Chapter 8: Human Papillomavirus and Skin Cancer

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A high prevalence of human papillomavirus (HPV) DNA, particularly in squamous cell skin carcinoma of immunosuppressed but also of immunocompetent patients, has renewed great interest in a possible etiologic role of HPV in nonmelanoma skin cancer. It is difficult, however, to interpret these findings against a background of low-level infections with multiple HPV types from supergroup B (HPV4-related and epidermodysplasia verruciformis [EV] HPV), probably acquired by everyone early in and throughout life. Thus far, no high-risk HPV types have been identified. Because of the low copy numbers of HPV DNA in skin cancers, probably not every tumor cell contains a viral genome, which is compatible with cutaneous HPV being possibly important for tumor initiation and progression, but not for maintenance of the malignant phenotype. The question with regard to high-risk types should, therefore, be readdressed in case–control studies on the basis of serology, which can reveal viral activities over years. The viruses lingering in all people are apparently activated by sunlight (UV) exposure, by immunosuppression, and by hyperproliferation of the epithelium (psoriasis) and/or in the specific genetic background of the host (EV). It is intriguing that most of these factors are established risk factors in skin carcinogenesis. The weak transforming activity of cutaneous HPV *in vitro* compared with the transforming activity of genital HPV may explain the need for activators and synergistic factors. The antiapoptotic activities of E6 proteins of cutaneous HPV could be relevant to oncogenesis in the interplay with UV exposure. Prospective studies should determine the kinetics of HPV activation relative to tumor development. [J Natl Cancer Inst Monogr 2003;31: 52–6]

State of the Art

Nonmelanoma skin cancer (NMSC) is the most common cancer among Caucasians. It outnumbers the total of all other cancers, and it is increasing in incidence in many areas. The two main histologic types of NMSC are squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). BCCs are about four times more common than SCCs in Caucasians, whereas SCCs are more frequent in blacks. The main risk factors for NMSC are exposure to UV radiation, fair skin, and the immune status of the host. Immunosuppressed organ-transplant recipients have an up to 100-fold increased risk of SCC and a 10-fold increased risk of BCC, resulting in a reversal of the normal ratio of SCC to BCC *(1,2).*

NMSC is rarely fatal, with cure rates approaching 99%. However, its impact on public health is nevertheless considerable. The annual cost of treating NMSC in the United States exceeds $500 million *(1).*

The involvement of HPV in human skin cancer has been demonstrated first in patients with the rare hereditary disease epidermodysplasia verruciformis (EV). This disease is characterized by disseminated, persistent, flat warts and macular lesions (more or less scaly, red, brown, or achromic plaques at skin level, with irregular outlines, sometimes forming large patches; and a histology that reveals large cells with pale-stained cytoplasm in the spinous and granular layers of the epidermis). The warts and macules arise during childhood, and there is a high risk of developing SCC later in life. The SCCs are most frequently localized in sun-exposed areas of the skin. The HPV types found in the macular lesions are commonly referred to as EV-HPV types, including, among others, HPV types 5, 8, 9, 12, 14, 15, 17, and 19–25. HPV DNA usually persists extrachromosomally in high copy numbers (100–300 per diploid host genome) in nearly all SCCs and is actively transcribed. According to early *in situ* hybridization experiments in EV patients, high copy numbers can be partially traced back to a few carcinoma cells in the tumor, supporting vegetative viral DNA replication. In contrast to multiple HPV types in benign lesions, mostly HPV type 5 or 8 and sometimes HPV types 14, 17, 20, or 47 are found in SCC and are regarded as high-risk types. In two patients, HPV5 was detected in the primary tumor and in the metastasis, which may point to an ongoing role for HPV5 in the cancer [reviewed in *(3,4)*].

Highly sensitive detection techniques, such as nested polymerase chain reaction (PCR), identified HPV DNA in a substantial proportion (30%–50%) of NMSCs in an immunocompetent population. In immunosuppressed patients, the HPV prevalence is generally higher in SCC than in BCC. Up to 90% of these SCCs contain viral DNA. A diverse spectrum of HPV types could be detected, mostly belonging to the subgenus of EV-associated HPVs. Multiple infections of individual tumors were frequently noted in immunosuppressed patients. No single HPV types predominate in skin cancers of non-EV patients and, so far, there is no evidence of high-risk types analogous to EV or cervical cancer [reviewed in *(2)* and *(5–8)*].

