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Cutaneous Squamous Cell Carcinoma and Human Papillomavirus: Is There An Association?

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Abstract

Background—The role of human papillomavirus (HPV) in the induction and maintenance of cervical, anogenital, and some oropharyngeal carcinomas is well recognized. However, its role in cutaneous squamous cell carcinoma (SCC) remains to be elucidated. HPV is thought to act as a possible co-carcinogen in the development of SCC.

Objective—To review the literature assessing the correlation and possible causation of HPV and cutaneous SCC in the immunocompetent and immunocompromised populations.

Methods—We reviewed HPV sampling and detection methods, epidemiologic studies examining HPV carriage in immunocompetent and immunosuppressed individuals, and evidence asserting an association between HPV and cutaneous SCC.

Results—Although an abundant body of evidence points toward a link between HPV and cutaneous SCC, many studies indicate otherwise. Recent studies have focused on viral activity in addition to DNA presence.

Conclusion—The possibility exists that HPV may play a role in the induction but not maintenance of cutaneous SCC.

Keywords

Squamous cell carcinoma; human papillomavirus

Introduction

Non-melanoma skin cancer (NMSC) is more common than lung, breast, prostate, and colon cancers combined and its rates are increasing by 4–8% yearly.[1] Although the majority of NMSC can be treated surgically, these cancers are associated with high morbidity and represent a significant burden on the healthcare system. Squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) are the most common types of NMSC. BCC is four times more common than SCC among fair-skinned individuals, although SCC is more prevalent among darker skin types. SCC is the second most common type of NMSC, after BCC.[2] Major risk factors for the development of SCC are ultraviolet radiation (UVR) exposure, fair skin, and immunosuppression.[3]

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In addition to sun exposure, the clinical behavior and epidemiology of SCC suggest a viral etiology. Organ transplant recipients (OTR) have a 65- to 100-fold increased risk of SCC compared to the general population,[4–7] an incidence ratio similar to other viral cancers including human herpesvirus-8 (HHV-8)-mediated Kaposi's sarcoma.[8] The keratoacanthoma subtype of SCC is able to spontaneously regress and may represent a midpoint between a viral wart and invasive SCC.[9] There are a variety of mechanisms by which oncoviruses lead to cancer (Table 1; reviewed by Arron[10]). Small DNA viruses and integrated retroviruses can express viral oncogenes that directly target cellular tumor suppressor genes and disrupt normal cell cycle controls, leading to transformation. Non-transforming retroviruses may integrate near cellular oncogenes, disrupting expression (somatic mutagenesis). Finally, some viruses trigger cancer by stimulating chronic inflammation, leading to carcinogenesis. Well-known examples of viral-associated NMSC include HHV-8 associated Kaposi's sarcoma and Merkel cell polyomavirus-associated Merkel cell carcinoma, both caused by DNA oncoviruses.

The search for a viral etiologic agent in SCC has focused on human papillomavirus (HPV). This virus is an attractive candidate given its etiologic role in verruca vulgaris, condyloma acuminata, anogenital, and some head and neck SCCs. β-genus HPV is also associated with cutaneous SCC in the genodermatosis epidermodysplasia verruciformis (EDV).[11]

The association between alpha HPV types and cervical cancer has been well established. Persistent infection with a high risk alpha HPV type is necessary, though not sufficient, for the development of cervical cancer,[12] and almost all cervical cancers test positive for HPV DNA.[12] Infection with high-risk HPVs (16, 18, 31, 33, 45), whether clinically evident or not, is the major risk factor for the development of cervical cancer.[13] In addition, the attributable risk of HPV for cervical cancer is higher than the risk associated with smoking and Hepatitis B for lung and liver cancer respectively.[12, 14] Certain environmental factors such as smoking, intake of oral contraceptive pills, co-infection with human immunodeficiency virus (HIV), herpes simplex virus, or chlamydia trachomatis, immunosuppression, and lack of male circumcision can further increase this risk.[12, 14, 15] Molecular studies have shown that HPV E6 and E7 proteins deregulate key controls in the cell cycle via interacting with p53 and Rb respectively.[16] As a **consequence**, HPV infected host cells acquire defects in differentiation and apoptosis leading to immortality and malignant potential.[17]

Based on this evidence, various groups have sought to examine the association of HPV with cutaneous SCC. However, before cancer causality can be attributed to a viral agent, epidemiologic evidence as well as a plausible biologic mechanism for oncogenesis must exist. The objective of this review is to update the reader on the epidemiologic association between HPV and SCC. In order to give the reader a framework with which to understand the field, we will briefly review relevant HPV biology and the array of sampling and detection techniques used in these studies. Finally, the clinical implications of an association between HPV and SCC will be discussed.

Human Papillomavirus

Papillomaviridae is a diverse family of non-enveloped DNA viruses that are highly host specific. They infect humans, other mammals, birds and reptile species. Papillomaviridae replicate exclusively in keratinocytes, and depend on keratinocyte differentiation to complete their life cycle.[18] HPV presumably gains entry through micro-abrasions of the skin or mucosal surface initially infecting follicular[19] or eccrine stem cells.[20]After initial entry, interactions between viral capsid proteins and cell surface glycosoaminoglycans and proteoglycans (such as heparan sulfate) allow viral attachment (Figure 1).[21–23] Specific

receptor interactions and endosomal transport aid in viral entry into the cell. The virus then disrupts the endosome and the viral genome is granted access to the cell nucleus, **where it replicates**. Once in the nucleus, HPV DNA exists in an episome separate from the host DNA, though high-risk HPV types can integrate into the host genome.[24]

The eight-kilobase viral genome is composed of genes expressed late (L) and early (E) in the life cycle. The L1 protein is the major capsid protein and is relatively well conserved between HPV types. It is the basis of prophylactic vaccines, and is used for classification and phylogenetic analysis[25]. In specific high-risk types of HPV such as 16 and 18, proteins E6 and E7 immortalize the cell preventing apoptosis and allowing the continuous replication of viral DNA. E6 is a multifunctional protein that binds and mediates the degradation of the tumor suppressor gene p53.[26] It also binds other cellular proteins affecting cellular metabolism driving the cell to immortality. E7 activates telomerase and inactivates retinoblastoma protein thus preventing cell cycle inhibition[26, 27]

Over 100 HPV types have been characterized to date, with approximately 90% being encompassed by the alpha, beta, and gamma genera (Figure 2).[25, 28] Classification is done by DNA sequence identity in the L1 capsid region, which can range from 40% for divergent genomes to >99% for closely related isolates. HPV in the same genus share at least 60% identity, and types share 76–89% identity.[29] Specific types have specific tropism, with the alpha genus primarily infecting mucosal epithelium and the beta, gamma, mu, and nu genera infecting cutaneous epithelium. The beta genus, which includes HPV 5 and 8, encompasses those types known as epidermodysplasia verruciformis (EV) types, due to the association of these types with that genodermatosis.[30]

While the significance of specific types of HPV is well recognized in cervical and anogenital SCC, as well as a subset of oropharyngeal SCC, its etiologic role in cutaneous oncogenesis is still debated.

