Basic and clinical features of cutaneous squamous cell carcinoma in organ transplant recipients

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CHAPTER 2

Update on our understanding of HPV as a risk factor for cutaneous squamous cell carcinoma in organ transplant recipients

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Abstract

Keratinocyte carcinomas are by far the most common malignancies seen in organ transplant recipients (OTR). Life-long immunosuppressive therapy is a major risk factor for developing squamous cell carcinoma (SCC) in OTR. In the years after transplantation, OTR develop numerous warts and wart-like lesions followed by the development of SCC. This resembles the clinical picture of epidermodysplasia verruciformis patients in which human papillomavirus (HPV) infections were associated with skin cancer. HPV can be divided into genera and cause several distinct benign and (pre-) malignant diseases.

There is evidence linking Beta-PV infection with the development of SCC in OTR. However, the role of Beta-PV in cutaneous squamous cell carcinoma carcinogenesis is still enigmatic. Unlike the carcinogenic Alpha-PV types, Beta-PV is not integrated in the human cellular DNA and is not necessary for the maintenance of the malignant phenotype of SCC.

The current view is that the carcinogenic effect of Beta-PV in OTR is subtle and probably exerted early in carcinogenesis.

Introduction

Organ transplant recipients, skin cancer and immunosuppressive therapies

Keratinocyte carcinomas are by far the most common malignancies seen in organ transplant recipients (OTR). The incidence of squamous cell carcinoma (SCC) is 60-250 times increased compared to the immunocompetent population, and for basal cell carcinoma (BCC) this is 10-40 times.[1-4]

Life-long immunosuppressive therapy is the most important risk factor for developing SCC in OTR. Other important risk factors include cumulative sun exposure, smoking and fair skin type with susceptibility to sunburn, which are risk factors similar to the immunocompetent population.[5]

Long term immunosuppressive therapy predisposes to the development of skin cancer and this is related to the type, duration and intensity of the immunosuppressive therapy. Azathioprine increases the photosensitivity of human skin to UVA radiation and when exposed to UVA radiation the active metabolite, methyl-thioinosine monophosphate (MeTIMP), which is incorporated into cellular DNA, generates reactive mutagenic oxygen species.[6,7] The carcinogenic effect of calcineurin inhibitors (cyclosporine and tacrolimus) is linked to aberrant production of cytokines that promote tumor growth, metastasis and angiogenesis.[8]

Immunosuppressive therapy with mammalian target of rapamycin (mTOR) inhibitors is possibly associated with a reduced risk of cutaneous SCC by antitumor and anti angiogenic properties, but seems only effective when the number of SCC is still low, and during the first year after conversion to mTOR inhibitor.[9-11]

Human papillomavirus infection

Human papillomaviruses (HPV) cause several distinct benign and (pre-) malignant diseases. HPV can be divided into Alpha, Beta, Gamma, Mu and Nu genera. Well known associations with benign lesions are with common skin warts (verruca vulgaris) and genital warts (condyloma accuminata). The most prevalent HPV types associated with common warts are the Alpha-PV types 2, 27 and 57 and the Gamma-PV type 4.[12-14] The majority of genital warts are caused by the mucosal Alpha-PV types 6 and 11, but other mucosal HPV types of the Alpha genera are also detected in genital warts.

The range of infections, precancers and malignancies associated with HPV continues to grow. The International Agency on Cancer Research IARC has classified mucosal

types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 as carcinogenic types (class 1), mainly causing cervical cancer; type 68 as probably carcinogenic (Class 2A); and types 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85 and 97 as possibly carcinogenic (Class 2B). HPV6 and HPV11 were not classifiable as to its carcinogenicity to humans, and the remaining mucosal HPV types were not taken into consideration by IARC (Class 3).[15]
The first time that HPV infection was linked with skin cancer was in patients with epidermodysplasia verruciformis (EV). EV is a rare autosomal recessive disease, initially described by Lewandowsky and Lutz in 1922, that has been proposed as a model for Beta-PV-mediated skin carcinogenesis.[16] EV patients have an increased susceptibility to widespread Beta-PV infections of the skin and develop pityriasis versicolor-like lesions and flat warts.[17,18] Skin cancers develop in one-third of the patients, mainly on sun-exposed sites in young to middle aged adult patients.[19]
In the years after transplantation, OTR develop numerous warts and wart-like lesions followed by the development of SCC (Figure 1,2), which resembles the clinical picture of EV patients. The association between wart-like lesions and SCC in OTR is an argument that HPV infection may play a role in SCC carcinogenesis.[20,21] This chapter will further focus on Beta-PV infection as a possible risk factor for cutaneous SCC carcinogenesis in OTR. In the following paragraphs laboratory and epidemiological evidence linking HPV infection with the development of SCC will be discussed. The HPV genome and taxonomy, replication and influence on cell cycle, and Beta-PV detection methods will be covered as well.