The amount of HPV DNA seems to be higher in skin tumors of immunosuppressed patients than in those of the general population. Viral load determinations in the author's laboratory revealed only one HPV DNA copy per 20–5000 cells in the tumors, indicating that probably not every tumor cell harbors an HPV genome (Weissenborn S, Wieland U, Pfister H: unpublished data). This finding explains the need for highly sensitive detection techniques to demonstrate HPV DNA. The frequently used, subgenus-specific PCRs differ in sensitivity with regard to individual types by more than one order of magnitude *(9,10).* In view of the low levels of viral DNA close to the detection limit, different sensitivities will affect both the rate of HPV DNA detection and the spectrum of HPV types identified. Adding HPV types 5- and 8-specific nested PCR to the EV-specific PCR with degenerate primers, for example, raised the percentage of HPV-positive SCCs by approximately one-third and showed that

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these high-risk types of EV patients are more prevalent than was anticipated earlier *(11)*.

SCCs of the distal digit and periungual skin in contrast to other skin areas are greatly associated with genital oncogenic HPV types, especially HPV16 *(12)*. This finding suggests a genital–digital mode of transmission. HPV16 E6 RNA transcripts have been detected in these cancers, suggesting that HPV has a role in their pathogenesis *(13)*.

HPV DNA is detected frequently in healthy skin samples or in plucked hairs from individuals with and without skin cancer *(7,8,14,15)*. When a few such samples were analyzed per individual, up to 50% of the immunocompetent volunteers and nearly all immunosuppressed patients turned out to be HPV DNA positive *(15)*. A study *(16)* of skin swabs from five different sites per individual showed 80% of the healthy control subjects to be HPV positive. HPV was detected more frequently on the forehead than on the arms or thighs, which may reflect a higher exposure of the forehead, but more likely it may be a result of local photoimmunosuppression due to sunlight exposure, causing a higher production of HPV. Within one individual, certain HPV types were detected on several skin sites. In one healthy volunteer, the detected HPV type showed up over a 5-month period at different sites, suggesting a persistent infection *(16)*.

With the use of various PCR protocols, many new partial HPV DNA sequences (350–430 nucleotides from the L1 gene) have been identified. On the phylogenetic tree of HPV, they could all be assigned to both subgroups of supergroup B (cutaneous/EV-HPV), namely, B1 or β or EV-HPV (HPV5 and related types) and B2 or γ (HPV types 4, 48, 50, 60, and 65). At the moment, it is not possible to say how many new HPV types are represented by the partial sequences, since amplification products of different PCRs are derived from different regions of the genome. However, judging from defined PCR systems, more than 25 new types could be added to B2/γ and more than 35 to B1/β.

HPV DNA seems to be acquired very early in life. Antonsson A, Karanfilovska S, Hasson BG (personal communication) reported at the 19th International Papillomavirus Conference that forehead swabs from 37% of 1-month-old children and 50% of 1-year-old children were HPV DNA positive. A virus on the skin surface of the mother and of close-contact persons appeared to be the most likely source of the infection. Because there are usually no apparent HPV-induced lesions in adults, one has to speculate that low levels of virus particles are produced within very inconspicuous lesions or even on the basis of clinically completely inapparent infections. The detection of viral DNA in skin swabs can reflect surface contamination and does not necessarily imply an established infection, unless supported by a demonstration of viral gene expression in the epithelium or by seroconversion in the host. Although the percentage of established infections is unclear at the moment, we can expect a high prevalence because antibodies against the bacterially expressed HPV8 capsid protein L1 could be demonstrated by western blot analysis in more than 15% of the sera from children up to 6 years of age *(17)*. Antibodies against HPV8 virus-like particles (VLPs) were detected by enzyme-linked immunosorbent assay (ELISA) in about 10% of the sera from children up to 10 years of age *(18)*. In the Western blot and the ELISA, two of four and four of seven sera from the patients with EV tested positive, respectively.

Probably everyone is infected throughout life with multiple HPV4-related and EV-associated HPV types. It is, therefore, not surprising that HPV DNA was detected in similar proportions of hair samples plucked from individuals with and without skin cancer. Only a slightly positive, nonsignificant association has been noted between EV-HPV DNA in plucked hairs and SCC (odds ratio [OR] = 2.00; 95% confidence interval [CI] = 0.50 to 8.0) *(19)*. However, a comparison of HPV DNA prevalence in single normal skin samples of control subjects and in normal skin from cancer patients with that in cancer tissue showed clear differences: 15% and 22% versus 86% *(20)*. Similar relationships were observed with the less sensitive Southern blot hybridization and type-specific PCR: positivity rates of 43% and 22% for SCCs of renal transplant recipients and immunocompetent patients versus 16% and 8% for uninvolved skin *(21)*. Negative results with the control tissue certainly do not exclude infections at other normal skin sites, but they may reflect the likelihood of identifying HPV DNA in a limited skin area and may strengthen to some degree the association of HPV with SCC. In contrast, there was hardly any difference between the positivity rates of BCC and paired healthy skin (32% versus 26%) *(8)*.