Immunity and HPV

The adaptive immune response to HPV is both antibody and cell-mediated, although the humoral response to natural HPV infection is weak.[31, 32] There is clinical evidence that a functional T-cell response remains a major mechanism of immunity against HPV. For example, the presence of a robust T-cell and macrophage infiltrate correlates with regression in genital warts.[33] Patients who have genetic immune defects in the quantity or quality of their T-cell repertoire often have susceptibility to viral infections as a distinct feature of their genetic disease.[34–36] Patients who have Dock8 deficiency or idiopathic T-cell lymphocytopenia can have extensive cutaneous verrucae. The degree of risk that these patients have to develop SCC is not yet clear, although SCC have been reported in these patients.[37] Neither is it known if the predisposition to developing SCC is directly related to an inability to mount an appropriate defense against HPV, or if it is a nonspecific consequence of the underlying immune defect. Finally, patients who have an acquired or iatrogenic cellular immune defect, such as those with HIV and patients who have undergone solid organ transplantation, have inefficient cell-based immunity to HPV. HIV infection reduces the likelihood of HPV clearance, [38, 39] and OTR can suffer from a variety of cutaneous viral infections, the most common of which is warts.[40, 41] Indeed, 50% of renal transplant patients with five-year graft survival have warts. The consequence of iatrogenic immunosuppression and the inability to clear viral infections can be far reaching, as solid organ transplantation increases the risk for other viral-associated malignancies.[42] Although this increase is presumed to be from the host's inadequate control of viral infection, the exact mechanism of tumor initiation and progression is still poorly understood. Given the

high incidence of cutaneous SCC among OTR, identifying a clear link between HPV and SCC would have important implications for therapy and prevention.[32]

Ultraviolet Light and HPV

The relationship between of ultraviolet (UV) light, HPV infection, and HPV-related carcinogenesis is not yet fully understood. In a case control study of cutaneous SCC in a Dutch population, a history of painful sunburn episodes was associated with increased recovery of EV–HPV DNA from plucked eyebrow hairs.[43] However, an increased lifetime sun exposure in the same cohort was associated with the opposite result. One reasonable hypothesis is that local immunosuppression by UV light promotes HPV infection.[43] UV light is known to suppress local cell-mediated immunity, can impede antigen presentation by depleting the Langerhan cells and interfering with antigen presentation.[44] UV also attenuates the effector memory response.[45] Given the dependence of the body's defense mechanism against HPV on an intact T-cell response, it is reasonable to infer that UV light promotes HPV infection through cutaneous immunosuppression. The destructive effects of UV light may also play a role in increased HPV infection. Salem *et al.* demonstrated an increase in the recovery of HPV in UVB and psoralen-UVA (PUVA) treated psoriatic plaques when compared to non-irradiated psoriatic plaques and normal controls.[46] That same study found that there was an even higher recovery among PUVA-treated psoriatic patients when compared to those treated with narrowband UVB (NB-UVB), suggesting that the UV wavelength and degree of damage is important in HPV infection. Furthermore, when the authors looked at UV-treated vitiligo patients, they found that HPV recovery in lesional skin among these patients was similar to the psoriasis-PUVA group, but significantly higher than the psoriasis-NB-UVB group. This contradicts the concept that psoriatic skin is more permissive than normal skin for the viral presence,[47] and strengthens the argument that UV treatment may facilitate HPV infection.

Detection Methods

Studies assessing the association of HPV (mainly the beta types) and SCC vary greatly in their methods of viral detection. Detection of HPV in the skin requires both a sampling strategy and a measurement method for viral nucleic acid, protein, or serum antibody response.

Sample Collection

The first step in assessing the presence of HPV is acquiring an adequate sample (Table 2). Skin biopsy is considered the gold standard in sampling for HPV testing. Skin biopsies can yield information about HPV in layers deep to the stratum corneum, which may reveal virus in the stem cell of the hair follicle or the eccrine ducts.[19, 20] Nonetheless, it is not known if HPV DNA detection from healthy skin represents viral particles, superficial, or deep viral infection. Some have advocated tape stripping biopsy sites to reduce surface contamination since the virus is shed from the skin, and this significantly reduces the detection of HPV DNA.[48] Biopsy is a minimally invasive procedure, but requires performer skill and moderate cost, which may make it impractical for large cohort studies. Skin swabs are a more convenient sampling method, allowing for quick, non-invasive, painless sampling that can be repeated multiple times with little risk. The disadvantage is that it is difficult to assess if a positive sample represents contamination, carriage, transient, or persistent infection. Presumably, multiple positive samples of the same HPV type over a period of time would be more likely due to persistent infection. Plucked hairs represent another tissue specimen that may be tested for HPV, as hair follicles are a reservoir for latent β-HPV.[7] This sampling method should have a lower risk of surface contamination than the skin swab.[49] Hair pluck is non-invasive, carries little risk, and can be performed multiple times. These less invasive

methods, though more feasible, may not be as clinically relevant as biopsy. Finally, blood samples can be used to identify serum antibodies to specific HPV types. Drawing blood is simple, quick, and can be repeated multiple times but information gathered is limited to serology.

Viral DNA Detection

Detection of HPV is frequently based on molecular assays for viral nucleic acid (Table 3). Most studies use PCR amplification with degenerate or multiplexed primers to amplify a broad range of viral types. Many published primer sets target the L1 capsid gene, as this is the most conserved area. Specific genotyping of amplified material is then performed with sequencing, blot, or bead-based readout.[50–57] Microarray detection of HPV has been used for readout of similarly amplified PCR products,[58–66] although comprehensive microarrays are in development that do not rely on targeted PCR amplification.[67]

Serology

The serologic response to different viral proteins is usually tested by enzyme linked immunosorbent assay (ELISA). Current ELISA techniques make use of virus-like particles (VLPs), which are non-infectious aggregates of capsid protein produced in the laboratory and do not contain viral DNA. Serologic responses are usually measured against the L1 and/or E6 proteins (Table 3). Some epidemiologic studies utilize serologic testing since most HPV-infections are rapidly cleared, and serology detects both current infection and prior viral exposure. In contrast, DNA detection methods are limited only to a specific body site and indicate only current infection.[68] Serology provides a useful epidemiologic tool to investigate the variety of HPV types and their distribution within the community. However, for cutaneous HPV infections, seroconversion may only appear months afterwards.[69] Not all hosts mount an antibody response to HPV and cross-reactive antibodies between different HPV types may complicate the picture.[70, 71] Although not sufficient to prove causality, serology may be used as a complement to other detection methods or to examine a population longitudinally.

HPV Carriage in Healthy Individuals

HPV is ubiquitous in the skin, and has been detected in newborns, young children, and adults.[72] With increasing age, more HPV types are isolated and seroreactivity to more types is seen.[72] HPV persistence with the same types is common, and family members often share the same types.[49, 73] Different studies using different sampling techniques (swab, hair-pluck, and/or serology, see Table 4) have shown that HPV types 5, 8, and 23 are most commonly detected, and persistence of the same HPV type is frequently seen. [49, 72, 74–78] Different ethnicities may harbor different HPV types.[79] The most prevalent type and the only type common to all countries studied (Japan, Bangladesh, Ethiopia, Zambia, Sweden) was HPV 5 (overall prevalence of 6.5%).