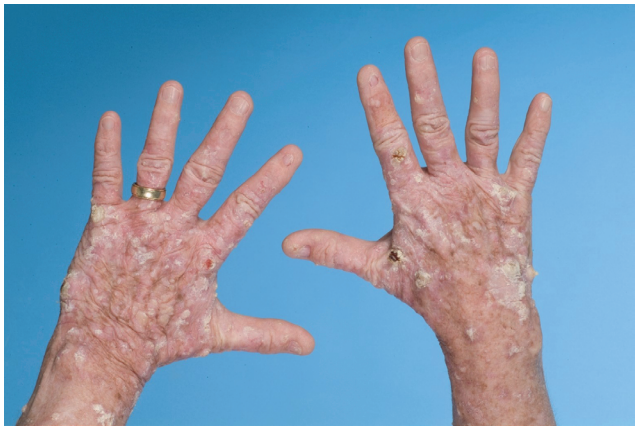

Figure 1.
Multiple wart-like lesions on dorsum of the hands

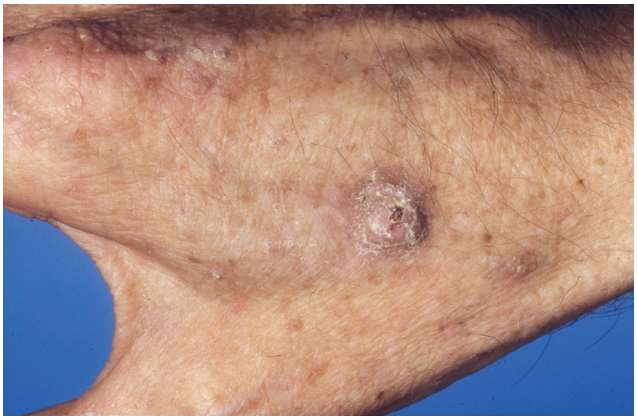

Figure 2.
Squamous cell carcinoma on dorsum of the hand

Human papillomaviruses

HPV genome and taxonomy

Papillomaviruses (PV) are non-enveloped circular double-stranded DNA viruses belonging to the family *Papillomaviridae*. The genome is approximately 8 kb and slightly varies in size between types. More than 150 types have been identified today and the number is still increasing (Figure 3). The genome is subdivided in an early (E) coding region, a late (L) coding region, and a long control region (LCR). The early region generally encodes for six non-structural viral regulatory proteins (E1, E2, E4, E5, E6 and E7 in most PV types and an additional E8 in some PV types) involved in several functions including transformation, transcription and viral adaptation to different cellular milieus.[22] The late region encodes for two structural proteins, namely L1 and L2.[23] Whereas all PV appear to have an E1, E2, L1 and L2 open reading frame (ORF) the other ORFs are not consistently present in every PV.[13] Beta-PV have E1, E2, E4, E6, E7, L1 and L2 ORFs, but lack E5.

The L1 ORF encoding for the major capsid protein L1 is relatively well conserved between HPV types. The L1 protein is the basis for currently registered prophylactic vaccines against HPV types 6, 11, 16 and 18 infections that cause genital warts and cervical cancer. The current papillomavirus classification system is based on DNA sequence homology of the L1 ORF and comprises a division in genus, species, type, subtype and variants. The phylogenetic tree is shown in Figure 3.[24]