A few preliminary seroepidemiologic studies *(17, 18 22)* have been used to link HPV infections and cutaneous tumors. In one study *(22),* the presence of antibodies against HPV8 VLPs was associated with the development of large numbers of actinic keratoses after adjusting for sex, age, eye and hair color, and sun exposure with an OR of 2.3 (95% CI = 1.0 to 5.3) and with the development of SCC with an OR of 3.1 (95% CI = 0.74 to 13.3). No association was observed with BCC (OR = 0.73; 95% CI = 0.23 to 2.4).

Attempts to identify mechanisms by which cutaneous HPV can contribute to NMSC development revealed a rather weak transforming potential *in vitro.* The E6 gene of EV-HPV seems to be the dominant oncogene in rodent cells, leading to morphologic transformation and anchorage-independent growth but not to tumorigenicity in nude mice. The E7 genes of HPV types 5 and 8 were able to transform rodent cells in collaboration with an activated H-ras gene. In contrast to the E6 proteins of HPV types 16 and 18, the E6 proteins of EV-HPV do not bind the cellular p53 protein and do not promote its proteolytic degradation. Furthermore, the E7 proteins of EV-HPV interact poorly with the retinoblastoma protein pRb [reviewed in *(4)*]. So far, it has not been possible to immortalize primary human foreskin keratinocytes with DNA of HPV type 5 or 8. Retroviral transduction of E6–7 only slightly altered keratinocyte differentiation in organotypic culture *(23)*.

An important contribution to NMSC development may be expected from the inhibition of apoptosis by E6 proteins of cutaneous HPV. Elimination of cells with heavy damage to DNA after exposure to the UVB component of sunlight is essential, since somatic mutations due to error-prone repair or oxidative damage may eventually lead to cancer. UVB radiation exposure of the skin leads to increased levels of the proapoptotic cellular Bak protein independent of p53 function. The E6 proteins of HPV types 5, 10, and 77, in turn, have been shown to target Bak for proteolytic degradation and to inhibit effectively UVB-induced apoptosis *(24,25)*.

High- and low-risk cutaneous HPV types may be distinguished by the biochemical properties of their oncoproteins (e.g., efficiency of targeting Bak) and/or the levels of oncogene

expression. The transcriptional E6 gene promoter of EV-HPV appears to be rather weak in human keratinocytes *(26,27).* With regard to a possible interplay between HPV and UV radiation exposure, it is interesting to note that a (UV-inducible) p53-responsive element has been found in HPV77 (isolated from an SCC of a transplant recipient), mediating activation of viral transcription *(28).* Promoter stimulation by UV irradiation was also described for HPV20 and some related EV-HPVs *(29).* This could imply particularly high levels of E6 when inhibition of Bak and apoptosis is the most deleterious because of heavy UV radiation damage.

Psoriasis and HPV

EV-HPV DNA, especially of types 5, 36, and 38, can be detected in up to 90% of the lesions and skin scrapings from patients with psoriasis *(30,31).* Virus-load determination revealed one copy of viral DNA per one to 20 000 cells (on average, about 1000 cells) (Table 1). The significantly higher prevalence of antibodies against HPV types 5 and 8 VLPs in patients with psoriasis compared with healthy donors (25%/4% and 43%/7%, respectively) also points to increased levels of productive infection *(18,30).* Photochemotherapy for psoriasis consists of administration of psoralen, followed by UVA radiation (PUVA). The overall prevalence of HPV DNA does not differ between PUVA-treated patients and untreated patients, but multiple HPV types are detected more frequently after photochemotherapy. One study *(31)* provided some indication that HPV types 5 and 8 are detected more frequently in patients exposed to high doses of PUVA (>2000 J/cm^2).

