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Mini-review

Associations between human herpesvirus-6, human papillomavirus and cervical cancer

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ABSTRACT

Cervical cancer (CxCa) is the second most common cancer among women globally. Human papillomavirus (HPV) infection is thought to be a necessary, but not sufficient, causal factor in CxCa development. Why some women are able to clear HPV infection with no adverse effects, whereas others develop cancer, remains unclear. HHV-6 has demonstrated transformative abilities and has been shown to be present in the genital tract. However, based on the current evidence, we cannot conclude that HHV-6 is a co-factor in HPV-associated carcinogenesis. Nonetheless, future research is warranted because of several crucial gaps in the literature.

1. Introduction

Cervical cancer (CxCa) is the second most common cancer among women globally [1–3]. In the US, there are over 12,000 new cases and about 4000 CxCa-related deaths per year [4]. Human papillomavirus (HPV) infection, which is the most common sexually transmitted infection in the US [5], is considered to be a necessary, but not sufficient, causal agent in CxCa development [6,7]. An estimated 27% of US women between the ages of 14 and 59 are HPV-positive, based on data from the National Health and Nutrition Examination Survey [5], and 70–75% of CxCa can be attributed to two high-risk HPV types, HPV 16 and 18 [5,8]. However, high-risk HPV types can often be detected in women with normal cytology, and the majority of these women will never develop CxCa [9,10].

The reason why some women are able to clear HPV infection with no adverse effects, while others develop CxCa is currently unknown. As a result, many studies have sought to identify potential cofactors or initiators (e.g., host characteristics, environmental factors, or co-infections) of CxCa oncogenesis in an attempt to explain this phenomenon [11,12]. Although co-infection with herpes simplex II virus has been implicated as a potential cofactor/initiator [11,13–17], other herpesviruses in the same family that maintain high levels of genetic homology have not been as well-studied [7,18]. Mounting evidence suggests that another member of the herpesvirus family, human herpesvirus-6 (HHV-6), may be an important player in cancer development [19–21]. HHV-6 is a beta herpesvirus with demonstrated transformative capabilities that infects about 90% of individuals before age three [19,22,23]. In recent years, this virus has garnered increasing attention as it has been implicated in the etiologies of brain cancers and lymphomas [19]. The purpose of this review is to summarize current knowledge on the role of HHV-6 as a potential cofactor in CxCa development.

2. HPV and cervical cancer

HPV 16 and HPV 18 were the first types of human papillomaviruses to be isolated directly from cervical cancer biospy tissues (in 1983 and 1984, respectively) [24–26]. Since then, a number of other HPV types have been identified. Currently, there are 120 different known HPV types [27–29], of which about a third can infect the anogenital region [30,31]. HPVs have been classified into low-risk (e.g., HPV 6, HPV 11, HPV 40) or high-risk (e.g., HPV 16, HPV 18, HPV 31) groups, depending on their likelihood of initiating carcinogenesis [27].

HPV has been established as the main causal factor in CxCa etiology [32,33]. In addition, it has been associated with lesions of the vulva, penis, anus, conjunctiva, and upper aero-digestive tract [34,35], and is strongly implicated in the etiology of head and neck cancers [36]. Two HPV oncoproteins, E6 and E7, can cause cell immortalization, both independently and synergistically [37], and are thus the most important players in HPV-associated carcinogenesis. Both these oncoproteins have been shown to be overexpressed in cervical carcinoma cells [30,38]. E6 interacts with and degrades both p53 and BAK, a key pro-apoptotic protein [39,40–

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43]. These interactions inhibit apoptosis and increase chromosomal instability. E6 is also involved in the activation of telomerase and in blocking the degradation of SRC-family kinases, possibly by stabilizing the activated forms of some of these kinases. However, E7, which binds to the pRB (retinoblastoma protein) and inhibits its function, is considered to be the major transforming protein [44]. The inhibition of pRB leads to a cascade of downstream effects which eventually results in the cell being able to bypass normal cell cycle check points. E7 also stimulates centriole amplification, which can lead to aneuploidy by causing multipolarity during metaphase and consequent unequal distribution of chromosomes [45].

Integration of HPV DNA into the host genome may potentially be an important event during neoplastic progression, as it allows for increased expression of E6 and E7 through interruption of the part of the E2 gene that produces a transcriptional regulatory protein (and, thus, normally inhibits the overexpression of these oncoproteins) [46]. Overexpression of E6 and E7 can, in turn, promote uncontrolled growth and malignant transformation. Therefore, the mechanisms by which HPV advances tumor development are well-characterized. However, it is unclear why not every woman infected with high-risk HPV goes on to develop CxCa.