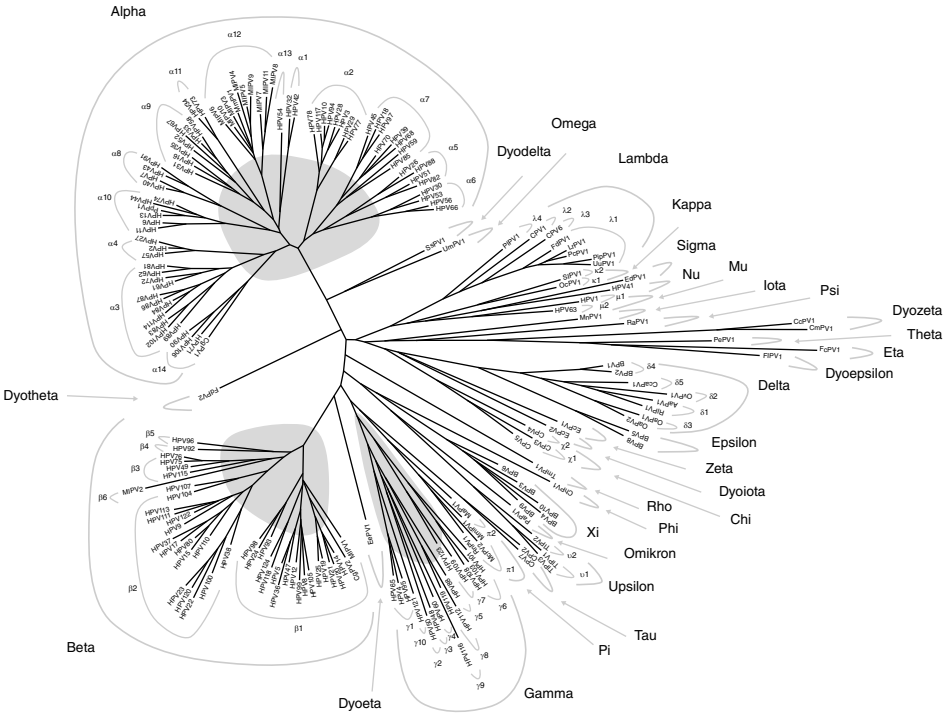

Figure 3. Phylogenetic Tree

As stated before, HPV can be divided into Alpha, Beta, Gamma, Mu and Nu genera. Species of the same genera share at least 60% homology of the L1 ORF. A new type is defined as one in which the complete nucleotide sequence ORF of the L1 gene differs by more than 10% from the most closely related known PV type. Table 1 shows all the HPV types according to the genus, based on the Papillomavirus Episteme database (http://pave. niaid. nih. gov/#home). The Beta, Gamma, Mu and Nu genera are cutaneous types. The Alpha genus contains all the mucosal types but also some cutaneous types (HPV2, 3, 10, 27, 28, 29, 57, 77, 94) and mucocutaneous types (HPV7, 40, 43, 91).[24]

HPV life cycle

HPV infection occurs when the virus enters the basal layer of the epithelium, supposedly achieved by small abrasions of the epithelium. However, the body-wide

Table 1. HPV types sorted by genus

genus	HPV type
Alpha	2,3,6,7,10,11,13,16,18,26,27,28,29,30,31,32,33,34,35,39,40,42,43,44,45,51,52,53, 54,55,56,57,58,59,61,62,66,67,68,69,70,71,72,73,74,77,78,81,82,83,84,85,86,87 ,89,90,91,94,97,102,106,114,117,125,160
Beta	5,8,9,12,14,15,17,19,20,21,22,23,24,25,36,37,38,47,49,75,76,80,92,93,96,98,99, 100,104,105,107, 110,111,113,115,118,120,122,124,143,145,150,151,159
Gamma	4,48,50,60,65,88,95,101,103,108,109,112,116,119,121,123,126,127,128,129, 130,131,132,133,134,135,136,137,138,139,140,141,142,144,146,147,148,149, 153,154,155,156,161,162,163,164,165,166,169,170
Mu	1,63
Nu	41

Source: http://pave. niaid. nih. gov/#home

distribution of especially Beta-PV infections suggests a direct route of infection, bypassing the requirement of epithelial injury or abrasion.[25-29] Life cycle studies have been mainly performed for the HPV types causing cervical cancer but the life cycle of several HPV types with a cutaneous tropism appears to be similar.[30]

E6 and E7 are mainly expressed in the (supra) basal layer and the granular layer of the epithelium and are associated with PV genome maintenance and cellular proliferation. E1 and E2 are involved in viral DNA replication and the regulation of E6 and E7 transcription. E4 of some cutaneous HPV types appears to be expressed throughout the epithelium except in the basal cell layer and presumably promotes viral DNA replication.[31,32] There is some variability in viral life cycle between PV types. However, the order of expression of viral genes throughout the differentiating epithelia is similar, although the localisation of E4 and L1 expression is variable and starts either in the lower part of the epithelium (HPV1 and 2) or in the upper part (HPV63 and 65).[33]

Beta-PV detection methods

There are different methods for Beta-PV detection, based on measurement of viral protein, viral DNA or serum antibody responses. Tissue specimens can be collected via several sampling methods like skin swabs, plucked hairs, biopsies and blood.[34] Several PCR-based methods targeting the HPV L1, E1 or E7 ORF have been developed to detect Beta-PV types in skin biopsies, plucked hairs and skin swabs.[25,26,35-45]

These PCR methods can be divided into I) type-specific PCRs, using HPV type-specific primers for the detection of a single HPV type, II) multiplex type-specific PCR

methods, in which multiple type-specific primer sets are combined in a single PCR reaction, III) broad-spectrum PCR methods using consensus primers, which permit simultaneous amplification of multiple types, and IV) quantitative PCR methods that allow viral DNA quantification.

Multiplex type-specific PCR and broad-spectrum PCR based methods amplify multiple HPV types in a single reaction. Subsequent genotyping in the case of amplification systems that target multiple HPV types is generally performed by sequence analysis, either directly or preceded by cloning of the amplimer. Sequencing, however, does not easily permit the identification of HPV types present in frequently occurring multiple infections.[39,46] Therefore, the more recent developed PCRs, are followed by reverse hybridisation technologies or APEX for the simultaneous identification of multiples HPV types.[39,41,45,47]

With virus-like particle (VLP) enzyme-linked immunoassay (ELISA) or multiplex technology (Luminex), antibodies against Beta-PV viral proteins can be detected, to determine a person's Beta-PV serological status. Serologic responses are usually measured against the major capsid protein L1 and the non-structural protein E6 using HPV virus-like particle (VLP) or GST-HPV fusion proteins in ELISA or with multiplex serology using GST-L1 fusion proteins, respectively.[48-50] The latter method (Luminex) is based on fluorescent bead technology that allows simultaneous detection of antibodies against up to 100 different in situ affinity-purified recombinant HPV proteins.[51] However, not all HPV infections induce an antibody response to HPV, and cross-reactive antibodies between different HPV types may be produced.[52] Nevertheless, serologic antibody responses provide a useful epidemiologic tool to explore HPV infections in certain populations.

