

Human papillomaviruses and non-melanoma skin cancer: Basic virology and clinical manifestations

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Abstract. Human papillomaviruses (HPV) infect cutaneous and mucosal epithelia and induce benign and malignant lesions. Non-melanoma skin cancer (NMSC), encompassing basal cell carcinoma and squamous cell carcinoma (SCC), is the most frequent cancer in the Caucasian population, and the incidence has increased dramatically worldwide. Ultraviolet (UV) radiation is a major risk factor for NMSC, and cutaneous HPV is also considered to play an active role during the pathogenesis of these cancers. The first evidence for the involvement of HPV in NMSC was reported in patients with Epidermodysplasia verruciformis (EV). HPV types detected in skin tumours of these patients are referred to as EV/cutaneous HPV types belonging to the beta- and gamma-papillomaviruses (PV). Epidemiological studies have shown a higher risk of several EV/cutaneous HPV types for NMSC. Furthermore, *in vitro* and animal models show transforming properties of some PV types. The anti-apoptotic activities, and the delay of DNA repair mechanism caused by some EV/cutaneous HPV E6 proteins in response to UV-induced mutations, may lead to the persistence of DNA-damaged keratinocytes. Thus, specific EV/cutaneous HPV types as co-factors in association with UV-radiation and the immune system seem to be involved in the early pathogenesis of cutaneous SCC.

Abbreviations: AK, actinic keratosis; EV, Epidermodysplasia verruciformis; HPV, human papillomavirus; LOH, loss of heterozygosity; NMSC, non-melanoma skin cancer; PV, papillomavirus; SCC, squamous cell carcinoma; OTR, organ transplant recipient; UV, Ultraviolet.

1. NMSC and ultraviolet radiation

Non-melanoma skin cancer (NMSC), comprising basal cell carcinoma (BCC), Bowen's disease, cutaneous squamous cell carcinoma (SCC), and its early stage actinic keratosis (AK), is the most frequent malignancy among populations of European origin [1–5]. The tumours are readily diagnosed on the basis of clinical and histopathological appearance (Fig. 1A–C). BCC rarely metastasises and shows ingrowths of epidermal keratinocytes adjacent to palisades of cells resembling those that constitute the lower basal layer [6,7]. They frequently occur on the face and are

characteristically indolent, small, pearly edged lesions with sometimes an ulcerated centre. On the contrary, SCC are invasive tumours, whose cells histologically appear like differentiated suprabasal keratinocytes, and approximately 3% metastasises [7]. They grow faster than BCC and produce a more indurated, hyperkeratotic lesion with ulceration. AK and cutaneous SCC frequently occur as multiple primary tumours in the same skin area ('field') in proximity to each other ('field cancerisation'; Fig. 1) [8].

During the last decade, worldwide incidence of NMSC has epidemically increased and now represents approximately 30% of total cancers [9–12]. Particularly, light-skinned and sun-sensitive individuals are affected, and the highest frequencies of the disease are found on sun-exposed sites of the body (Fig. 1G–J) [13]. Furthermore, skin cancer risk is relatively increased in