Studies Utilizing Skin Swab

A small study (n=16) of newborns and young children showed that 45% of newborns who underwent forehead skin swabs were shown to harbor HPV DNA within the first few days of life, and some shared the same types with their mothers.[72] By four years, HPV was detected in 70% of children.[72] Family members may share some of the same HPV types.[80] Another study arguing for intra-familial transmission showed the vast majority of HPV types in children are also found in one or both parents.[49] One-third of HPV types that persisted in parents also persisted in their children although no specific type predominated. Type-specific persistence was generally more common with adults (92%) than children (66%).[49] The same HPV types have been isolated from 48% of healthy adults after a period

of six years indicating chronic persistence.[73] Persistence has not been significantly associated with age, sex, history of warts, or HPV genus.[73] Viral transmission, although not systematically studied, conceivably occurs vis-à-vis direct contact with skin or its remnants.[81]

A great multiplicity in HPV genotypes has been observed in healthy, non-immunosuppressed adults with positivity ranging from 42%–87%.[49, 50, 73, 79, 80, 82–85] Some have investigated different HPV profiles among different ethnicities and climates.[79] HPV DNA prevalence is lower in Zambian samples compared to those from Sweden ($P<0.01$) and Bangladesh ($P<0.05$).[79] HPV-5 was the only type found in samples from all 5 countries (Bangladesh, Japan, Ethiopia, Zambia, and Sweden) studied.[79]

Sun-exposure,[84] history of skin cancer,[84] and increasing age[82, 83] have been implicated as risk factors for HPV detection. One study using serial sampling in 40 adults and children (484 total samples) showed that HPV positivity was on average higher from samples of the forehead (79%) and back of the hand (81%) than buttocks (64%) implicating UV radiation as a possible risk factor, though environmental exposure unrelated to sun is also a possibility.[49]

Studies Utilizing Hair-Pluck

A high rate of detection of various HPV types has been found in hair-pluck samples from healthy individual with certain types predominating. Earlier studies may show a lower rate but that may be due to less sensitive detection methods.[86] Detection ranges from 84–91% in larger studies and HPV 23 is the most commonly detected.[75, 87, 88] This may indicate that HPV 23 has a greater propensity to infect or maintain infection in the follicle than other types. However, HPV 23 DNA in hair follicles has not been shown to be significantly associated with seropositivity, whereas other HPV types have.[75] Thus HPV 23 may only cause transient infection or has the ability to evade the host immune system. HPV 24 and HPV 36 have also been reported to be common.[75, 87] β-HPV detection increases over time and is associated with age, but not with gender, skin type, sunburn or sun exposure.[87, 88]

Studies Utilizing Serology

Serologic analysis allows for larger scale studies. In addition, it allows tracking a population over time, analyzes trends in HPV infection, and complements other detection methods. A cross-sectional study from Germany examined the prevalence of the L1 (major capsid protein) antibody given 29 cutaneous and 5 mucosal HPV types from all five genera (alpha, beta, gamma, mu, nu) in 1,797 sera from the general population.[68] The overall seroprevalence for any of the 34 HPV types was about 60% but the type specificity depended on gender and age. For all types and both sexes, antibody reactivity was lowest in children.[68] Antibodies to the high risk HPV 16 were elevated after puberty in women but not in men.[68] Antibodies to HPV 8 increased with age, with prevalence peaks at 40 and 60 years in women and 50 and 70 years in men.[68] A London-based study of 928 subjects observed statistically significant differences between Caucasians and non-Caucasians, with higher β-HPV 93 in non-Caucasians and higher μ-HPV 1 and HPV 4 in Caucasians.[89] This association held for both immunocompetent and immunosuppressed subjects. No HPV seroprevalence difference was observed by immune status.[89]

Sun-exposure risk has also been assessed by examining populations from different geographic latitudes. Overall HPV prevalence (β-HPV types 50–57%, γ-HPV types 40–48%) and the most frequent β-HPV (HPV 8 followed by 15, 17, 38 and 49) and γ-HPV (HPV 4 and 65) were similar among healthy individuals living in the Netherlands, Italy and Australia.[90] These results are consistent with others looking at β-HPV types showing HPV

8, 15, and 38 to be the most prevalent in healthy controls.[74, 78] This may imply that the distribution of β-HPV types is similar amongst those of European ancestry and may be less attributable to sun exposure. An even higher rate of HPV seropositivity was found in 411 patients undergoing skin cancer screening in Florida (91.2% and 78.8% were positive to ≥1 and ≥2 cutaneous HPV types respectively).[91] Interestingly, antibodies to the L1 (the major capsid protein) and E6 viral proteins (a non-structural intracellular protein) are rarely found concomitantly.[92]

Is HPV involved in the development of SCC in immunocompetent individuals?

Tissue Evidence Indicating a Positive Correlation Between HPV Detection in SCC

Numerous studies have examined the presence of HPV in NMSC, pre-malignant and benign lesions as well as normal, uninvolved skin (Table 3). Some of these studies do not distinguish SCC from actinic keratoses (AK), Bowen's disease, or BCC. Others assess "tumors" or NMSC rather than SCC specifically. The inclusion of non-SCC tumors may mask or give a false association with HPV. Thus, for future studies, separation of the different NMSC subtypes should be performed.

Multiple studies have indicated a relationship between SCC and various HPV types in immunocompetent patients.[74, 82, 84, 93–99] When skin lesions and control skin are tape "stripped" (n=349), SCC and benign lesions are more often positive for HPV DNA than healthy skin samples.[94] However, HPV 1 was more common in benign lesions (odds ratio (OR) 3.47) while HPV 2 was more common in SCC than benign lesions (OR 4.40).[94] In general, HPV DNA was more associated with sun exposure (OR 4.28) in both lesions and healthy skin.[94] Localized immunosuppression from UV light, thus, may be contributing to higher HPV loads. Others that have shown higher HPV 2 detection in SCC (OR 4.0) did not find a link with sun exposure.[93] Interestingly, some have shown that the history of NMSC has been correlated with higher HPV detection in normal skin (OR 6.41), but higher HPV detection did not correlate to sun exposure.[95] To complement skin biopsy, Andersson also examined serum samples from the same immunocompetent individuals (n = 434).[74] When compared to benign lesions SCC showed increased HPV DNA (OR 2.08) in contrast to BCC and AK.[74] Nonetheless, seroreactivity to HPV was similar among groups, the only significant difference being higher in SCC than BCC patients.[74]

While most positive associations between SCC and HPV have been of the cutaneous beta types, high-risk mucosal types have been implicated in some reports.[97, 98, 100] Zheng *et al.* showed a higher percentage of extragenital Bowen's disease positive for HPV DNA than control skin with mucosal types detected in higher amounts through a semi-quantitative PCR.[98] Others have shown that HPV detection was the same in extragenital Bowen's disease from both non-and sun-exposed skin.[100]

Skin swabs from immunocompetent individuals have shown increased detection of HPV DNA from those who spent more time outdoors, those with a history of NMSC or those who actively have AK or SCC.[82, 84] In the Netherlands, presence of beta-HPV in plucked eyebrow hairs was associated with a three-fold risk for SCC, however this was not the case in Australia or Italy.[75]

Tissue Evidence Indicating a Lack of Correlation Between HPV Detection in SCC

Just as an abundant amount of data suggests a link between HPV and SCC, a great body of evidence suggests otherwise.[50, 85, 93, 94, 101–106] Although some studies do not find a significant difference in HPV detection rates, others note a predilection of certain HPV types

for SCC.[85, 93, 94, 102, 103] Newer studies examining viral messenger RNA levels, which correlate with viral activity, have not supported a transcriptional mechanism to link HPV with SCC.[107] The first study employing tape stripping of tumors found decreased HPV positivity in all samples (benign, premalignant, BCC, and SCC), however SCC were not significantly more positive than other tumors.[48]