Detection of papillomavirus proteins and DNA in paraffin-embedded tissue sections is possible by combining in situ hybridization and fluorescence detection methods,(FISH=Fluorescent DNA in situ hybridization).[53] Also laser capture microdissection can be used to specifically test a particular part of an histological specimen for HPV presence and viral load.[54]

Beta-PV replication, cell transformation and carcinogenesis

After infection, Beta-PV will start to replicate in the epidermal keratinocytes. As for mucosal HPV, keratinocyte differentiation is probably also crucial for Beta-PV to fulfill their life cycle.[55,56] It is expected that in the basal epidermal layers a low level of replication of Beta-PV DNA is maintained to ensure viral episome distribution

among daughter cells, and especially the early (E) genes will be expressed.[57]
Transformation of human cells has been studied in great detail for the HPV types causing cervical cancer (e. g. HPV16 and HPV18). In general, transformation requires long-term and deregulated expression of viral oncogenes E6 and E7. This process is facilitated by integration of the viral episome into the cellular DNA, disturbing the E2 ORF, thereby causing a lack of control of E6 and E7 expression.[32] In general, Beta-PV are found episomal, also in actinic keratoses and SCC, although (integrated) HPV has been occasionally isolated from an SCC metastatic lesion in an OTR.[43,58]
Since papillomaviruses depend on (unscheduled) host cell DNA replication in order to produce progeny, the virus tends to keep its host cell in a replicative state (S-phase) as long as possible. This is particularly relevant as terminal keratinocyte differentiation is a dead-end road resembling programmed cell death (apoptosis).[59,60] For high-risk mucosal HPV types it is known that the E7 early gene product drives cells into S-phase by binding and degrading the tumor suppressor protein pRb.[61] For HPV38, a Beta-PV type that has been associated with SCC in some studies, this has been shown as well, but it does not seem to be a mechanism exploited by all Beta-PV types. For the high-risk mucosal HPVs, such as HPV16 and HPV18, this mechanism acts through ubiquitination and subsequent degradation of the tumor suppressors p53 and pRb. Presumably, persistent infections are needed to accumulate sufficient mutations in the host cellular genome to generate a malignant cell. The proliferative phenotype of these malignant cells remains dependent on E6/E7 expression.[62]
HPV38 E7 was shown to bind and degrade the tumor suppressor pRb similar as HPV16 E7[63,64]. Furthermore, HPV38 E6 and E7 are sufficient to deregulate the cell cycle and senescence programs in primary human keratinocytes, thereby increasing the lifespan of human skin keratinocytes.[63,65,66] HPV38 E6, possibly in combination with E7 induces telomerase activity, which plays a key role in transformation of human keratinocytes.[67]
Another mechanism behind the lack of cell cycle arrest in Beta-PV expressing cells might be the upregulation of delta-Np73 as a result of p53 accumulation.[68,69] This upregulation prevents p53 to induce the transcription of genes involved in apoptosis and growth suppression, altering the regulation of cell cycle checkpoints that are normally activated by UV radiation.[69]
Several studies investigating the effect of Beta-PV E6 and E7 in organotypic (raft) cultures showed that Beta-PV could delay cell differentiation and disturb keratinocyte outgrowth.[66,70-72]