Extensive keratinocyte proliferation in psoriasis will support increased replication of latently persisting EV-HPV and, consequently, will enhance the detection rate of viral DNA and stimulate antibody responses. Antibodies to HPV5 are also generated under other conditions associated with rapid keratinocyte growth, such as epidermal repair in patients with extensive burns or with autoimmune bullous diseases *(32).* The relationship between HPV and these diseases—passenger or driver—is unclear. It has been speculated that HPV proteins act as "autoantigens" involved in the autoimmune pathogenesis of psoriasis *(33).* On the other hand, HPV may support epidermal regeneration and may even be beneficial to the host in epidermal repair *(32).*

It is generally accepted that the risk of skin SCC is increased in patients undergoing long-term PUVA therapy. There is some evidence that the introduction of methotrexate in patients undergoing long-term PUVA treatment further enhances the risk of SCC development and even progression to metastatic SCC *(34).* EV-HPV DNA, including HPV5, has been detected in 75% of SCC *(35).*

The EV Paradigm

The ubiquity of EV-HPV, originally believed to be associated exclusively with tumors of EV patients, raises the question about the specific properties of these patients. Only in EV patients and exceptionally immunosuppressed patients do these infections result in pathognomonic pityriasis versicolor-like lesions or red plaques with characteristic cytopathic effects linked to highly active viral replication in differentiating keratinocytes. SCCs arise 10–30 years after the first appearance of the benign, macular lesions in 30%–60% of the Caucasian and Japanese EV patients *(3).*

Table 1. Viral load in psoriasis skin lesions*

1 HPV copy/total No. of cells	HPV5 (n = 19)	HPV20 (n = 11)	HPV36 (n = 41)
Range	0.6–218 000	0.6–10 000	0.7–50 000
Average	16 800	1800	5000
Average without outliers	530	100	1050

*Weissenborn S, Wieland U, Pfister H: unpublished data. HPV = human papillomavirus.

Genetic linkage analyses on EV consanguineous families have recently identified susceptibility loci on chromosomes 17q25 (EV1) and 2p21–24 (EV2) *(36).* It is of particular interest that EV1 overlaps with a major susceptibility locus for familial psoriasis *(37).* One may speculate that proteins encoded at these sites are involved in the control of EV-HPV infection and that defects in these genes abolish or relax the usually efficient host restriction of EV-HPV replication and/or gene expression.

Genital HPV, at least HPV types 16 and 18 and related types, may be less well controlled in general. Be that as it may, it is tempting to speculate that susceptibility loci, which are crucial for the natural course of persistent infection, also exist for non-EV-HPV.

Speculations on the Role of HPV in Skin Carcinogenesis

When contrasting EV, where a role of HPV in skin carcinogenesis is widely accepted (*see* above "State of the Art") against NMSC in general, one becomes concerned about finding no high-risk HPV types within supergroup B specifically associated with malignant tumors and about the low copy number of HPV DNA in skin cancers, suggesting that only a minority of the tumor cells contain HPV DNA. One has to realize, however, that, even in EV–SCC, large amounts of viral DNA are confined to only a few cancer cells. The majority of tumor cells could not be shown to contain HPV DNA by *in situ* hybridization, and no HPV DNA-positive cell line could be established from an EV–SCC, so far. Thus, there is no proof that HPV DNA and viral activities are necessary for the maintenance of the malignant phenotype. The data, particularly on NMSCs in general, are more compatible with cutaneous HPV being possibly important for tumor initiation and progression—in other words, with a "hit-and-run" mechanism of carcinogenesis. There is reasonably good evidence for a hit-and-run mechanism in bovine papillomavirus type 4 (BPV4) infections of the alimentary canal in cattle and esophageal cancer. Carcinomas grow in close association with BPV4-induced papillomas, and transformation of papillomas to carcinomas has been observed, but no viral DNA is usually detectable in the cancer tissue *(38).* Malignant conversion occurs in animals feeding on bracken fern, which contains mutagenic and immunosuppressing chemicals. Experimental reproduction of the papilloma–carcinoma complex suggested that immunosuppression is important for the persistence and the spread of BPV4-induced papillomas and that fern mutagens are responsible for neoplastic conversion *(39).* However, no malignancies of the alimentary tract were detected in animals fed bracken fern but not inoculated with BPV4.

There is an urgent need for longitudinal studies of the natural history of cutaneous HPV infections to determine the time of persistence and the activity of individual HPV types during the many years preceding cancer development. After this concept, attempts to identify high-risk HPV types should rely more on case–control studies of the type-specific humoral immune re-

sponse against early and late viral proteins as a reflection of viral activities over years than on HPV DNA, which may just be a remnant in cancers of past activities or evidence of transient infections of tumors and control subjects.

Classical oncogenic activities of cutaneous HPV are weak, as estimated from *in vitro* studies. This finding explains the special need for activators and the additional risk factors. Of interest, cutaneous HPV infections are activated by established risk factors in skin carcinogenesis, such as sunlight (UV) exposure and immunosuppression.