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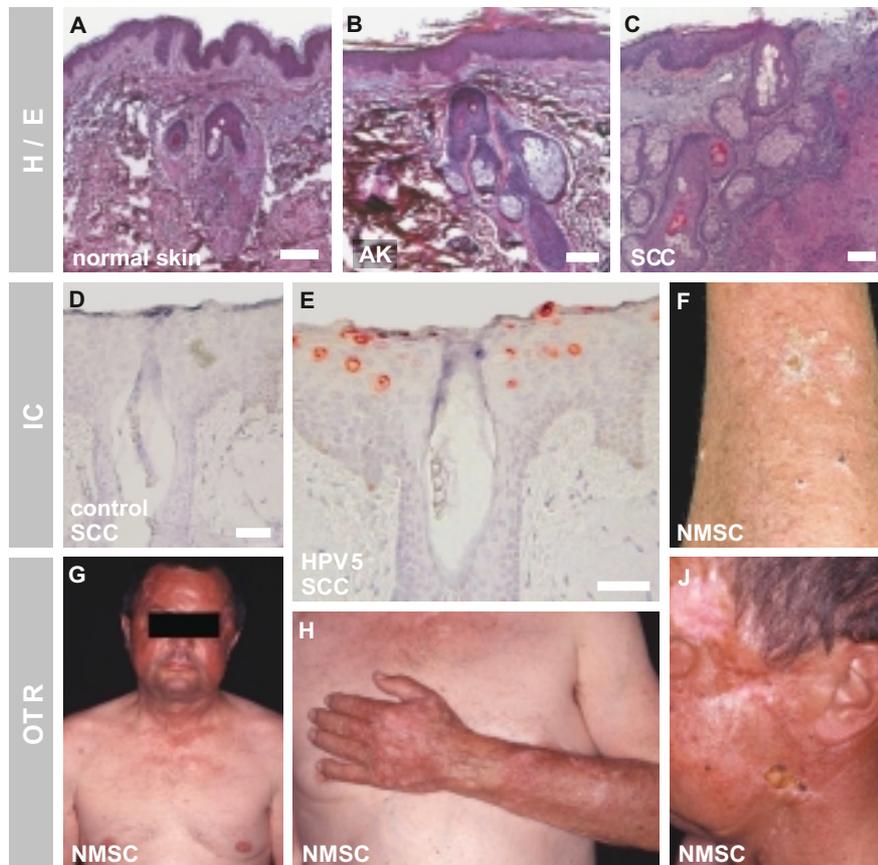


Fig. 1. Non-melanoma skin cancer (NMSC) from an immunocompetent (IC) patient and an immunosuppressed organ transplant recipient (OTR). Sections of paraffin embedded skin tissues stained with haematoxylin and eosin (H / E) for histology (A-C). Normal skin (A), actinic keratosis (AK) (B), and cutaneous squamous cell carcinoma (SCC) (C). Presence of beta-human papillomavirus (HPV) DNA in dysplastic cells of cutaneous SCC (IC patient, 86 years old female) shown by *in situ* hybridisation (ISH) (A. Köhler and I. Nindl, unpublished data) (D,E). Negative control using the HPV 16 genome (D), tyramide signal amplification after ISH, red staining indicate HPV 5 DNA in the nucleus. HPV 5 DNA was not detected in all dysplastic cells, and note the enlarged nuclei in the HPV positive dysplastic cells (E). Field cancerisation with multiple AK and SCC is shown on the forearm of an IC patient (F). Development of multiple AK and SCC exclusively on sun-exposed areas as demonstrated with an immunosuppressed OTR (G-J). Head and chest (G), trunk and left arm (H), left lateral face (J). Originals, magnification scale bars indicate 50 μ m.

Caucasians who have been born in countries close to the equator, compared to those who have migrated to them after birth, indicating that the exposure to solar radiation early in life has importance for tumourgenesis [14]. This highlights the role of ultraviolet- (UV) rays as a major environmental risk factor for the development of epithelial skin cancers [15–18]. The major physical adaptation to UV-radiation, which is the most ubiquitous human carcinogen, is pigmentation of the skin, whose degree is largely based on inheritance.

UV-radiation is a component of sunlight and comprises three wavelength classes, UV-C (ranging from 190 to 280 nm), UV-B (280 to 320 nm), and UV-A (320 to 400 nm). The non-ionising radiation cumulatively induces NMSC by at least two interacting rather than

separate mechanisms, causing permanent DNA damage and preventing local immunologic recognition of mutant cells [19]. UV-A induces photooxidative stress and secondarily characteristic genome mutations, while UV-B irradiation results directly in the formation of cyclobutane (thymidine) dimers and transitions in DNA. Because of the absence of effective repair mechanisms, such genetic changes lead to permanent mutations in keratinocytes, which subsequently have the potential to progress into epithelial skin cancer [15,18,20,21].

The development of BCC shows a different etiology compared to SCC and is rather marginally subjected to the immune system. It is considered a hair follicle-derived tumour [22] resulting from relatively few genetic alterations that are largely well understood [17].