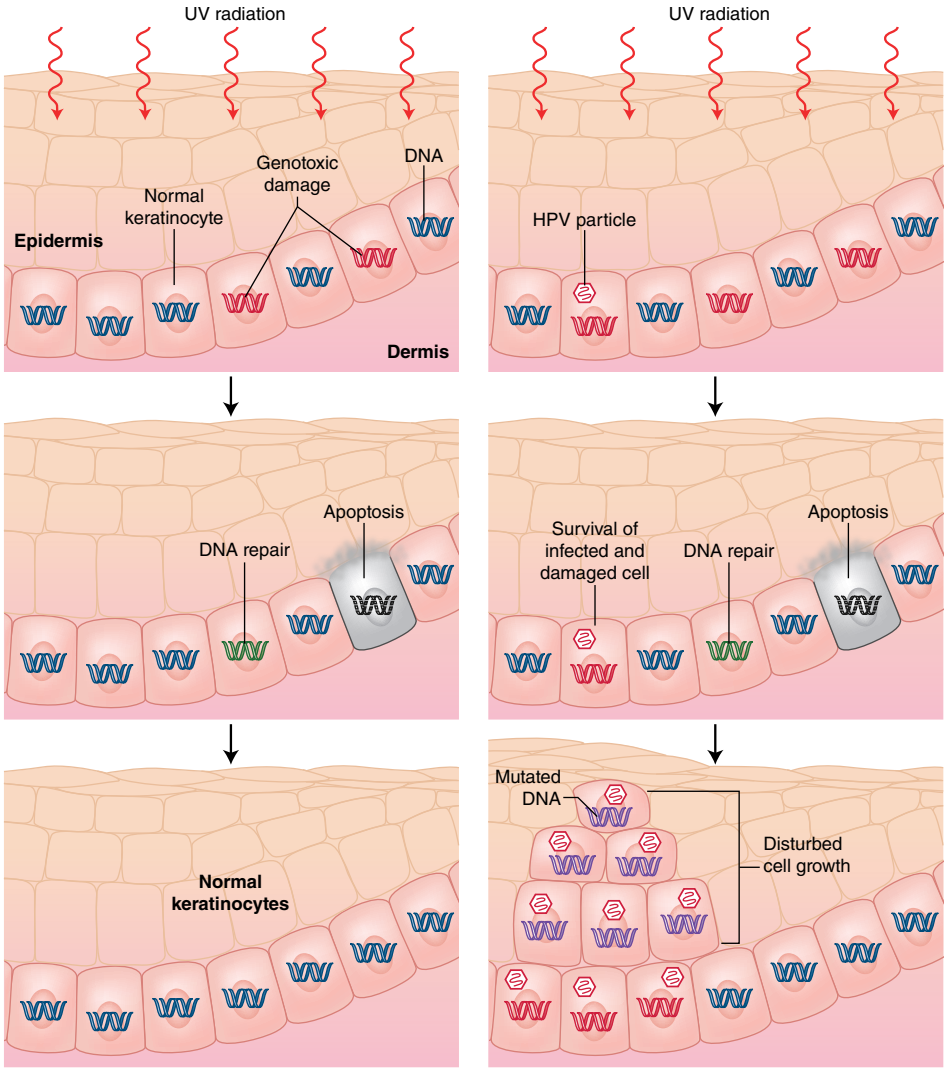

Figure 4. Ultraviolet radiation causes genotoxic damage in the keratinocytes in the basal layer of the epidermis. Panels on the left illustrate DNA repair in the cells or dying due to apoptosis. The panels on the right show the situation when a keratinocyte is infected with HPV. The infected keratinocyte has low ability for DNA repair and is less sensitive to apoptosis. The DNA mutated cell can proliferate and multiply and lead to dysplastic keratinocytes. Currently it is believed that not every mutated cell carries a virus episome. Permission for reprint accepted by NEJM (rights JNBB).

As the vast majority of SCC occurs on sun-exposed sites, it is generally believed that Beta-PV, if involved in skin cancer development, somehow cooperates with UV-radiation in transforming cutaneous epithelial keratinocytes. Beta-PV could confer its possible carcinogenic effect via inhibition of DNA repair and apoptosis in UV-damaged cells which is illustrated in Figure 4.[73]

HPV5 E6 appeared to exert this effect via the degradation of Bak, a protein involved in induction of apoptosis.[74-76] The E6 of HPV8, 20, 22, 38, 76, 92 and 96 can protect UV-treated keratinocytes from apoptosis.[77,78] The oncoproteins E6 and E7 from Beta-PV38 significantly contribute to SCC development in the skin rendering keratinocytes more susceptible to UV-induced carcinogenesis in mice.[79,80] Interference with UV-induced DNA repair and apoptosis by Beta-PV might occur, and epidemiological studies seem suggestive of a joint effect of UV-radiation and Beta-PV infection as well.[50,73,81]

Still other studies have shown that the E6 proteins of HPV5 and HPV8 inhibit the transforming growth factor Beta (TGF-Beta) signaling pathway by the degradation of the SMAD3 transcription factor.[82] TGF-Beta-triggered pathways lead to the synthesis of inhibitors (p16, p17, p21, and p27) of the cyclin-dependent kinases that play a crucial role in the cell cycle. It can be postulated that specific degradation of SMAD3 could negatively regulate inhibitors of the cell cycle and favor cell transition from the G1 to the S phase, allowing viral DNA vegetative replication and, as a side effect, cell transformation.

Additionally, there is evidence that the association of HPV5 and HPV8 E6 proteins with MAML1 inhibits Notch signaling and that Notch signaling plays a role in both virus–host perturbations and tumor genesis.[83]

In HPV positive skin cancers, not all tumor cells contain HPV DNA and the viral load is higher in actinic keratoses.[84] If Beta-PV are involved in the development of skin cancer, they probably play a role in tumor initiation and progression, not in maintenance of the malignant phenotype, through a “hit-and-run” mechanism of viral carcinogenesis.[79]

Epidemiology of HPV infection and skin cancer

HPV carriage in humans

HPV DNA can frequently be detected in skin swabs and plucked hairs both in newborns, children and adults.[29,34,50,85-89] Family members may share some of the same HPV types.[29,90] The prevalence of HPV DNA ranges between 42 and 87%, and 84 and

91%, respectively, using skin swab and plucked hair sampling, and varies also between geographical regions and ethnicities.[25,29,46,90-95]
Viral DNA is persistent in/on the skin.[86,89,95] Increasing age, sun exposure, sunburn, skin type and a medical history of skin cancer are factors which are associated with an increased prevalence of HPV DNA.[25,46,91,96,97] Duration of immunosuppression and immunosuppression itself in OTR is also associated with an increased prevalence of HPV DNA in plucked hair samples compared with the immunocompetent population.[46]
Seropositivity for one or more HPV types is approximately 60% in the general population (most frequently for HPV8 followed by HPV15, 17, 38 and 49), but can be higher in certain subpopulations.[97-99] Serological responses to HPV are also more common in OTR and vary between 80 and 90% to at least one HPV type. Between 45 and 56% of these positive seroresponses are against Beta-PV types. Most commonly detected Beta-PV types are HPV8, 15, 17, 38 and 49.[25,100,101]