In addition to a possible stimulation of oncogenic transformation by HPV, it is tempting to speculate that the antiapoptotic activities of E6 proteins of these HPVs are particularly relevant to oncogenesis in the interplay with UV exposure, because the antiapoptotic activities will favor accumulation of somatic mutations, e.g., in the p53 gene, which is affected in more than one half of NMSCs in the general population and in EV patients *(40–42).* With such a scenario in mind, HPV might be regarded more as an auxiliary factor to sunlight. However, SCCs and BCCs are also found in less UV-exposed or non-exposed sites, such as the trunk and the feet; of interest, four such SCCs were recently shown to be HPV positive *(7).* This finding suggests that HPV may be an independent risk factor in specific cases.

Attention should also be paid to potential immunomodulatory functions of the E6 and E7 proteins as revealed in the past for genital HPV *(43).* These functions may impair the immunologic elimination of cancer cells. Secretion of anti-inflammatory cytokines, even by a few HPV-positive cells, could protect the whole tumor by affecting the local immune response.

If the contributions of HPV to skin carcinogenesis discussed above are substantiated by future research, it should be possible to reduce the risk of skin cancer by interference with HPV infection (regardless of whether HPV is a necessary or an auxiliary factor in carcinogenesis). Patients at high risk of skin cancer, such as immunosuppressed and PUVA-treated psoriasis patients, could benefit from a vaccine and/or therapeutic interference with, for example, antiapoptotic activities of viral proteins.

How to Measure Skin HPV in the Future

A plethora of HPV types seems to persist at low levels all over the skin. We, therefore, need highly sensitive, broad-spectrum PCRs for initial amplification. Experience with established protocols clearly shows that no single PCR allows a satisfying coverage of all types. Combined use of about five protocols will be necessary for comprehensive analysis. Infections with multiple HPV types are a particular challenge for typing. The current strategy of PCR, the cloning of amplification products and the sequencing of many clones, is not feasible in large epidemiologic studies. One possibility is to newly develop PCR–reverse hybridization line probe assays for the simultaneous identification of all types on one nylon strip. The so-called subgenus-specific PCRs only show preferential sensitivity for the respective subgenus and may also amplify some HPV sequences from other subgenera. Therefore, they cannot replace exact typing, even if only classing with a subgenus was intended.

Overexpression of p16(INK4) recently turned out to be an easy-to-use biomarker for the screening of high-risk genital HPV infections *(44).* Unfortunately, a comparable marker cannot be envisioned yet for epidemiologic studies of cutaneous HPV infections.

There is preliminary evidence that case patients and control subjects do not really differ in the presence versus the absence of HPV but rather they differ in the extent of HPV replication. Quantitative techniques are, therefore, needed to determine the viral load.

Sampling of tissue, swabs, or hairs for HPV DNA detection is a particular problem in epidemiologic studies of skin infections in view of the size of this organ. The skin cannot be completely screened all of the time. Serologic analysis, in contrast, does not depend on a precise sampling, and serum antibodies are an important marker of past and present HPV infection. VLPs have been prepared for several EV-associated HPV types in the meantime and have been used in ELISA. There seems to be low or no cross-reactivity between different types, but only a small subset of EV-associated types has been tested so far. There are hardly any tests available for antibodies against early antigens of cutaneous HPV. Overall, much additional work has to be done to provide the appropriate tools for the epidemiologic studies of skin HPV, and these assays remain to be validated.

Directions for Future Epidemiologic Studies

Well-recognized risk factors for NMSCs are UV light exposure and immunosuppression. Epidemiologic studies of the role of HPV in cutaneous carcinogenesis have to take these factors into account. HPVs are the most strongly associated with SCC in immunosuppressed patients, and one should focus on SCC rather than on BCC. The rapid development of precancerous actinic keratoses and skin cancer after the onset of immunosuppression in transplant recipients, particularly in countries with a sunny climate (keratoses start about 9 months and SCC start about 5 years after transplantation), allows prospective studies to start at the transplantation to determine the kinetics of HPV infection–replication–gene expression in relation to tumor development. With the educated guess that most or all of the transplant recipients are already infected in the beginning by several HPV types, there is a strong need for precise typing to differentiate the role of individual HPV types and for quantitative tests to monitor the activity of HPV rather than infection *per se.* One should look for viral load and gene expression as well as for seroconversion as a result of increased stimulation of the immune system due to higher protein expression.

Viral load and, particularly, serologic status also have to be tested in case–control studies, which should be carried out in countries with different sun exposure.

Attempts to fulfill the criteria of Bradford-Hill *(45)* to establish a causal role of HPV in skin carcinogenesis may fail with regard to a strong association with infection even by specific types in view of the widespread occurrence of HPV. It will, therefore, be most important to demonstrate the temporal sequence of virus activation and tumor development and the quantitative relationship between virus activation and disease.

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