More than 70% of hereditary and non-hereditary BCC carry a loss of heterozygosity (LOH) at 9q, and loss of 9q22-31 is associated with the Gorlin's syndrome (nevoid BCC syndrome) [23]. In turn, patients suffering from that syndrome exhibit a high risk to develop BCC during the entire lifetime, but are frequently affected by the presence of multiple BCC already at early age [24,25]. The autosomal dominant disorder is based on a germline mutation and persistent inactivation of the tumour suppressor gene *PTCH1* located at chromosome 9q [26–28] that is involved in the sonic hedgehog (SHH) signalling pathway resulting in activation of the transcription factor *Gli1*.

Despite increasing knowledge of BCC development, little is known about the causal alterations of cutaneous SCC. However, the development of SCC clearly results from the mutual interaction of multiple factors that is reflected by, for example, distinct LOH patterns of both various chromosomes and many genes in this disease [29]. LOH has been specifically identified on chromosome 3 (25%), 9 (40%), and 17 (40%), with an overall fractional allelic loss of 30% [30,31]. Recently, 118 differentially expressed genes have been identified by the expression profile of 22,283 genes in normal human skin biopsies compared with AK and cutaneous SCC [32]. Cutaneous SCC is not associated with any hereditary disease, but aneuploidy is frequent occurring in between 20 to 80% of patients [33,34]. UV-B fingerprint type of mutations in NMSC, comprising characteristic C→T and CC→TT substitutions, have been particularly identified in the target genes *p53* (chromosome 17p13.1) [35,36], *p16^{INK4a}* (chromosome 9p21) [37–40], and *Ha-ras* (chromosome 11p15.5) [17,41,42]. *p53*-mutant cells fail to undergo apoptosis because of UV-induced cellular injury and might also have a selective growth advantage to repopulate injured areas after sunburn events [36,43]. Thus, genomic instability is a driving force in skin cancer development in general, but *p53* mutations rather than *p16^{INK4a}* and/or *Ha-ras* mutations seem to be early events during the development of SCC (Nindl et al., unpublished data) [17].

The number of NMSC is remarkably increased in organ transplant recipients (OTR) underscoring the correlation between skin cancer and immune status [44]. Cumulatively, the main risk factors for cutaneous SCC are long-term exposure to UV-radiation, fairly pigmented skin, and immune status. Furthermore, several studies reveal a role of human papillomaviruses (HPV) in the multi-step process of skin carcinogenesis as a co-factor with UV-radiation [45].

2. Human Papillomaviruses

Papillomaviruses (PV) are small DNA viruses with a genome of nearly 8,000 base pairs. They are host specific and have been found in more than 20 different mammalian and avian species. HPV infect keratinocytes of skin and mucosa (Fig. 1D–E), and their lifecycle is adapted to the differentiation of the host cells [46]. Presently, about 100 HPV types have been completely sequenced and are classified into mucosal/genital and cutaneous types based on sequence analyses and clinical manifestation. Phylogenetic analysis of complete L1 gene sequences identify a series of taxonomic units such as alpha-, beta-, and gamma-PV [47]. The human mucosal/genital types belong to the alpha-PV and can be divided into low-risk and high-risk types as inferred from their association with benign and malignant cervical lesions [48–50]. Low-risk types such as HPV 6 and HPV 11 have the potential to induce genital warts (*Condylomata acuminata*), whereas high-risk types such as HPV 16 and HPV 18 are causally involved in carcinogenesis of the uterine cervix and other mucosae of anogenital and oropharyngeal sites [49,51]. Cutaneous HPV are phylogenetically heterogeneous, and the classical cutaneous warts-associated types (species 2 and 4 of alpha-PV, mu-, and nu-PV) can be distinguished from the *Epidermodysplasia verruciformis* (EV)-associated or EV/cutaneous types (beta- and gamma-PV) [47,52].