Over 5 million women across the world are infected with HPV every year, and yet, the vast majority (~80%) will clear the infection within two years [30,47]. About 1600 out of every million (0.2%) HPV-positive women will eventually develop invasive CxCa [30,48,49]. Attempts to explain the discrepancy between the high prevalence of HPV infection and the frequency of CxCa cases have been minimally fruitful [50]. Suggested cofactors include host genetic polymorphisms, immunological or hormonal differences, lifestyle factors (e.g., diet, smoking, oral contraceptive use), and concomitant viral infections [18,32,51–56]. While other herpesviruses, such as herpes simplex II, Cytomegalovirus (CMV), and Epstein-Barr virus, have been studied as potential cofactors for several decades [7,12,13,18,30], exploring the role of HHV-6 in CxCa development is a newer, understudied area of research.

3. HHV-6 and carcinogenesis

HHV-6 is a ubiquitous virus that was first discovered in 1986 [57,58]. It has since been implicated in a number of conditions with varying degrees of evidence; these conditions include roseola, epilepsy, encephalitis, multiple sclerosis, chronic fatigue syndrome, glioma, and certain leukemias and lymphomas [19,59,60–71]. Although the oncogenicity of this virus has not yet been firmly established, a preponderance of evidence supports the hypothesis that HHV-6 has several attributes that can promote the carcinogenic process [19–21,72]. Previous studies have shown that HHV-6 open reading frame-1 (ORF-1) can bind to and inactivate p53, thus impeding tumor suppression [20,21]. HHV-6 U95 can bind to and dysregulate members of the NF-κB (nuclear factor kappa B) protein complex [73,74]. NF-κB proteins have a wide range of functions that may be relevant to cancer development, including regulation of inflammatory and apoptotic pathways [75]. HHV-6 may also be capable of influencing inflammation and anti-cancer immunosurveillance by altering cytokine production [76,77]. Specifically, HHV-6 infection has been associated with higher levels of proinflammatory cytokines, IL-6 and IL-8, in astrocyte cultures (although it may also be promoting the production of the anti-proliferative cytokine, TGF-β, in these cultures) [78].

In addition, unlike other herpesviruses, HHV-6 is able to integrate into the subtelomeric regions of the host's chromosomes, likely through homologous recombination [79–82]. A major mode of transmission for integrated HHV-6 is believed to be through germline inheritance [23,82]. Because inheriting CI-HHV-6 would result in there being one copy of the viral genome per cell of the host's body, there is usually a much higher viral load among those with chromosomally-integrated HHV-6 than in individuals with active HHV-6 infection, which is in contrast to HPV. Although the full spectrum of effects related to HHV-6 remains unknown, it is hypothesized that integration may contribute to telomeric elongation, which may help the cell avoid apoptosis [19]. Furthermore, through interruption of the subtelomeric sites of the host's chromosome, integration may present another indirect mechanism (telomeric instability) by which carcinogenesis may be instigated.

4. HHV-6 presence in the genital tract

Although HHV-6 variants (HHV-6A and HHV-6B) have been shown to be T-cell- and neurotropic, some evidence suggests they can also infect and replicate in human epithelial cells [14,58,83,84]. Furthermore, HHV-6 DNA has been detected in peripheral blood [85], cord blood [86,87], saliva [88–90], cystic fluid from glioma patients [78], and genital tract secretions from pregnant and non-pregnant women [86,91,92]. Various studies have reported low-level HHV-6 shedding from the genital tract in up to 25% of female participants [91–94]. Pregnant women generally seem to demonstrate higher prevalences of shedding [95,96], although this is not always the case, and there has been some implication that women who are more sexually active may account for some of the higher estimates [94]. Discrepancies in these estimates may also potentially be due to differences in methods used for viral detection. Interestingly, one of the higher estimates (19%) came from a study that had collected cervical, rather than vaginal, swabs from pregnant women in Japan [91].

Cumulatively, the majority of these studies indicate the presence of HHV-6 in the female genital tract. As most of these studies did not culture virus from the swabs, the presence versus absence of viral DNA may not provide definitive evidence of an active infection [94], especially because HHV-6 is capable of integrating its DNA into host chromosomes (which may be a viral mechanism for establishing long-term latency) [19]. Although one study detected HHV-6 specifically in acellular vaginal fluid [94], whether viral DNA detected from swabs originated from a cellular or acellular source remains largely unclear in most of these studies. It has been suggested that the HHV-6 DNA detected in a proportion of studies may be attributable to infected lymphocytes that were picked up along with cervical or vaginal cells by the swabs [12]. If this is the case, HHV-6 detection from genital swabs could simply be indicative of latent systemic infection with this ubiquitous virus. Alternatively, the presence of HHV-6 DNA in every cell of the body is also possible per inheritance of the chromosomally-integrated form of the virus [19,23]. However, HHV-6 has also been shown to be capable of infecting cervical carcinoma cell lines and HPV-immortalized genital cell lines *in vitro* [83,97,98], and a few studies have been able to localize the virus to epithelial tumor cells [14,99,100].