Some studies have found HPV to be more common in AK rather than SCC, BCC, benign lesions, or normal skin.[85, 103, 105, 106] In fact, since HPV 2 had been previously linked to SCC but not benign lesions (see above), Vasiljevic *et al.* examined four HPV types of species 2 via real time-PCR in SCC, BCC, seborrheic keratoses, AK, and normal skin. The members of HPV species 2 were preferentially found in AK albeit in low copy numbers.[105] Previous to this study, Weissenborn *et al.* also examined HPV via real time-PCR and detected a higher load in AK.[106] HPV 5 and 8 (oncogenic types), as well as HPV 38 have also been implicated in AK.[85, 103] While oncoviruses are usually detected in the corresponding tumor tissue, some have argued that HPV may be involved in the early steps of oncogenesis but not in tumor maintenance, thus explaining its presence in pre-malignant lesions. If this were the case, then an explanation for tumoral loss of HPV infection after malignant degeneration is needed. Additionally, the studies that have examined AK and SCC did not take into account sun exposure, which may have been the reason for higher HPV detection in AK. In turn, HPV may not be involved in cutaneous oncogenesis but rather better adept at infecting normal or pre-malignant cells than cancerous cells.

Two studies (n= 85 and n=82 SCC), utilizing lesional, perilesional, and healthy skin biopsies from the same patient or other individuals did not show any difference in overall HPV DNA among SCC, benign lesions or healthy skin, but as indicated above, these studies did find HPV species 2 to be more common in SCC.[93, 94] Another large study (n = 101 SCC, 101 BCC) did not find any difference in HPV detection in SCC versus BCC but found SCC to be more often infected with HPV genus β-species 1 (including type 5 and 8), multiple HPV types, and certain HPV types reported to be important in skin cancer (5, 8, 15, 20, 24, 36, and/or 38).[102] Two smaller studies also did not find any difference in HPV detection between SCC and BCC.[101, 104] However, these studies did not examine normal skin controls.[101, 102, 104]

While previous studies have examined HPV DNA levels, messenger RNA transcriptome sequencing has recently been employed to assess β-HPV gene expression in SCC from both immunocompetent and immunosuppressed individuals. Interestingly, sequencing did not identify β-HPV expression in any of the SCC studied.[107] Although 30% of SCC tumors and 28% of normal skin samples did contain HPV DNA by nested PCR, the viral load was found to be extremely low, with most tumors having less than 1 HPV copy per cell.[107]

While the previously described studies examined skin biopsies, a few have examined eyebrow hair. Two studies (n= 64 SCC and 25 SCC) that examined plucked eyebrow hair in those with SCC did not find a significant association with HPV DNA when compared to those with normal skin, BCC, or AK.[92, 108]

Serologic Evidence

Several relatively large studies and some smaller ones have examined the association of SCC with serum antibodies against HPV (Table 5). Most studies indicate a positive relationship between antibody response to HPV (whether against a single or specific type or multiple types) and SCC development, [75, 78, 92, 109–111] with only one study failing to find any correlation.[74]

The two largest serology-based studies have both indicated a positive correlation. An international, multi-center, epidemiologic study (689 SCC cases, 845 controls) showed that a positive antibody response to four or more β-HPV types was associated with SCC (OR 1.8), especially among fair skinned individuals.[75] Antibodies against certain types correlated with eyebrow HPV presence. [75] These may indicate that some HPV types cause only transient infection or may avoid detection by the host's immune system. In the other large study (663 SCC cases, 898 BCC cases, 805 controls), Karagas et al showed a correlation with an antibody response against HPV and SCC but not BCC, with the odds ratio for SCC increasing with increasing number of β-HPV types.[112] These findings confirm the results of an earlier study by the same group (252 SCC cases, 525 BCC cases, and 461 controls) which detected HPV antibodies more frequently in SCC patients (OR = 1.6) than in controls; multiple β-HPV antibodies further increasing that risk.[78] Seropositivity to β-HPV types 5,[78] 8,[92, 109, 111, 113] 15,[110] 17,[110] 20,[113] and 38,[110, 113] and gamma HPV 50[110] have all been implicated in SCC development. In 107 patients, HPV seropositivity at baseline has been associated with a risk of developing a second SCC after five years for a number of beta, gamma types and one nu type (HPV 5, 9, 15, 17, 23, 24, 41, 76).[114] Interestingly, one study actually found a negative correlation between HPV 15 serology and SCC.[111]

Those with SCC on chronically sun-exposed sites were more likely to have antibodies to β-HPV types than those with SCC at sun-protected sites.[78] In an interesting, albeit small study, HPV L1 protein seroreactivity was shown to increase and E6 seroreactivity to decrease with the severity of the skin lesion (control, AK, SCC). The authors speculated that SCC patients may not have the ability to mount a response to HPV E6 proteins or that antibodies to E6 may be protective.[92] Antibodies to β HPV have been detected close to or at the time of SCC occurrence but not before, and more frequently in those with SCC of sun-exposed sites.[78] Seropositivity to HPV 8 viral like particles has been associated with the development of actinic keratoses.[115]

Not all serologic studies have found a positive correlation with HPV and SCC. Andersson *et al.* examined both tissue and serum samples and reported that seroprevalence was the same for patients with SCC versus controls but higher in SCC patients versus BCC patients.[74] The presence of HPV DNA in tissue and antibodies to the same HPV type in the serum were not significantly correlated, but seropositivity to any HPV type was more common among those positive for cutaneous HPV DNA of any type. Plasmeijer *et al.* followed a cohort of 1,311 participants who had serologic testing for β- HPV types from 1992–2007 for the development of new SCC (n = 150). No correlation was found between HPV serology and the later development of SCC even with stratification by sex, skin color, and sunburn propensity. However, when a sample subset (cases collected in 1992) was stratified, a two-fold increased risk in SCC was found in HPV serologically positive individuals.[116]

HPV Carriage and Immunosuppression

As with the immunocompetent, HPV carriage is common in immunosuppressed individuals with HPV 5, 20, 23, and 24 being most prevalent.[73, 76, 83, 87] In a large (n = 351) biopsy-based study, HPV positivity was evident in one-third of normal skin samples from renal-transplant recipients (RTR).[117] Harwood *et al.* examined skin samples from normal, sun exposed and non-sun exposed sites in 39 immunocompetent individuals and 38 RTR showing an HPV prevalence of 86% in RTR, twice as much compared to their immunocompetent counterparts.[95] Sun-exposure, duration of transplantation, age, sex, history of NMSC did not increase risk for HPV detection.[95] However, after adjusting for a history or presence of NMSC, β-HPV types were more common on the skin of RTR (OR 21); mixed infections with different HPV types were also more common.[95] In contrast to that study[95], a case control study from Germany showed that HPV prevalence from benign

lesions and normal skin was similar in both transplant and non-transplant groups.[118] Another study analyzing benign keratotic lesions in RTR with and without a history of skin cancer found similar prevalence rates of 55% and 53% respectively.[119] A large variety of β-HPV types were seen with higher prevalence in sun exposed areas of those with a history of skin cancer.[119]