The E5 proteins are considered to access oncogenic potential and are specifically present in alpha- and delta-PV but are absent in EV/cutaneous types. The alpha-PV E5 proteins are classified into four groups based on the clinical manifestations of the corresponding viral infection: the presence of E5-alpha that is found in high-risk types correlates with cervical cancer, E5-gamma and/or E5-delta are associated with mucosal benign lesions, and E5-beta corresponds to benign cutaneous lesions [53,54]. Recent studies reveal a pathogenic role of EV/cutaneous HPV types in the development of NMSC (Fig. 2). In order to prove the causal involvement of HPV in the development of malignancies, specific criteria have to be fulfilled such as the presence and activity of the virus in cells of human skin cancer lesions and evidence on transforming properties of viral genes.

3. HPV and NMSC

3.1. *Epidermodysplasia verruciformis*

The rare heritable disorder EV was initially described in 1922 by the Swiss physician Felix Lewandowsky

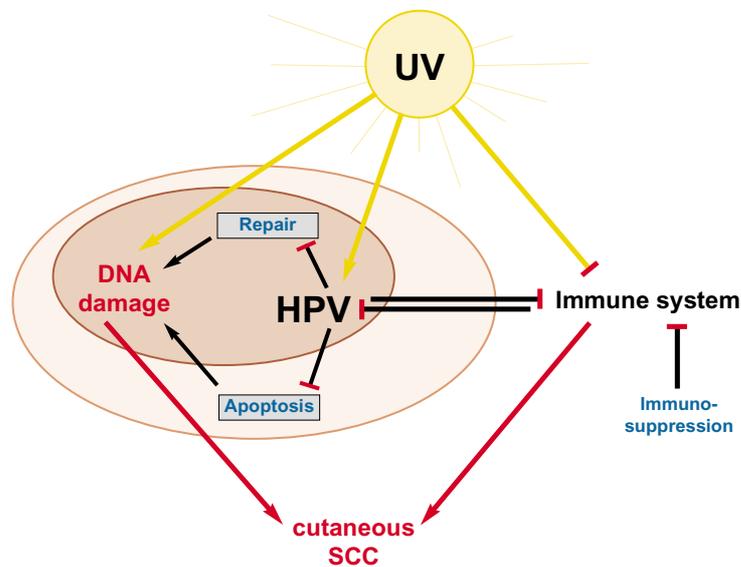


Fig. 2. Multiple risk factors during skin carcinogenesis. Important risk factors for cutaneous squamous cell carcinoma (SCC) are (i) UV-radiation, (ii) EV/cutaneous HPV, and (iii) the immune status. UV-radiation increases the expression of viral genes, induces DNA-damage in keratinocytes, and locally causes immunosuppression. The anti-apoptotic activities, and the delay of DNA repair mechanisms, influenced by HPV in response to UV-induced mutations may lead to the persistence of DNA-damaged keratinocytes and subsequently to skin cancer. Additionally, a low immune status is associated with an increased risk for the development of cutaneous SCC.

under the assistance of Wilhelm Lutz [55]. The disease exhibits extensive polymorphous warts mainly located on sun-exposed sites, which proceed into cutaneous SCC in about 30% of the patients [56,57]. The first evidence for the involvement of HPV in skin cancer was reported from EV patients. The EV types HPV 5 and HPV 8 have been detected in 90% of cutaneous SCC in these patients, whereas HPV 14, HPV 17, HPV 20, and HPV 47 are rarely found in this carcinoma [58].

The genome of EV/cutaneous HPV types is mostly not integrated into the host genome and persists extrachromosomally [59]. This is in contrast to cervical cancer-associated high-risk alpha-HPV, whose viral genome is usually integrated into the host chromosome(s) [60]. HPV DNA persist in high copy numbers in cutaneous SCC (100–300 viral copies per cell equivalent), but not all cancerous cells are HPV-infected [61]. EV/cutaneous HPV types are transcriptionally active in both AK and cutaneous SCC [62,63]. Moreover, persistent HPV infections in cutaneous primary and metastasised SCC from EV patients have been reported [64,65], which may result from a defect of the innate or adaptive immune system by a still unknown mechanism [66].

