

HPV and skin carcinogenesis

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ABSTRACT

Epidemiological and biological studies provide several lines of evidence for the involvement of cutaneous beta human papillomaviruses (HPVs), together with ultraviolet (UV) radiation, in the development of cutaneous squamous cell carcinoma. These viruses appear to act with a hit-and-run mechanism, being necessary at an early stage of carcinogenesis and being dispensable for the maintenance of the malignant phenotype. Studies in experimental models show that beta HPVs, mainly via the E6 and E7 oncoproteins, are able to promote proliferation and to circumvent cellular stresses induced by UV radiation. These findings support a model of skin carcinogenesis in which beta HPV-infected keratinocytes remain alive despite the accumulation of UV-induced DNA mutations. In this manner, these cells become highly susceptible to progression towards malignancy. Thus, UV radiation is the main driver of skin cancer development, while beta HPVs act as facilitators of the accumulation of UV-induced DNA mutations.

1. Mucosal high-risk and beta HPV types appear to promote carcinogenesis by distinct mechanisms

Mucosal high-risk (HR) human papillomavirus (HPV) types have been clearly associated with different types of anogenital cancers as well as a subgroup of oropharyngeal cancers. In addition, other HPV types, phylogenetically classified as genus beta, appear to be involved in human carcinogenesis [1]. Findings support their role in the development of cutaneous squamous cell carcinoma (cSCC), together with ultraviolet (UV) radiation.

Because the first beta HPV types, 5 and 8, were isolated from the skin of patients with epidermodysplasia verruciformis (EV), who are highly susceptible to beta HPV infections and UV-induced cSCC [2], the possible link between beta HPV types and skin carcinogenesis was initially well accepted by the scientific community. The fact that organ transplant recipients, due to the status of their immune systems, are at high risk of beta HPV infection and of cSCC development provides indications that beta HPV infection plays a role in cSCC development also in non-EV individuals [3,4]. Finally, with the development of high-sensitivity PCR-based assays, it became clear that beta HPV types are abundantly present in the skin, raising the question of whether they could also promote cSCC in the general population (reviewed in Ref. [1]). However, case-control studies in immunocompromised and immunocompetent individuals did not reproduce the same scenario observed in cervical cancer, which is associated with persistent mucosal HR HPV infections. In cervical cancer, HR HPV DNA is detected in all cancer cells. This is explained by the fact that persistent mucosal HR

HPV infections, via the expression of the E6 and E7 oncogenes, promote chromosomal instabilities, facilitating cellular transformation. However, after cancer development, the presence of E6 and E7 in the cancer cells continues to be essential for the maintenance of the transformed phenotype (reviewed in Ref. [5]). In contrast, beta HPV DNA is weakly detected in cSCC. In addition, viral load appears to decrease with the severity of the skin lesions, being higher in the pre-malignant lesions, actinic keratosis [6]. Thus in case-control studies, the odds ratio for beta HPV DNA positivity and risk of cSCC was much less than the odds ratio for mucosal HR HPV DNA and risk of cervical cancer (reviewed in Ref. [7]). The ubiquity of beta HPV and its high abundance in the skin of healthy individuals is also responsible for the low odds ratios obtained in these case-control studies. The use of multiple biomarkers for past or present beta HPV infections, e.g. seroreactivity, viral DNA in eyebrow hairs, and viral DNA in skin swabs, was found to be essential to observe a weak, but significant, association between viral infection and history of cSCC [1,8–14]. A possible explanation for the different scenarios in cervical cancers and cSCC is that in the skin beta HPV types act only as co-factors together with UV radiation. They may play a role at the beginning of the tumorigenic process, facilitating the accumulation of UV-induced DNA mutations. Once crucial cellular genes, e.g. TP53, are mutated, the infected cell can progress towards transformation, and the expression of the viral oncogenes, E6 and E7, is dispensable for the maintenance of the malignant phenotype.

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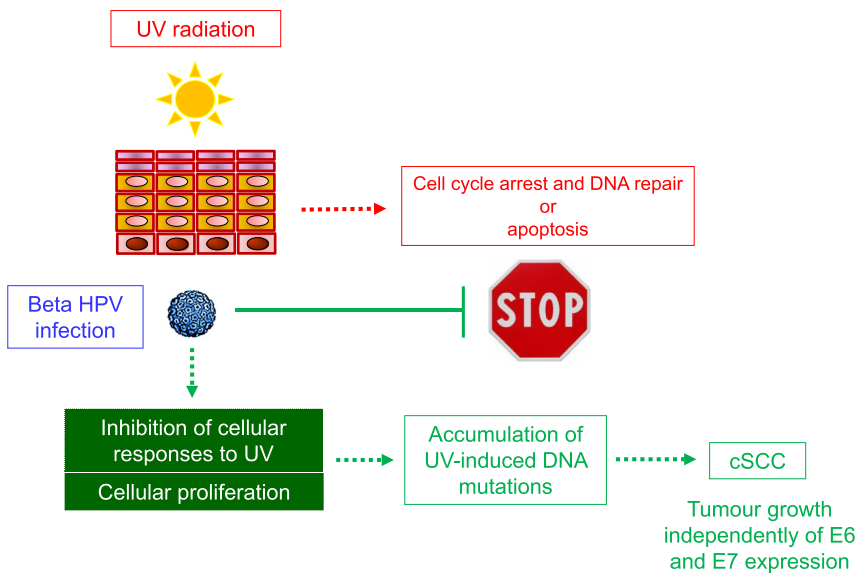


Fig. 1. Working model for cooperation between beta HPV types and UV radiation in promoting cSCC. Under normal conditions, UV irradiation of the skin induces DNA mutations in keratinocytes in the basal layer, with consequent (i) cell cycle arrest and repair of DNA mutations or (ii) apoptosis, if the DNA damage is unreparable. Upon beta HPV infection, E6 and E7 expression inhibits the cellular response to UV-induced stress. As a consequence, DNA-damaged cells continue to proliferate, with a high risk of evolving into cancer cells. After inactivation of tumour suppressor genes or activation of cellular oncogenes by DNA mutations, the expression of the viral genes becomes dispensable.