Antonsson *et al.* examined skin swabs from RTR, dialysis patients, and healthy controls revealing that RTR had increased HPV positivity when compared to either group (Table 4).[83] However, around 12% of the RTR had a history of skin cancer, whereas no skin cancer was reported in the other groups. HPV DNA was also more prevalent on the forehead when compared to other sample sites,[83] consistent with other studies indicating increased HPV detection in sun-exposed areas. Others have shown similar initial HPV rates between immunocompetent subjects and RTR (69% vs. 71% respectively) but higher rates of persistence in RTR (71% vs. 90%) six years later.[73] Most persistent infections were β-HPV types with HPV 20 predominating.[73]

A few studies have examined HPV DNA in plucked hairs from the immunosuppressed.[87, 117] The largest of these examined 560 OTR from five European centers and found that HPV detection prevalence of 81%–98% and HPV 23 to be the first or second most common type.[87] HPV 24, 36, 38 were also highly prevalent. The results were very similar to immunocompetent individuals. [87] As indicated earlier, this may indicate greater HPV 23 maintenance of infection in the hair follicle when compared to other HPV types. Another hair pluck study looking specifically at HPV 5, a high risk type associated with EDV, found increased among RTR although there were no differences in samples from individuals with and without skin cancer in either group.[76] For the most part seroprevalence in this population is similar to the immunocompetent, with the only difference being that reactivity to HPV 8 is increased in the immunosuppressed.[89, 120, 121]

Antibodies against HPV 8 (high-risk EV type) viral particles have been found to be significantly higher ($p < 0.001$) in RTR regardless of history of skin cancer (21.1% vs. 7.6% controls).[122] Several studies have examined antibodies to HPV in the immunosuppressed without history of skin cancer, including two large UK-based studies.[89, 120] These studies indicated no difference in seroprevalence between immunocompetent or immunosuppressed individuals.[89] Seroprevalence to at least one HPV type in OTR varied from 86%–90%.[89, 120] Seropositivity to high-risk mucosal types was higher in women, younger patients, and non-Caucasians.[89, 120] Immunocompetent individuals with a history of NMSC also had higher seroreactivity (45%) when compared to healthy controls.[122]

Is HPV involved in the development of SCC in immunosuppressed individuals?

The evidence implicating HPV in the pathogenesis of NMSC in immunosuppressed individuals is mixed (Table 5). Several studies have linked HPV with SCC but many studies have been small and others uncontrolled. Newer studies have shown low transcriptional activity of the virus. Thus even though HPV DNA has been detected in prior studies, the low rate of DNA transcription may indicate that HPV is not involved in cutaneous oncogenesis.

The largest of these studies (210 SCC cases, 394 controls) showed that individuals with both β-HPV DNA (from hair pluck samples) and antibodies against the same type (concordant DNA and antibodies) were at significantly increased risk of SCC, even if each measure did not independently show an association.[123]

Biopsy-based studies comparing lesions from OTR versus those from non-immunosuppressed individuals showed a higher rate of HPV detection in SCC from OTR but not BCC, warts, or pre-malignant lesions.[118, 124] These studies have shown HPV 5 and 8 to be more frequent in SCC of OTR.[118, 124] Another study (n =148) revealed a difference of HPV detection in SCC (84% vs. 27%), BCC (75% vs. 36%), and pre-malignant lesions (88% vs. 54%) between the two groups.[125] This result is consistent with earlier, smaller studies.[104, 121] However, one may argue that this finding may simply be the result of a higher viral load in the immunosuppressed and does not necessarily imply a role for HPV in cutaneous oncogenesis. This could potentially be controlled for by assessing uninvolved skin from OTR with and without a history of skin cancer and OTR SCC lesions. Longitudinal studies of viral load and number of types in OTR and the subsequent development of SCC would also be helpful.

Uncontrolled studies have indicated high HPV detection in both premalignant lesions and SCC from RTR (81–91%).[119, 126] An earlier, uncontrolled *in situ* hybridization (ISH) study showed around 50% HPV detection in SCC from RTR.[127] A biopsy based study examining normal skin in OTR with and without history of NMSC did not find a significant difference of HPV positivity between the two groups, although the group without history of NMSC was small.[95]

Not all studies point toward an HPV association with SCC in immunocompromised patients. Two studies indicated a similar HPV DNA load in OTR SCC, BCC, pre-malignant, and benign lesions.[125, 128] Some have shown a significant association only when the analysis combined BCC and SCC.[85] A very early small study using RT-PCR failed to detect any HPV DNA in 28 non-genital SCC from immunosuppressed RTR.[129] This may have been technique dependent, as using more sensitive primer sets, Southern blotting or RT-PCR could have increased the positive yield.[129, 130] A more recent study showed that a high percentage (75%) of OTR SCC were positive for HPV when screened by PCR; when examining transcriptional activity and DNA localization via RNA and DNA ISH positivity was 38% and 36% respectively.[131] The authors feel that the detection of transcripts in keratinocytes suggests that HPV has an active, rather than passive presence in these lesions. The same authors found relatively low transcriptional activity in warts, and suggested that the ISH technique used may lack sensitivity and therefore the low levels in SCC may be an underestimate.[131] Although PCR HPV positivity may have been higher, the authors claim that ISH may be more biologically relevant (infection versus carriage or contamination).

A newer method employing transcriptome sequencing of HPV in SCC from both RTR and immunocompetent patients noted an absence of HPV gene expression (see above).[107] A study analyzing lesions from OTR demonstrated low viral DNA load (for HPV 8, 9, 15) in SCC and transcriptional activity (at low levels) of E6/7 (for HPV 5, 8, 9, 15 and 20) in only one of seven SCC.[132] Analysis of serial dilutions of HPV DNA from NMSCs revealed that HPV DNA could only be detected in undiluted DNA solutions, and based on the dilution of β-globin genes, it was estimated that HPV DNA was less than one viral genome per cell.[133] Studies of HPV seroprevalence in immunocompromised individuals with history of SCC are scant. Longitudinal examination of different HPV type seropositivity in this population and the correlation with new SCC would be a beneficial complement to tissue studies.

Conclusion

Although it is clear that UVR and immunosuppression predispose to SCC, the exact role of HPV in cutaneous oncogenesis remains to be elucidated. The evidence remains mixed for both immunocompromised and immunocompetent populations. Challenges include the abundance of HPV types, high rate of carriage in normal skin, co-occurrence of multiple

types,[134] immunologic cross-reactivity between types, and possibility that HPV may be involved in initiation of oncogenesis but not in tumor maintenance.

To demonstrate that a pathogen causes cancer, we must have convincing epidemiologic evidence of association and a plausible biologic mechanism for oncogenesis. This has been demonstrated for the high-risk alpha-papillomaviruses and cervical cancer, but we have not yet satisfied these requirements to demonstrate that beta-papillomaviruses causes cutaneous SCC. HPV appears to be ubiquitous in the skin, with increased prevalence in the setting of sun exposure or immunosuppression. The studies described here used multiple sampling and detection techniques, relatively small sample sizes (with some exceptions), and detection restricted to specific HPV types. Due to this heterogeneity in study design, a meta-analysis to power a true summary estimate of association might prove difficult.