The eight vertebrate transmembrane cochlear (TMC) expressed genes are evolutionary conserved and encode proteins that constitute three subfamilies. The proteins

comprise at least eight membrane-spanning domains, and are located in the endoplasmatic reticulum [67]. The first susceptibility gene locus associated with EV (i.e., EV1) is mapped to chromosome 17q25 [68] and the second locus (EV2) to chromosome 2p21-p24 [69]. Two homologous genes, *TMC6* (*EVER1*) and *TMC8* (*EVER2*), have been identified at the EV1 locus, and nonsense mutations of these genes increase the susceptibility to infection with particular beta-HPV types resulting in a higher risk for cutaneous SCC [70,71]. They appear to be involved in the regulation of the immune system, and additionally in the replication of EV/cutaneous HPV, but the precise function has still to be determined. Moreover, antibodies against beta-HPV types have been found at young ages from EV patients, but age-matched sera from the general population are usually HPV-seronegative (M. Pawlita, personal communication) indicating that viral replication is perturbed in EV patients. Future studies are highly desirable, since knowledge about the mechanism will provide further insights in how EV/cutaneous HPV are controlled at the cellular level and how weak control may contribute to the increased risk for skin cancer.

3.2. General population

Among children from 1 month to 4 years, the prevalence of cutaneous HPV DNA in forehead swabs vary

between 50 to 70% [72]. Asymptomatic cutaneous HPV infections of normal skin thus occur very early in infancy caused by a high multiplicity of HPV. Boxman and colleagues [73] firstly detected EV/cutaneous HPV types in hair follicles from eyebrows, arms, and/or legs in 10 of 22 healthy volunteers (45%). Furthermore, cutaneous HPV types have been found in plucked eyebrow hairs in up to 67% of immunocompetent individuals [73–75] and hair follicles are probably a reservoir of EV/cutaneous types. An individual spectrum of EV/cutaneous HPV types is present in plucked hairs from immunocompetent individuals, and similar types are found in different skin areas from both sun-exposed and non sun-exposed sites [76]. Possibly, the skin of each human is infected with an individual pattern of cutaneous HPV types, whose replication is controlled by the immune system. Genital alpha-HPV has been rarely detected in skin samples indicating the high specificity of HPV types to the host tissue.

Over the last two decades, several PCR methods using degenerate, nested and/or type-specific primers followed by sequencing, hybridisation, or cloning and sequencing have been developed to detect a broad range of cutaneous HPV types [73,77–81]. Different prevalence rates of EV/cutaneous HPV types have been reported in NMSC from immunocompetent patients, most likely due to the differences in the detection system used. This assumption has been confirmed by studies reporting increased prevalence rates when a combination of different primer sets have been applied [82, 83]. Recently, two high-throughput PCR-based HPV detection systems have been developed in order to identify EV/cutaneous HPV types with a high sensitivity and both systems are applicable for future large epidemiological studies [84,85].

Overall, the number of EV/cutaneous HPV types in NMSC is higher (27 to 85%) compared to normal skin (15 to 35%) [86–92]. The highest prevalence rate of EV/cutaneous HPV types have been detected in 97 of 114 immunocompetent AK patients (85%) [90]. Moreover, the virus load in skin tumours is low, and a single HPV copy can be detected in only 10 to 1,000 dysplastic cells [88,93,94]. The highest virus load of 6 EV/cutaneous HPV types examined was found in 20 AK, followed by 6 SCC, and has been least in 1 metastasis [95]. Thus, the virus load of EV/cutaneous HPV in cutaneous SCC is very low, and the number of HPV infected cells may decrease during skin carcinogenesis.

An increased risk of SCC, but not BCC, has been associated with EV/cutaneous HPV types in two epidemiological studies investigating viral DNA in eye-

