estrogen and/or progesterone, have been proposed to promote cervical carcinogenesis by upregulating the expression of HPV viral oncogenes, thereby promoting persistent infection and reduced clearance [39]. It is understood that E6 and E7 HPV oncogenes possess sex hormone responsive elements where estrogen as well as progesterone are able to bind to. Upon activation of these responsive elements, transcription for the oncogenes may be upregulated. This then leads to inhibition of the normal p53 tumor-suppressing function [40]. Moreover, women
who consume oral contraceptives are also more likely to develop cervical ectopy also known as ectropion, a condition in which the columnar epithelium extends outward onto the ectocervix under the influence of estrogen. Cervical ectopy may increase the exposure of squamocolumnar junction to HPV infection and carcinogens [32,41,42]. Nevertheless, while some studies found a direct significant association between oral contraceptive use and persistent HPV infection, others did not find any [28,32]. On the contrary, previous studies suggested that the use of copper intrauterine devices may lower the risk of persistent HPV infection and cervical cancer as they can augment the local immune response in the cervix [32]. Local immune response is believed to be crucial in maintaining viral clearance [43]. Not only copper intrauterine devices, but the levonorgestrel intrauterine system (LNG-IUS) also produces higher levels of inflammatory cytokines and chemokines including TNF-α and IFN-γ compared to non-users in the endocervical fluid [44]. Nevertheless, future investigations are required to elucidate this matter.

A summary of how several molecular and host lifestyle factors are associated with persistent HR HPV infection, which acts as the intermediate phenotype before cervical neoplasia, can be found in Figure 1.

**Limitations**

Although this study presents a comprehensive review of various molecular and host lifestyle risk factors that have been associated with persistent HPV infection and cervical cancer, it has several limitations. First, there were some difficulties in obtaining newer studies regarding the molecular mechanisms underlying persistent HPV infection and its progression into cervical neoplasia. In contrast, there has been an exponential increase in the number of studies that investigated the relationship between numerous lifestyle factors and persistent HPV infection. Therefore, we hope that studies regarding molecular mechanisms responsible for persistent HPV infection and progression can be updated in the future. Second, the generalisability of findings related to host lifestyle factors associated with persistent HPV infection acts as the intermediate phenotype before cervical neoplasia.

**Figure 1.** Persistent HPV infection acts as the intermediate phenotype before cervical neoplasia.
HPV infection may be of concern as the primary studies extracted were based in different countries under different circumstances. Future systematic reviews and meta-analyses focusing on these lifestyle factors should be able to address this concern.

CONCLUSIONS

Persistent HR HPV infection remains a major factor implicated in cervical carcinogenesis. The persistence of HPV infection is multifactorial as it is influenced by molecular factors and various host lifestyle factors. Understanding the molecular factors underlying persistent HPV infection may enable insight into potential therapeutic targets. On the other hand, identifying host lifestyle factors associated with persistent HPV infection can provide updated knowledge which is necessary for better prevention and education programs. Future research is needed to clarify the exact mechanisms underlying the persistence of HPV infection.

DECLARATIONS

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