5. HHV-6 as a potential co-factor

A series of papers from the 1990s provided the rationale and biologic plausibility behind the idea that HHV-6 should be considered as a potential candidate in the search for co-factors of HPV-associated cervical carcinogenesis. In 1994, Chen et al. reported that HHV-6 co-infection increased expression of HPV E6 and E7 RNA *in vitro*, particularly in the C4-1 cervical cancer cell line [98]. They also found that HHV-6 molecular clones, pZVB-70 and pZVH-14, affected transcriptional regulation of HPV-18 by transactivating its long control region. To examine the impact of HHV-6 *in vivo*, nude mice were inoculated with infected and uninfected

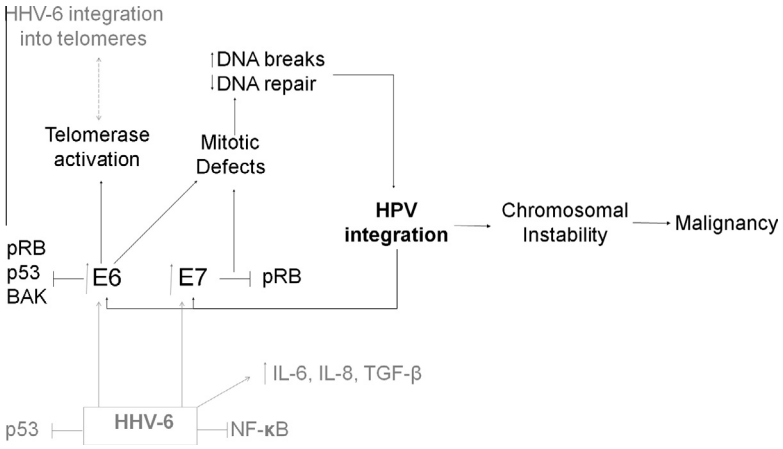

Fig. 1. Tentative model of potential interactions between HHV-6 and HPV in cervical carcinogenesis.

CxCa cells. Mice that were inoculated with HPV and HHV-6 co-infected CxCa cells developed tumors in half the time it took for control mice (inoculated with only HPV-infected CxCa cells) to develop tumors (3 versus 6 weeks).

A follow-up study by Chen et al. examined whether HHV-6 was present in surgical specimens or biopsy tissues of women with squamous cervical carcinoma (SCC), cervical intraepithelial neoplasia III (CIN III), cervicitis, or normal cervices (post-hysterectomy) [100]. They detected HHV-6 in 8% of SCC or CIN III patients, but none of the cervicitis or normal specimens were found to be positive for HHV-6. Another early study by Wang et al. found that 2 out of 8 (25%) paraffin-embedded SCC specimens were HHV-6 positive by PCR [58], whereas Yadav et al. used *in situ* hybridization to identify HHV-6 DNA in 8 out of 20 (40.0%) paraffin-embedded SCC specimens and 1 of 8 normal tissue samples (12.5%) [99]. Interestingly, HHV-6 was localized to the transformed cells of the CxCa specimens. By contrast, an individual with low-grade squamous epithelial lesions (LGSIL) was the only HHV-6-positive participant detected by nested PCR in a study of 109 total women with normal and abnormal (specifically, LGSIL, high-grade SIL, and invasive squamous carcinoma) cytological smears [96]. Although these early studies provided important *in vivo* data on the prevalence of HHV-6 and HPV co-infection in women with and without CxCa, inferences that can be drawn from these studies are limited by the small numbers of women in each subgroup.

More recently, a number of studies have also provided somewhat mixed results on the role of HHV-6 in HPV-associated cervical oncogenesis. In a study of 388 women, Chan et al. found that about 3.6% of cervical samples were positive for HHV-6 [18]. Proportions of HHV-6 positivity were similar between normal and abnormal samples. Only 2 of 24 invasive CxCa specimens were HHV-6 positive, and there were no differences in distribution of HHV-6 positivity by high-risk HPV type distributions. The odds ratio for the association between HHV-6/HPV co-infection and risk for a high-grade lesion was 0.98 (95% CI: 0.12–6.31), although this estimate was severely underpowered. Similarly, Tran-Thanh et al. found no significant association between HHV-6 and HSIL, controlling for socioeconomic status, age, and HPV presence (OR: 6.4; 95% CI: 0.3–128.5) [95]. Although their regression model was also underpowered, the pattern of HHV-6 positivity among their HPV-infected participants also did not suggest a correlation between HHV-6 positivity and higher-grade lesions (1.5% HHV-6 positive among LSIL, 6.2% among HSIL, and 2.7% among SCC). Furthermore, although Lanham et al. reported a higher prevalence of HHV-6 positivity among female patients of a colposcopy clinic, their findings were more suggestive of an association between high-grade CIN and HHV-7, rather than HHV-6 [101].