More recent studies have focused on the biologic mechanism for oncogenesis (reviewed by Arron[10]). While a complete review of this topic is beyond the scope of this review, it is important to recognize that evidence for low DNA viral loads and minimal gene expression in tumor tissue argue against HPV involvement in tumor maintenance. *In vitro* research has revealed that inflammatory gene expression by E6/E7 differs among alpha and beta genera[135] and that beta-papillomaviruses E6/E7 proteins do not interfere in UVB-induced apoptosis.[136] However, HPV may have a different, subtler involvement in cutaneous oncogenesis. The traditional notion that induction, proliferation, and the malignant phenotype must depend on the persistence of viral nucleic acid for an agent to be regarded causative may not apply in this setting. If HPV promotes cutaneous oncogenesis through the "hit-and-run" phenomenon, acting as a co-carcinogen with other environmental factors such as UV radiation,[137] then high risk patients may have to be followed temporally by tracking rates of HPV infection through various detection methods. Future SCC development in these patients will then have to be correlated with previous infection, since at the time of tumor development some HPV types may have been cleared.

The establishment of HPV involvement in SCC formation will have important implications for screening and prognosis. HPV may only be involved in a subset of SCC or in a high-risk population. Detection of specific HPV types in cutaneous SCC may indicate a better prognosis as is the case for HNSCC.[138] Conversely, it may carry a worse prognosis. In either case, clarifying the nature of this association, if any, would help tailor treatment. If HPV infection is associated with SCC in certain high-risk individuals, early vaccination against certain types may decrease future disease burden. This would be particularly relevant to the OTR population. Clarification of any viral mechanism in cutaneous SCC development would lead to enhanced treatment options as well as a reduction of the significant healthcare costs associated with this cancer.

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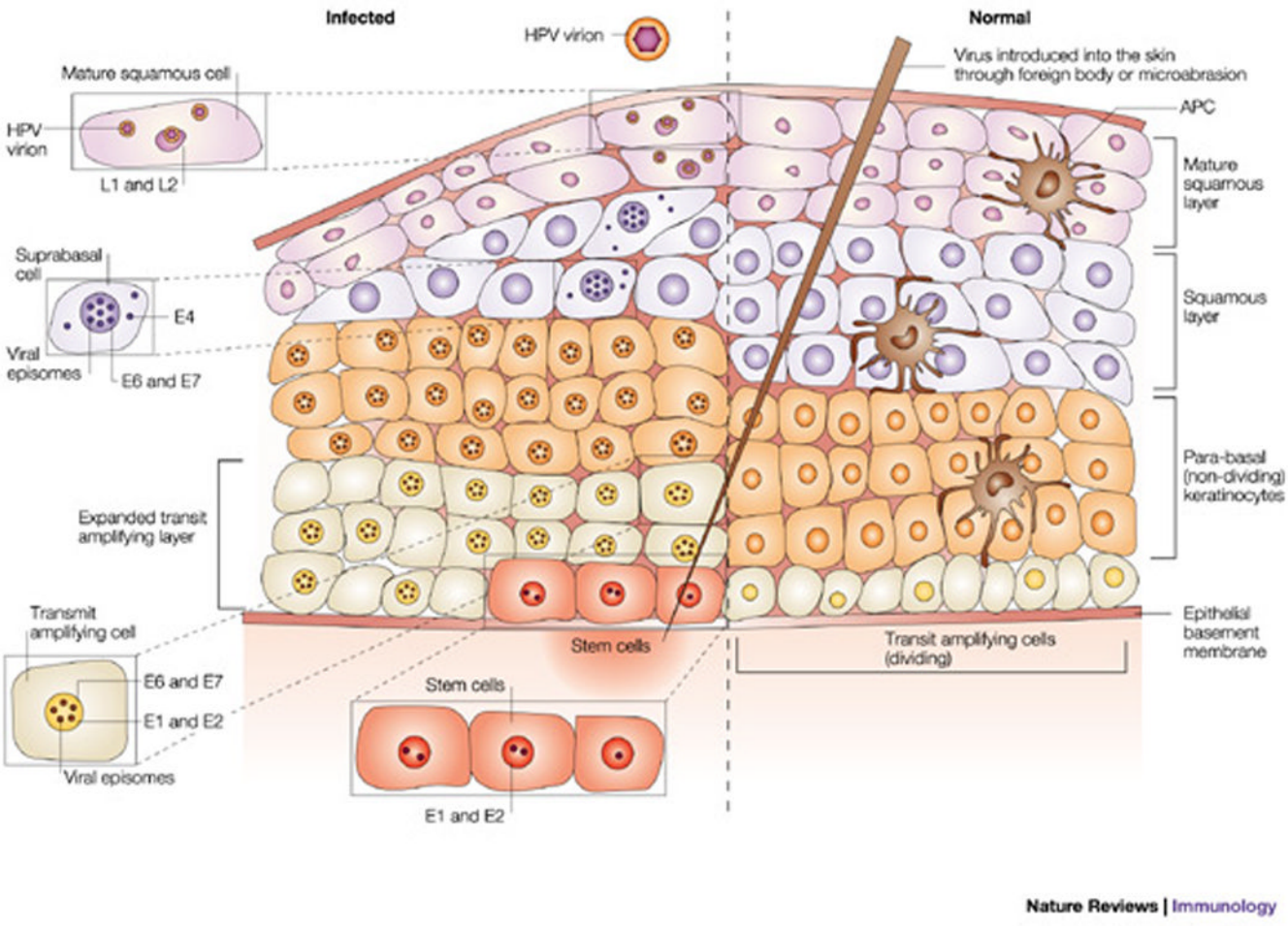

Figure 1. Cervical stratified squamous epithelial cell architecture and the expression of human papillomavirus (HPV) proteins after infection

Daughter cells of epithelial stem cells divide along the basement membrane and then mature vertically through the epithelium without further division (right side). After introduction of HPV into stem cells in the basal layer of the epithelium, expression of viral non-structural proteins occurs. Under the regulation of these proteins, the dividing-cell population expands vertically and epithelial cell differentiation is delayed and is less complete. Viral proteins are expressed sequentially with differentiation as shown, and mature virions are produced only in the most superficial layers of the epithelium. Intraepithelial antigen-presenting cells (APCs) are depleted in the HPV infected epithelium. Reproduced with permission from[21].