brow hairs [75,96]. EV/cutaneous HPV types were detected in hairs from 17 of 25 SCC (68%) *versus* 14 of 25 controls (56%) resulting in a non-significant positive association of HPV with SCC (odds ratio 2.00, 95% confidence interval 0.50–8.0) [75]. In another case-control study, a positive association of EV/cutaneous HPV and SCC was found (odds ratio 1.7, 95% confidence interval 1.1–2.7), with prevalence rates of 75% in SCC (116 of 155) *versus* 58% in controls (216 of 371) [96]. However, an association of a specific HPV type with SCC has not been determined to date. In a few, small epidemiological studies investigating seroreactivity of EV/cutaneous HPV an increased risk of HPV 8 and SCC has been reported [97–99]. A larger study has examined antibody response to six EV/cutaneous types (i.e., HPV types 5, 8, 15, 20, 24, and 38) in 161 SCC, 454 BCC, and 386 control patients [100]. Seroreactivity of HPV 8 and HPV 38 was significantly increased in SCC *versus* controls. In a recent large epidemiological case-control study, antibodies against 16 HPV types (HPV 1, 2, 3, 5, 6, 8, 9, 10, 15, 16, 20, 24, 32, 36, 38, and 57) belonging to beta-, alpha-, and mu-PV have been investigated in 252 SCC, 525 BCC, and 461 control patients using a newly developed multiplex serology [101]. An increased risk of beta-HPV, particularly HPV 5 (odds ratio 1.8, 95% confidence interval 1.0 to 3.1) and SCC, but no association of HPV seropositivity and BCC was found. In conclusion, clinical and epidemiological data support a role of EV/cutaneous HPV in the development of SCC and not BCC.

3.3. Immunosuppressed organ transplant recipients

Persistent HPV-induced warts, AK, and cutaneous SCC, which mainly arise on sun-exposed sites, are the main types of cutaneous tumours in immunosuppressed OTR [4,102,103]. Within 15 years after transplantation, up to 40% of renal transplant recipients have developed NMSC [104]. These cancers predominately represent SCC, but only to a lesser extent BCC. Compared to the general population, the incidence of cutaneous SCC and BCC is approximately 150-fold and 10-fold higher in transplant recipients, respectively, thus leading to a reversal of the SCC to BCC ratio of 1:4 in the general population to 5:1 in transplant recipients [16,44,105,106]. Cutaneous SCC in OTR shows characteristic clinical and morphological features with HPV-induced warts [107]. An important observation is the association and co-localisation of cutaneous SCC with HPV-induced warts in OTR indicating that per-

sistent warts may be progress into skin cancer [108]. Overall, the EV/cutaneous HPV prevalence rate, and the number of multiple types, was higher in cutaneous SCC from immunosuppressed OTR in comparison to the general population. Moreover, OTR have a higher HPV prevalence rate of up to 90% in cutaneous SCC compared to normal skin (11 to 32%) [79,87, 88,92,109–113]. In up to 92% of plucked eyebrow hairs, EV/cutaneous HPV types have been detected [73, 74] leading to low differences in comparison to HPV prevalence of patients with cutaneous SCC. A large European epidemiological study examined the risk of EV/cutaneous HPV types for SCC in 219 OTR with and 550 without a history of cutaneous SCC. An increased risk of one or more EV/cutaneous HPV types in eyebrow hairs for cutaneous SCC have been found in OTR (odds ratio 3.00, 95% confidence interval 1.5–6.2) (J.N. Bouwes Bavinck and the EPI-HPV-UV-CA group, abstract, 4th HPV and Cancer Meeting, November 2006, Turin, Italy). Moreover, a higher prevalence of seroreactivity against beta-HPV was found in OTR with SCC (60%) versus in OTR without SCC (55%). Persistent EV/cutaneous HPV infection with the same variant in primary skin SCC, recurrence, and metastases in organ transplant recipients has been reported [94]. Two studies report viral activity of EV/cutaneous HPV types in cutaneous SCC [114,115]. E2 and E4 transcripts have been detected in 20% of warts and 38% of SCC, and E6/E7 transcripts have been found in a single SCC only [114]. Transcriptional activity of the EV/cutaneous HPV types 8, 9, and 15 of E6/E7 has been detected in 4 of 10 viral positive AK and cutaneous SCC from immunosuppressed OTR [115]. Thus, viral activity of early genes seems to be present in at least a subset of AK and cutaneous SCC suggesting a role for HPV during skin carcinogenesis.