Presence of HPV DNA in skin cancer and precursor lesions

Several studies have investigated the prevalence of HPV DNA in SCC and precursor lesions in both immunocompetent individuals and OTR. HPV carriage was nicely summarized by Aldabagh et al.[34] The percentages of HPV DNA positivity vary between immunocompetent individuals and OTR and depend on the detection methods used. The prevalences of HPV DNA in SCC in the immunocompetent population range between 26 and 69%. HPV DNA can also be found in benign lesions, actinic keratosis and normal and sun-exposed skin from both patients with and without SCC.[84,94,102-105] Frequently, Beta-PV types, including HPV5 and 8, are detected in higher rates in actinic keratoses compared to SCC.[106] Viral load of HPV infection was found to be significantly higher in actinic keratoses compared to SCC.[84] In a small study Beta-PV DNA was found to be more often present in perilesional skin than in SCC and mirror site healthy skin, but there are also studies reporting that Beta-PV species 2 is more likely to be identified in SCC than in adjacent healthy skin.[102,103,107].
Numerous studies were carried out in OTR to assess the presence of HPV DNA in keratinocyte carcinomas. De Villiers et al found HPV DNA positivity in 91% of SCC and in situ SCC in 25 OTR, the most prevalent types being among the Beta-PV types.[38] Berkhout et al found similar frequencies and distribution of Beta-PV types in hyperkeratotic papillomas, actinic keratosis and SCC, but lower in BCC, benign lesions and normal skin.[108]

Some studies have compared the prevalence of HPV DNA in SCC and precursor lesions of immunocompetent individuals with OTR. In a German study, HPV DNA was detected more frequently in SCC of OTR (75%) than in immunocompetent patients (47%).[109] Similar HPV prevalences were found in cutaneous warts (91% vs. 94%), pre-malignant skin tumors (38% vs. 36%) and normal skin specimens (17% vs. 16%) for both patient populations. HPV types 5 and 8 were found more frequently in SCC and only in the SCC of the OTR.[109] In a Dutch group of OTR with and without skin cancer, the prevalence of Beta-PV DNA in benign keratotic skin lesions was equally high, around 50%.[108-110] A higher prevalence of Beta-PV DNA was found in lesions from sun-exposed sites with a history of skin cancer.[110] In a Scottish study no difference was detected between lesions from immunocompetent individuals and OTR with HPV15, 24 and 38 as the most frequently detected types. In this study multiple infections were more common in tumors from immunocompetent individuals (70%) compared with those from OTR (26%).[111]

Association between HPV DNA and skin cancer

Case-control studies investigating the association between the presence of Beta-PV DNA and SCC are summarized in Table 2. Most studies were performed with immunocompetent patients and show a statistically significant association between the presence of Beta-PV DNA and SCC or its precursor actinic keratoses, or a non-significant trend in the same direction (Table 2). However, in a large international case-control study, the presence of Beta-PV DNA in eyebrow hairs was only significantly associated with an increased risk of SCC in the Netherlands, but not in Italy and Australia.[87] Overall the Beta-PV DNA positivity was more than 90% for all participants.[87]So far, only one large study was performed with European OTR showing a 2.4 times increased risk of SCC in OTR with Beta-PV DNA in eyebrow hairs.[116]

High viral load of Beta-PV DNA in eyebrow hairs was reported to be associated with increased risk of cutaneous SCC in immunocompetent Australian patients and in OTR, with total load seemingly more important than the load of any specific type.[117] Exposure to individual cutaneous HPV types and the risk of SCC was recently summarized.[118] Many Beta-PV types are associated with an increased risk of SCC, but no specific type stands out. HPV9, 24, 36, 76 and 92 showed the strongest association with SCC.[118]

Table 2. Epidemiological studies summarizing the association between Beta-PV DNA and SCC development

Author (ref)	Study type	Population / country	Infection marker	Method	HPV-types	Cases	Controls	Adjusted odds ratio (95% CI)	Comments
Boxman [36]	Nested case-control/	IC / Australia	DNA in eyebrow hairs	Nested PCR	Beta	64 KC* 51 BCC 25 SCC	64 51 25	0. 8 (0. 3-1. 8) 0. 6 (0. 2-1. 5) 2. 0 (0. 5-8. 0)	*BCC/SCC/intra-epithelial carcinoma/ KC undefined
Boxman [112]	Cross-sectional	IC / Australia	DNA in eyebrow hairs	Nested PCR	Beta	276 AK	231	3. 4 (1. 8-6. 5) (M) 1. 0 (0. 6-1. 8) (F)	Significant association between Beta-PV and AK only in men
Struijk [113]	Case-control	IC / the Netherlands	DNA in eyebrow hairs	Type-specific PCR	2, 5, 8, 15, 16, 20, 24, 38	155 SCC	371	1. 7 (1. 1-2. 7)	Association between Beta-PV and SCC with increasing age and male sex
Harwood [114]	Case-control	IC / U.K.	DNA in normal skin biopsies	Degenerate/ Nested PCR	Beta	10 KC*	29	6. 4 (1. 8-22. 9)	*BCC/SCC
Struijk [49]	Case-control	IC / Australia	DNA in eyebrow hairs	Type-specific PCR	5, 8, 15, 20, 24, 38	126 AK 64 SCC	57	1. 6 (0. 8-3. 0) 0. 9 (0. 4-2. 0)	
McBride [115]	Prospective	IC / Australia	DNA in eyebrow hairs	Nested PCR	Beta	71 1-10 AK 41 > 10 AK	179	1. 8 (0. 7-4. 4)	Association with having more than 10 AK. Significant associations with age over 60 years, fair skin color, high sun exposure
Bouwes Bavinck [87]	Case control	IC / Australia (AU), Italy (I), the Netherlands (NL)	DNA in eyebrow hairs	PCR-reverse hybridization assay	Beta	689 SCC	845	2. 8 (1. 3-5-8) NL 1. 7 (0. 79-3. 6) I 0. 91 (0. 53-1. 6) AU	
Proby [116]	Case-control	IS / France, Italy, the Netherlands, U.K.	DNA in eyebrow hairs	PCR-reverse hybridization assay	Beta	210 SCC	394	2. 4 (1-5. 4)	For HPV5, 9, 24 and 36