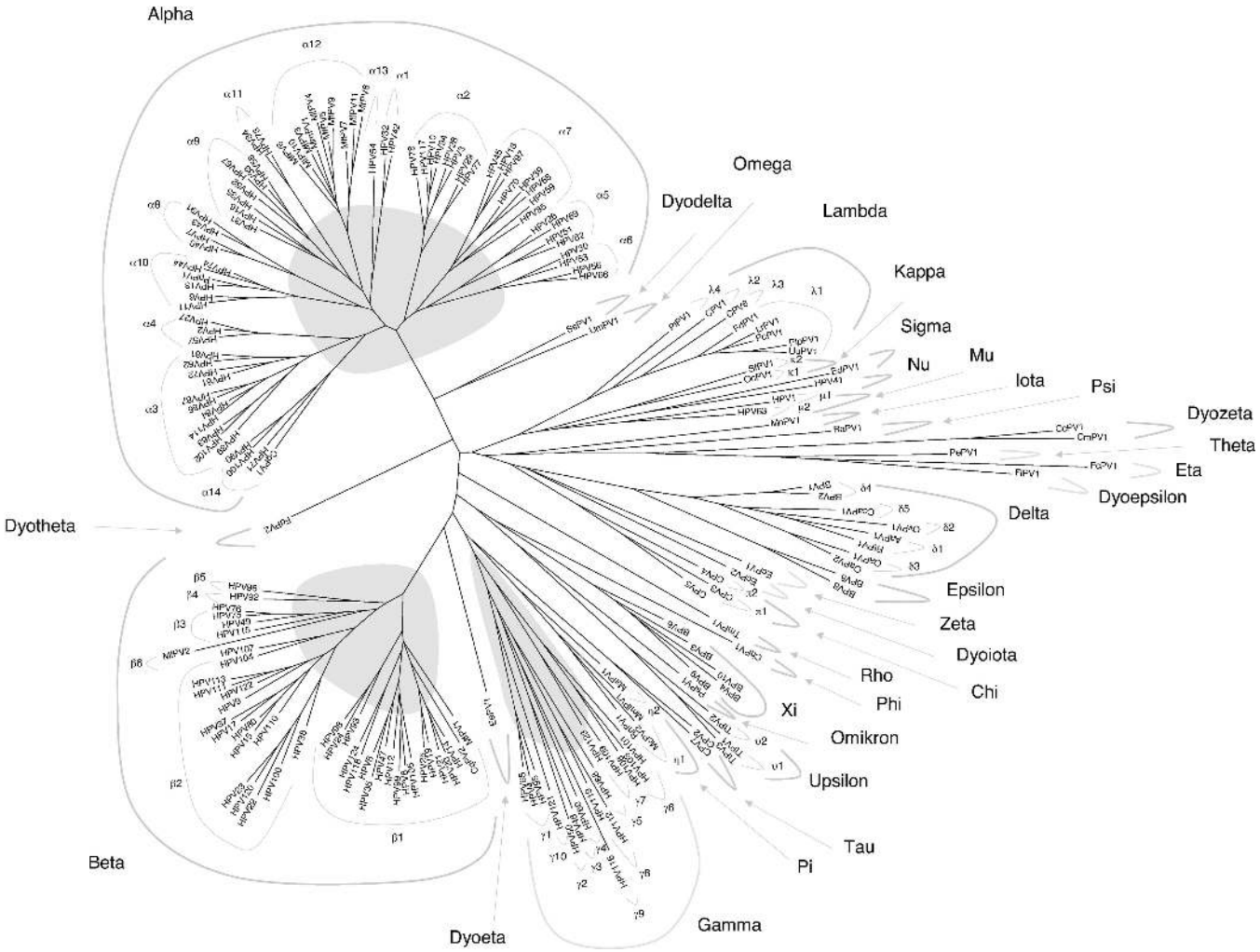

Figure 2. Phylogenetic tree inferred from the L1 nucleotide sequences of 189 papillomaviruses
Reproduced with permission from[25].

Table 1

Mechanisms of Viral Oncogenesis

HPV: human papillomavirus, EBV: Epstein-Barr virus, HHV-8: human herpesvirus-8, MCV: Merkel cell polyomavirus, HTLV-1: human T-lymphotropic virus-1, HBV: Hepatitis B Virus, HCV: Hepatitis C Virus.

Mechanism	Virus Type	Example and Associated Human Malignancy
Direct		
Viral encoded oncoproteins target cellular tumor suppressor genes	DNA oncoviruses	HPV: Cervical carcinoma EBV: Burkitt's lymphoma HHV-8: Kaposi's sarcoma MCV: Merkel cell carcinoma
Retroviral encoded oncogenes are re-incorporated into host genomic DNA and are aberrantly expressed	Transforming retroviruses	HTLV-1: Adult T-cell leukemia/lymphoma
Indirect		
Retroviral integration near cellular oncogenes disrupts their expression	Nontransforming retroviruses	None Described
Chronic inflammation and progressive fibrosis	Various	HBV, HCV: Hepatocellular carcinoma

Table 2
Sampling methods for detection of HPV

Sampling Method	Advantages	Disadvantages
Skin Swab	• Painless • Multiple samples at one time • Inexpensive • Allows study of large populations • Allows study of individuals and populations over time • Minimal risk	• Doesn't distinguish between contamination versus true infection • Doesn't distinguish between transient or lasting infection • Limited to a specific body site • Normal hosts are highly positive • Doesn't analyze deeper infection
Hair Pluck	• Less invasive than biopsy • HPV reservoir may be follicle • Multiple samples at one time • Minimal risk • Allows study over time	• Doesn't analyze tumor • Limited to specific body site
Biopsy	• Viral DNA and expression within deeper layers of tumor or sample. • "Stripped" biopsies further reduce contamination	• Invasive • Limited samples • Limited to a specific body site
Blood Draw	• Simple to obtain • Inexpensive • Allows study of large populations • Allows study of individuals and populations over time	• Only serology can be obtained

Table 3

Selected Detection Methods for HPV

PCR: Polymerase chain reaction. DNA: Deoxyribonucleic acid.

Detection Method (material detected)	Advantages	Disadvantages
Type-specific PCR (DNA)	• Excellent sensitivity • May be nested for additional sensitivity • May be used for viral load quantitation (qPCR)	• Limited to a single type • Requires significant labor and input material to detect multiple types
Degenerate PCR (DNA)	• Coverage of multiple types, typically within one genus • Good sensitivity • May be nested for additional sensitivity	• Requires secondary readout to determine specific types detected
Microarray (DNA)	• Broad coverage of multiple types • May be used as secondary readout of degenerate PCR	• Less sensitive than PCR when used as a primary detection method • More expensive than PCR • Requires specialized equipment
Serology (Antibody)	• Not limited to specific body sites • Non-invasive • Allows study of large populations • Allows study over time	• Not all hosts mount an immune response • Not all viruses detected by immune system • May have cross-reactive antibodies between HPV types • Seroconversion may happen weeks to months after infection • Indicates cumulative exposure, not necessarily relevant exposure

Table 4
HPV is frequently detected in the absence of SCC

Selected studies assessing HPV carriage in normal skin or HPV antibodies in the serum of subjects with no history of SCC. Studies limited to those of sample size ≥75. NR: not reported.

Study	Cases	Sampling Method	Immune status	Findings
Michael [68] 2008	1,797 samples from 1,797 German adults and children (758 males and 1,039 females)	Serology	NR	• Antibodies to μ- and ν- PV appear early in life, those to mucosal α-PV in women after puberty, and antibodies to β- as well as to γ-PV accumulate later in life.
de Koning[87] 2009	1405 SCC-free immunocompetent (n=845) and immunosuppressed (n=560) individuals from six countries of different latitudes	Hair pluck	Immunocompetent and Immunosuppressed	• The frequency of β-PV-positive participants ranged from 84 to 91% in the immunocompetent population with HPV23 as the most prevalent type, and from 81 to 98% in the Immunosuppressed population with HPV23 as the most or the second most prevalent type.
Casabonne[89] 2009	928 samples; 409 OTR patients without skin cancer, 367 individuals with end stage renal failure on dialysis, 152 immunocompetent individuals without skin cancer	Serology	Immunocompetent and Immunosuppressed	• No difference between HPV seroprevalence by immune status was observed • Significant differences in HPV seroprevalence were identified according to ethnicity
Waterboer[90] 2009	807 samples from 807 healthy individuals from the Netherlands, Italy and Australia	Serology	Immunocompetent	• Overall HPV seroprevalence was similar across the three countries (50–57% for β-PV types, 40–48% for γ-PV types), and the most frequent β-PV and γ-PV types were the same in all countries.
Weissenborn[49] 2009	484 samples from 40 individuals: 14 children, 10 mothers, 10 fathers, and 6 grandparents	Skin swab	Immunocompetent	• More than 75% of the HPV types in babies were also detected in their parents • Type-specific persistence for at least 9 months was more prevalent in parents (92%) than in children (66%)
Casabonne[120] 2009	425 samples from 425 individuals	Serology	Immunosuppressed	• 86% of participants were seropositive to at