2. Biological properties of E6 and E7 from beta HPV types

2.1. *In vitro* models

Biological findings in *in vitro* experimental models, e.g. cell lines and/or primary human keratinocytes, demonstrated the transforming properties of E6 and E7 of some beta HPV types (reviewed in Ref. [15]). For instance, as previously shown for mucosal HR HPV types, E6 and E7 from beta-2 HPV38 immortalize primary human keratinocytes [16], an event associated with inactivation of the tumour suppressors p53 and retinoblastoma (pRb). Accordingly, it has been shown that HPV38 E6 and E7 alter the properties of both cellular proteins [16,17]. Due to this activity, several studies were focused on HPV38. However, the transforming properties of E6 and E7 in primary human keratinocytes appear not to be conserved in all beta HPV types. For instance, it has been reported that E6 and E7 from beta HPV14, 22, 23, 24, and 36 were only able to extend the life span of primary keratinocytes [18].

Many more studies provided additional findings that highlighted the ability of some beta HPV types, e.g. HPV5, 8, and 38, to deregulate fundamental events that are normally lost in cancer cells, such as cell cycle, DNA repair, apoptosis, and activation of immune-related pathways [2,15]. Importantly, beta HPV oncoproteins are able to target proteins/pathways that are well known to be deregulated during the development of cSCC, such as p53 and Notch. In skin keratinocytes, Notch signalling has tumour suppressor functions and plays a key role in cell–cell communication as well as in the switch between proliferation and differentiation of keratinocytes (reviewed in Ref. [19]). E6 from several beta HPV types interacts with Mastermind-like 1 (MAML1) [20–22], a core component of the complex that regulates the canonical Notch signalling pathway [23]. Interaction of MAML1 with the viral protein resulted in loss of the ability of Notch to activate transcription.

2.2. HPV transgenic mouse models

Studies in animal models fully supported the epidemiological findings, demonstrating the ability of the viral oncoproteins to synergize with UV radiation in promoting cSCC [24–26]. Due to the fact that HPV38 E6 and E7 were able to transform primary human keratinocytes, a transgenic (Tg) mouse model for this beta HPV was developed, which expresses the viral genes in the skin under the control of a keratinocyte-specific promoter. These Tg animals showed elevated susceptibility to skin carcinogenesis upon long-term UV exposure, which closely correlates with the accumulation of DNA mutations with the typical UV

signature [26]. In addition, analyses of the mutated genes in the skin lesions of UV-irradiated HPV38 E6/E7 Tg animals revealed a similarity to the mutated gene pattern in human cSCC, with the highest mutation rate in p53 and Notch genes. In the same model, silencing the expression of beta HPV38 E6/E7 genes before the development of any UV-induced skin lesions prevented development of cSCC after long-term UV irradiation. In contrast, loss of the viral genes after lesion development did not affect tumour growth [26].

2.3. PV animal models

The scenario characterized in HPV Tg mice is in agreement with findings obtained in mouse models that have an additional advantage of being naturally infected by murine PVs, e.g. *Mus musculus* PV 1 (MmuPV1) and *Mastomys natalensis* PV (MnPV) [25]. Studies in *in vitro* models highlighted functional similarities between E6 from beta-1 HPV8 and MmuPV1 [27,28].

In addition, MnPV infection of the African multimammate mouse *Mastomys coucha* significantly increases the susceptibility to UV-mediated skin carcinogenesis. Interestingly, two different types of cSCC were detected after infection and long-term UV radiation: (i) well-differentiated keratinizing SCCs with high viral loads and viral gene transcriptional activity, and (ii) poorly differentiated non-keratinizing SCCs almost lacking in MnPV DNA. The loss of viral DNA in the latter type of cSCC resembles the situation in humans, in which beta HPV viral load is very low after development of cSCC [25].

3. Conclusions

Epidemiological and biological data support the model that beta HPV types act only at an initial stage of carcinogenesis by directly targeting important cellular proteins, such as pRb, p53, and Notch. A plausible hypothesis is that beta HPV types, in order to efficiently complete their life cycle in the skin, have developed strategies to maintain infected cells in a proliferative status, even if they have been damaged by UV radiation. By doing so, they strongly increase the probability of infected cells progressing towards malignancy. Due to the irreversible UV-induced DNA damage, the expression of the viral genes may become dispensable for the maintenance of cSCC [26] (Fig. 1).

A future challenge for HPV research is to evaluate whether a similar synergistic model, as observed for UV radiation and beta HPV types in the skin, may also exist for other HPV types and environmental factors at other anatomical sites. For instance, can well-characterized oncogenic

HPV types, such as the mucosal HR HPV types, synergize with oral carcinogens and act with a hit-and-run mechanism in development of a subset of head and neck cancers? Although this hypothesis is highly speculative and no findings are available in humans, studies in animal models provide evidence that mucosal HR and beta HPV types strongly cooperate with a carcinogen that mimics tobacco-induced DNA mutations [29,30].

Other future studies could be focused on the large group of gamma HPV types, of which very little is known about their biological properties and potential association with diseases.

The establishment of new associations between HPV infections and human cancers will clearly have a positive impact on the development of new preventive strategies for these diseases.

Conflicts of interest

I have no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.pvr.2019.04.003>.

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