IC;immunocompetent, IS;immunosuppressed/transplant patients, KC; keratinocyte cancer, AK; actinic keratosis, BCC; basal cell carcinoma, SCC; squamous cell carcinoma

Association between HPV serology and skin cancer

Most studies investigating the association between serologic antibody responses to HPV and SCC were also carried out in the immunocompetent population and show a statistically significant association between the presence of Beta-PV antibodies and SCC, or a non-significant trend in the same direction (Table 2 and 3).

The odds ratios are increasing when there are more positive serological responses against increasing numbers of Beta-PV types.[48,50,121,124,125]The association with SCC is exclusively found for Beta-PV types, particularly HPV5, 8, 9, 15, 17, 20, 24, 36, 38, 49, 75, 76 and 92, but not for Alpha, Gamma, Mu or Nu HPV types.[118]

In an international case-control study a positive antibody response against 4 or more Beta-PV types was associated with a doubled risk for SCC in Australia and the Netherlands.[87] A population-based cohort study in Australia, however, revealed no associations between the presence of any Beta-PV antibodies and the occurrence of SCC. However, among people who were less than 50 years old in 1992, the presence of Beta-PV antibodies was associated with a two-fold increased risk of SCC.[126] In the United Kingdom in a prospective pilot study among 39 SCC cases and 80 controls also no statistically significant differences were found in the seroprevalence of antibodies against any of 38 HPV types.[128] HPV seropositivity was strongly associated with the risk of developing a second SCC after 5 years for both Beta and Gamma HPV types.[127]

With respect to the presence of Beta-PV DNA in (pre-)malignant and benign skin tumors, no correlation was found with serum antibodies to the same HPV type measured. However, seropositivity to any Beta-PV type was significantly more common among patients positive for HPV DNA of any HPV type and seroprevalences were higher for SCC patients compared to BCC patients.[85]

Viral transcriptional activity and skin cancer

Viral transcriptional activity was investigated in SCC from both immunocompetent individuals and OTR. Only a portion of SCC was positive for cutaneous HPV using in situ hybridization (122), but similarly low transcriptional activity in warts in OTR was observed.[129] The biological activity of HPV types found in tumor tissues was assessed by examinating HPV E6/E7 RNA expression for cutaneous HPV types. HPV DNA was detected in 25 of 31 tissue samples. E6/E7 transcripts of HPV8, 9 and 15 were found in low copy numbers in one SCC and three AKs, but not in normal skin or verrucae vulgaris.[130]

Table 3 Epidemiological studies summarizing the association between Beta-PV seroprevalence and SCC development

Author (ref)	Study type	Population / country	Infection marker	Method	HPV-types	Cases	Controls	Adjusted odds ratio (95% CI)	Comments
Steger [119]	Case-control	IC / Germany	L1 serology	Western blot	8	11	445	10. 7 (2. 5-63. 2)	
Stark [120]	Case-control	IC / Germany	L1 serology	ELISA	8	14 SCC	210	30. 3 (7. 4-142. 5)	
Bouwes Bavinck [121]	Case-control	IC / the Netherlands	L1 serology	ELISA	8	13 SCC	82	3. 1 (0. 7-13. 3)	
Feltkamp [48]	Case-control	IC / the Netherlands	L1 serology	ELISA	5, 8, 15, 20, 24, 38	540 SCC	333	1. 4 (0. 8-2. 5)	
Masini [122]	Case-control	IC / Italy	L1 serology	ELISA	8, 15, 23, 36	46 SCC	84	3. 2 (1. 3-7. 9) (HPV8) 0. 4 (0. 2-0. 9) (HPV15) 1. 0 (0. 3-3. 3) HPV23) 2. 8 (0. 8-10. 0) (HPV36)	
Karagas [50]	Case-control	IC / U.S.A.	L1 serology	multiplex	Beta	252 SCC	461	1. 5 (1. 1-2. 1)	
Struijk [49]	Case-control	IC / Australia	L1/E6 serology	ELISA	5, 8, 15, 16, 20, 24, 38	126 AK 64 SCC	57	2. 3 (0. 9-4. 9)(L1) 0. 6 (0. 3-1. 3) (E6) 3. 9 (1. 4-10. 7) (L1) 0. 5 (0. 2-1. 1) (E6)	Associations between Beta-PV L1 and E6 serology and AK/SCC
Casabonne [123]	Nested case-control	IC / U.K.	L1 serology	multiplex	Beta	39 SCC	80	0. 5 (0. 1-1. 7)* 1. 0 (0. 4-2. 5) **	Association between 1* or 2+** Beta-PV type(s) and SCC
Karagas [124]	Case-control	IC / U.S.A.	L1 serology	multiplex	Beta	663 SCC	805	1. 0 (0. 7-1. 3)* 1. 4 (1. 0-2. 0)** 1. 5 (1. 0-2. 2)*** 1. 7 (1. 1-2. 6)****	Association between 1* or 2-3**, 4-8*** or >8**** Beta-PV type(s) and SCC
Proby [116]	Case-control	IS / France, Italy, the Netherlands, U.K.	L1 serology	multiplex	Beta	210 SCC	394	1. 6 (1. 1-2. 5)	Association for concordant detection of both antibodies and DNA for at least one Beta-PV type