4. Molecular mechanisms and transforming properties of EV/cutaneous HPV

4.1. In vitro models

4.1.1. Monolayer

Using monolayer cultures, the E6 gene of HPV 8 shows transforming properties in rodent fibroblast lines (C127, Rat 1), whereas HPV 8 E7 is involved in the replication of the viral DNA [116]. HPV 47 E6 is required and sufficient for the transformation of the rat fibroblastic cell line (3Y1) [117]. The E6 genes of the EV/cutaneous HPV types 5, 8, 14, 20, 21, 25, and 47 in-

duce morphological transformation in 3Y1 cells [118]. The oncogenic potential of HPV types 5, 8, and 47 is higher than those of HPV types 14, 20, 21, and 25. The E7 genes of HPV 8 and HPV 47 fail to induce morphological transformation in rodent fibroblast lines suggesting that E6 rather than E7 is correlated to malignant conversion [116,119]. However, HPV 8 E7 is able to transform rodent cells concertedly with the activated *Ha-ras* oncogene [120]. The first study analysing the ability of cutaneous HPV types to immortalise human foreskin keratinocytes was performed with HPV 1 and HPV 8 [121]. Furthermore, the E6 or E7 gene of HPV 1, showing a transformed phenotype in rodent cells, is not able to immortalise human keratinocytes, and only a weak immortalising potential for HPV 8 E7 has been observed. The E6/E7 protein of EV/cutaneous HPV 38, but not HPV 10 and HPV 20, displays transforming activity by increasing the life span of human primary keratinocytes [122]. Additionally, HPV 38 E7 is able to degrade and inactivate the tumour suppressor gene *pRb*.

Purdie and colleagues [123] were the first to demonstrate UV-induced activation of the viral promoter of the cutaneous type HPV 77 that was identified in cutaneous lesions from OTR. The promoter contains a *p53* binding site, and the response is mediated through the UV-induced expression of *p53*. The influence of UV-radiation varies on viral gene expression of warts-associated types (HPV types 1, 2, 3, 7, 27, 41, and 77) and EV/cutaneous types (HPV types 5, 20, 23, and 38) [124]. The presence or absence of wild-type or mutated *p53* influences the gene expression of HPV types 20, 27, and 77, but not of HPV 41, suggesting that *p53* is not an exclusive factor involved in the interaction between UV-radiation and HPV. UV-induced cytokines activate the promoter of HPV 20, but inhibit the promoter of the warts-associated type HPV 27 [125].

Independently from *p53* degradation, the E6 protein of HPV 5 inhibits apoptosis in response to UV-damage [126] by inactivation of the pro-apoptotic protein *Bak* [127]. Moreover, HPV-negative skin cancers express *Bak* that is in contrast to HPV-positive cancers, in which *Bak* has not been detected. This underscores the importance and clinical relevance of the interaction between the UV-induced *Bak* protein and EV/cutaneous HPV types. E6 of both HPV 1 and HPV 8 binds *XRCC1*, a protein that is required for DNA repair suggesting a link between EV/cutaneous HPV and the according host pathways [128]. The E6 protein of HPV 5 compromises the repair of UV-induced thymine dimers [129], and E6 of HPV 77 additional-

ly forces keratinocytes into the S1-phase by inhibiting *p53*-activated, pro-apoptotic genes [130]. However, the E6 expression of beta-HPV 5 does not interfere with such pro-apoptotic genes indicating that this is probably not the favoured mechanism of EV/cutaneous HPV types in the cell. The anti-apoptotic activity, and the delay of the DNA-repair mechanism, may lead to the persistence of UV-damaged keratinocytes suggesting that cutaneous/EV HPV may be involved in the early stages of skin carcinogenesis.

4.1.2. Organotypic keratinocytes cultures

Functional analyses of EV/cutaneous HPV types have been retarded for a long time because of both the relation between viral development and host cell differentiation, in addition to the lack of a system with continuously differentiating keratinocytes that constitute a stratified epithelium (i.e., the natural target cells of PV). However, the organotypic raft culture partly has such properties and consists of human primary keratinocytes, which stratify and differentiate into a squamous epithelium [131]. Growth and differentiation markers are expressed similarly to the human epithelium making this system a key technology for the investigation of human skin carcinogenesis. The E6/E7 genes of the EV/cutaneous HPV types 5, 12, 15, 17, 20, and 38 have strong effects on the growth and differentiation in organotypic cultures of human primary keratinocytes, but an invasive phenotype have not been observed [132]. HPV 8 E7 promotes invasion of keratinocytes into the dermis [133]. This immortal phenotype was associated with the disruption of the basement membrane and the overexpression of metalloproteinases *MMP-1*, *MMP-8*, and *MT-1-MMP*.