By contrast, in a cross-sectional study of 208 Italian women, HHV-6 was detected among 25% of cervical samples, and its prevalence was found to be significantly higher among HPV-infected women with HSIL, compared to HPV-infected normal women (41% versus 0%, respectively) [7]. Additionally, this study found high HHV-6 viral loads among some women with severe disease, but generally, did not observe higher viral loads with increasing disease severity. Nonetheless, as high HHV-6 viral loads may be indicative of chromosomally-integrated HHV-6 [102,103], this finding may warrant further investigation. The authors concluded that HHV-6 may potentially play a role as a cofactor in CxCa development [7].

To our knowledge, HHV-6 integration status has not yet been assessed in studies evaluating the relationship between HPV and HHV-6 co-infections in CxCa development. However, a more recent study did examine HPV-16 integration status in relation to HHV-6 prevalence among 60 women with LSIL, HSIL, or CaCx [30]. There was no correlation between HHV-6 presence and HPV integration status, or between HHV-6 positivity and severity of the lesion. However, additional studies with larger sample sizes that take into account the integration status of both HPV and HHV-6 are needed to corroborate these results.

6. Conclusions

Although it seems that most evidence to date suggests that HHV-6 is more likely to be acting as a "bystander" rather than a cofactor in HPV-associated oncogenesis [18], these studies do not yet provide a consensus on the role of HHV-6 in CxCa development. It is clear that HHV-6 detection in the genital tract is relatively rare, with most estimates that are based on larger study populations falling under 15% [95]. Its rarity does not, *per se*, eliminate HHV-6 as a possible etiologic factor. If HHV-6 is a sufficient, rather than necessary, causal factor in CxCa development, its presence may only be detectable in a small subset of CxCa patients. The sample sizes of most studies to date have not been large enough to maintain adequate statistical power for the detection of small differences in HHV-6 prevalence between groups of LSIL, HSIL, and SCC patients.

Additionally, several crucial gaps in knowledge need to be filled by future studies before HHV-6 can decisively be evaluated as a potential cofactor. Among CxCa patients who do have HHV-6/HPV co-infections, few studies have attempted to determine whether both viruses are present within the same cells or whether they are present within different cells of the same cervical specimen [58,98]. If the two viruses are not located within the same cell, it is less likely

that HHV-6 would be able to contribute to HPV E6/E7 overexpression *in vivo*, as it was found to do *in vitro*. However, lack of localization to the same cell would not necessarily exclude HHV-6 as a potential initiator or cofactor, as this virus could also influence HPV-associated cervical carcinogenesis more indirectly, possibly through its plethora of other effects, including the ones depicted in Fig. 1. For example, HHV-6 and HPV both inhibit p53 [20,43]. Additionally, HPV E6 activates telomerase [42], whereas HHV-6 may potentially contribute to telomeric elongation or instability by integrating into the host genome [79,82]. Such joint effects may increase the likelihood that HHV-6 and HPV could act synergistically in carcinogenesis, but based on what is currently known, such statements cannot be made definitively.

Another gap in knowledge that remains to be addressed is whether the detection of HHV-6 DNA in cervical specimens may be the result of a latent systemic infection, germline inheritance of chromosomally-integrated HHV-6, sexually-transmitted viral infection, contamination of the cervical specimen by infected lymphocytes, or a yet unidentified HHV-6 tropism toward dysplastic cells [12,95]. Studies that consider HHV-6 integration status and evaluate the presence and viral load of HHV-6 in blood compared to cervical specimens could lend insight into whether the infection is systemic, inherited, or limited to the cervix. Such studies could also potentially help assess whether a "hit and run" type strategy, which has been suggested as an initiating mechanism for other herpesviruses such as CMV [18,30,101], could be at play.

HHV-6 is an interesting candidate to consider as a possible cofactor in CxCa development because it has been shown to be capable of transforming keratinocytes and has been detected in CxCa specimens [72,97,100,104,105]. The genome of this ubiquitous virus contains at least one putative oncogene, and previous research has implied that HHV-6 may be involved in cancer susceptibility, particularly with regard to leukemias, lymphomas, and brain tumors [19,59,66,70,71]. However, larger, more comprehensive epidemiologic studies are necessary before conclusions can be drawn about whether HHV-6 is any more likely to be a co-factor in HPV-associated cervical oncogenesis than other herpesviruses or environmental exposures.

Conflict of interest statement

No conflicts of interest to declare.

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