Waterboer [125]	Case-control	IC / Italy	L1 serology	multiplex	Beta / Gamma	43 SCC	77	2. 8 (1. 1-7. 1)* 2. 6 (1. 0-6. 5)** 3. 0 (1. 2-7. 9)*** 3. 4 (1. 2-9. 5)****	*HPV15 **HPV17 ***HPV38 ****HPV50
Bouwes Bavinck [87]	Case control	IC / Australia (AU), Italy (I), the Netherlands (NL)	L1 serology	multiplex	Beta	689 SCC	845	>= 4 types postive 2. 0 (1. 2-3. 4) * 2. 2 (1. 4-3. 3) ** 1. 6 (0. 94-2. 7) ***	 *NL **AU ***I fair skinned
Plasmeijer [126]	Prospective	IC / Australia	L1 serology	multiplex	Beta	150 SCC	1161	1. 0 (0. 7-1. 4)* 2. 1 (1. 0-4. 6)**	*HR for being positive for at least 1 Beta-PV type **HR in subset of people <50y
Paradisi [127]	Case-control	IC / Italy	L1 serology	multiplex	Beta / Gamma	16 SCC	48	4. 25 (1. 22-15. 58)* 8. 08 (1. 05-62. 42)** 3. 01 (1. 01-8. 97)*** 3. 46 (1. 08-11. 5)****	*HR HPV24 **HR HPV15 ***HR HPV95 ****HR HPV60

IC;immunocompetent, IS;immunosuppressed/transplant patients, KC; keratinocyte cancer, AK; actinic keratosis, BCC; basal cell carcinoma, SCC; squamous cell carcinoma

Transcriptome sequencing, performed on 31 SCC, failed to identify HPV expression in any of the skin tumors.[131]

Human papillomavirus and basal cell carcinoma

A recent systematic review showed no association between Beta-PV DNA or Beta-PV serological responses and BCC.[118] In a subgroup of patients with Beta-PV DNA positive BCC, however, an association between a serological response against Beta-PV and BCC could be observed.[132] Some Beta-PV types could up-regulate the p16INK4a and Akt/P13K pathway and might play a role in the carcinogenesis of BCC.[133] The evidence that HPV is involved in BCC carcinogenesis is clearly much weaker than the possible involvement of HPV in cutaneous SCC carcinogenesis.

Conclusions

The role of Beta-PV in cutaneous SCC carcinogenesis is still enigmatic and clearly different from the role of mucosal Alpha-PV types in cervical cancer. Despite experimental evidence and a plausible hypothesis of a biological mechanism supporting a carcinogenic role of Beta-PV in SCC carcinogenesis, the epidemiological studies are still difficult to interpret. The viral load and the plurality of Beta-PV types are an important obstacle to identify the responsible HPV types in epidemiological studies. Several case-control studies observed an association between Beta-PV antibodies in the serum and/or Beta-PV DNA in plucked eyebrow hairs and development of cutaneous SCC, but so far no specific high risk Beta-PV types could be identified. Possibly much larger studies will be needed to identify the responsible HPV types in between the massive number of bystander HPV types. It is also not clear what the implications are in daily practice for Beta-PV infections in OTR.

Since Beta-PV is not integrated in the human cellular DNA and is not necessary for the maintenance of the malignant phenotype of cutaneous SCC, the carcinogenic effect, if present, is subtle and probably exerted early in carcinogenesis. This fits with observations that premalignant lesions such as actinic keratoses often contain a higher load of Beta-PV compared to SCC. By impairing DNA repair and UV-induced apoptosis, these persistent skin viruses may, after a long period, lead to cellular instability and sequentially cause field changes, actinic keratoses and finally

cutaneous SCC. Whether different Beta-PV types have different effects on cellular mechanisms and a combination of these HPV types may further increase the risk of cutaneous SCC is unknown. Further research is clearly needed to unravel the secrets of Beta-PV in SCC carcinogenesis.

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