Study	Cases	Sampling Method	Immune status	Findings
				least one HPV: 41% to mucosal α types, 33% to cutaneous α types, 57% to α types, 56% to β, 47% to γ types and 45% to other types
Iannacone[91] 2010	411 samples from 411 individuals	Serology	NR	• The seroprevalence of 1 or more cutaneous HPV type was 96% from men and 90% for women. • Seroprevalence was highest for HPV types 4 (46%), 1 (37%), and 8 (31%) in men and for types 4 (47%), 63 (34%), and 1 (33%) in women.
Antonsson[79] 2003	248 samples: 50 individuals from, Bangladesh; Sweden; Ethiopia; Zambia; and from 48 individuals from Japan	Skin swab	Immunocompetent	• The prevalence of HPV DNA in the material from Bangladesh was 68 %, Japan 54 %, Ethiopia 52 %, Zambia 42% and Sweden 70 %. • A great multiplicity of genotypes was demonstrated • Most prevalent HPV type was HPV-5, with an overall prevalence of 6.5%.
de Koning[88] 2007	184 serial samples from 23 individuals	Hair pluck	Immunocompetent	• 61% of the individuals were β-PV DNA positive for one or more types at intake, whereas during follow-up this percentage rose to 96 %. • HPV23 was the most frequently detected β-PV type. • Type-specific β-PV DNA was detected over 6 months or longer in 74% of the individuals. • In 57% of the individuals,DNA from multiple β-PV types was detected simultaneously for 6 months or longer.
Boxman[76] 1999	135 samples from immunocompetent individuals (69 with history of NMSC and 66 without), 31 samples from	Hair pluck	Both	• HPV 5 was detected in hairs derived from 14 of 31 (45%) immunosuppressed patients and 21 of 135 (16%)

Study	Cases	Sampling Method	Immune status	Findings
	immunosuppressed individuals			immunocompetent individuals. • HPV 5 DNA was detected in similar proportions of hair samples plucked from individuals with and without skin cancer in either group.
Hazard[73] 2006	126 samples: 42 healthy individuals, 21 renal transplant recepients	Skin swab	Immunocompetent and Immunosuppressed	• Among the healthy individuals, the prevalence of HPV was 69% (29/42) at baseline and 71% (30/42) at follow-up. Among those positive at baseline, 48% (14/29) had a persistent infection. • Among the RTRs, 71% (15/21) were positive for HPV at baseline and 90% (19/21) at follow-up. Persistent infection was detected in 33% (5/15).
Harwood[95] 2004	124 samples from sun-exposed and nonsun-exposed sites form 39 immunocompetent individuals and 38 renal transplant recepients, both with and without NMSC	Biopsy	Immunocompetent and Immunosuppressed	• HPV DNA was detected in 58 / 67 (87%) and 20 / 57 (35%) samples from renal transplant and immunocompetent patients, respectively. • There was no difference in either the prevalence or spectrum of HPV types found in sun-exposed and non sun-exposed normal skin. • Significant association between NMSC and the presence of EV HPV DNA in normal skin
Antonsson[72] 2003	115 samples from 38 infants and 31 parents	Skin swab	Immunocompetent	• HPV infections of normal skin are acquired very early in infancy and are caused by a great multiplicity of HPV types
Chen[84] 2008	100 immunocompetent individuals, 50 with history of frequent sun exposure and 50 without	Skin swab	Immunocompetent	• HPV prevalence was higher in individuals who spent more time outdoors and in individuals with a history of skin cancers (p=0.044 and p=0.04, respectively). • Furthermore, individuals wearing

Study	Cases	Sampling Method	Immune status	Findings
				sunglasses as a means of sun protection had a lower prevalence of HPV (p=0.018). • HPV-76 was only detected in the group without frequent sun-exposure

Table 5

HPV is frequently correlated with SCC

Selected studies examining the association between HPV and SCC as compared to normal skin, benign skin lesions, or non-SCC tumors. Studies limited to those of sample size ≥75. NR: not reported.

Study	Cases	Controls	Sampling Method	Correlation	Immune status	Finding
Bouwes Bavinck[75] 2010	689	845	Hair pluck and serology	Positive in some subsets	NR	• A positive antibody response against 4 or more β-PV types associated with SCC Australia, the Netherlands, and fair-skinned Italians • β-PV infection was significantly higher in cases than controls in the Netherlands but not in Italy or Australia
Karagas[112] 2010	663 SCCs 898 BCC	805	Serology	Positive in SCC but not BCC	Immunocompetent	• SCC cases, but not BCC cases, showed a higher prevalence of each of the individual β-HPVs tested a compared with controls. • The odds ratios for SCC increased with the increased number of β types positive.
Karagas[78] 2006	252	461	Serology	Positive	NR	• HPV antibodies more frequently in SCC patients than in controls • Highest SCC risk was associated with positivity for multiple HPV types
Proby[123] 2011	210	394	Hairpluck and serology	Positive with concordance	Immuno-suppressed	• β-HPV DNA was highly prevalent (>94%) with multiple types frequently detected in both groups. • Individuals with both β-HPV DNA and antibodies present for the same HPV type (concordant DNA and antibodies) were at significantly increased risk of SCC, even if each measure did not independently show an association.
Feltkamp[113] 2003	161	333	Serology	Positive	Immuno-competent	• SCC relative risk was significantly increased in those seropositive for HPV8 and HPV38
Plasmeijer[116] 2011	150	None	Serology	None but positive in a subset	NR	• No association found between the presence of any β-HPV L1 antibody and the occurrence of SCC, even with stratification by sex, skin color, and sunburn propensity. • No significant association between antibodies to any individual β-HPV type examined and the later development of SCC.

Study	Cases	Controls	Sampling Method	Correlation	Immune status	Finding
						• Set of samples from 1992: Individuals who were less than 50 years the presence of β-HPV antibodies was associated with a two-fold increased risk of SCC.
Paradisi[114] 2011	107 (SCC and BCC)	None	Serology	Positive for SCC, Negative for BCC	NR	• HPV seropositivity at baseline was strongly associated with the risk of developing a second SCC after 5 years for a number of beta and gamma HPV types (• No association between serological status for any HPV type tested and an increased risk of BCC was found.
Patel[102] 2008	101	101 BCC cases	Biopsy	Positive	Immuno-competent	• SCC lesions were significantly more likely to be infected with HPV genus β-species 1 (includes types 5 and 8), than BCC samples
Asgari[93] 2008	85	95	Biopsy	Positive and Negative	Immuno-competent	• No difference in HPV detection across various HPV species in case versus control tissue. • HPV DNA from β-HPV species 2 was more likely to be identified in tumors than in adjacent healthy tissue among cases.
Forslund[94] 2007	82	92	Biopsy	Positive and Negative	Immuno-competent	• Similar HPV detection rates in SCC and benign lesions. • β-PV species 2 predominate in squamous cell carcinomas; species 1 are primarily found in benign lesions
Berkhout[117] 2000	81	31	Biopsy	Positive	Immuno-suppressed	• HPV DNA more common in SCC than normal skin