4.2. Animal models

4.2.1. Cattle and horses

Using animal models, the role of PV in skin carcinogenesis has been studied at three different levels, namely in naturally occurring lesions, in colonies of animals that have been collected from the field, and in transgenic animals. Most non-human PV have been isolated and sequenced from severe epithelial diseases of warm-blooded vertebrates including many gregarious animals such as cattle, dog, and rabbit. Bovine papillomavirus type 4 (BPV 4) from the xi-PV is the etiological agent of epithelial papilloma of the upper alimentary canal in cattle [134]. Carcinoma shows characteristic morphological features with papilloma, which can progress into cancer. However, BPV 4 DNA is found in early pa-

pilloma of the alimentary tract, but not in advanced papilloma, oesophageal carcinoma, or adenoma and adenocarcinoma of the lower intestine [135,136]. Therefore, the presence of BPV 4 is restricted to the early transformation stages during carcinogenesis ('hit-and-run mechanism'). Decrease of virus load has also been shown in a study investigating AK through SCC developmental stages of human cancers [95]. Thus, the multiple factors including BPV 4 infection that concertedly lead to carcinoma in cattle [137] may serve as animal model for an improved knowledge about the complex role of HPV in human skin cancer.

BPV 1 and BPV 2 belong to the delta-PV exhibiting an E5 open reading frame (ORF), which may have been independently developed from that of the alpha-PV [53]. The virus is able to infect horses and play an important role in the development of equine sarcoids [138–140]. Roughly half of the healthy horses having contact with infected fellows carry BPV 1 in their skin [141]. This indicates that the usually strict specificity of PV to their hosts [142] is occasionally dismantled and that interspecies transmission may rarely occur in PV (although without replicative establishment of the virus). However, the underlying mechanisms and the possible impact on the malignant developments of novel host invasion have not been seriously addressed for PV to date [52]. On the contrary, their presence in humans has exclusively been regarded as old primate inheritance [142].

4.2.2. Cottontail rabbit

Anatomical sites, in which PV cannot complete the productive life cycle, appear particularly involved in the development of skin cancers. This has also been suggested for malignancies caused by cottontail rabbit PV (CRPV) [46] from the kappa-PV. Viral DNA easily induces papillomata making the cottontail rabbit model a powerful system, which identifies the molecular mechanisms that are required for the induction and progression of epithelial neoplasia. Benign papillomata are induced in nearly all rabbits that are infected by CRPV, and 80% of the initially benign epithelial tumours progress into carcinoma within 6 to 14 months [143]. The virus thus plays an active role during skin carcinogenesis of domestic rabbits interacting with chemical carcinogens [144]. Mutation analysis of viral DNA shows that only the E7 gene, but not the E4 gene, is involved in the development of papilloma [145]. However, the E4 gene remains an important factor for viral DNA amplification and expression of the late structural proteins [46]. Mutation of the highly conserved amino acids results in replication-competent but transactivation-deficient E2 proteins [146].

4.2.3. Multimammate mouse

A colony of *Mastomys coucha* (Smith, 1834) [initially determined as *M. natalensis* (Smith, 1834)] has been established from animals collected in South Africa, which spontaneously develop multiple benign skin tumours such as papilloma and keratoacanthoma during aging. The animals are latently infected by the first *Mastomys natalensis* papillomavirus (MnPV 1) [147], which is the etiological agent of the lesions [148–150]. MnPV 1 belongs to the iota-PV and does not contain any E5 ORF. Analogously to HPV 8-induced NMSC in EV patients, MnPV 1 DNA persists episomally, without any evidence of integration [147]. Virus-induced tumours do not regress, but proceed after topical application of carcinogens and tumour promoters of cutaneous SCC [151], which is in contrast to the human keratoacanthoma development. MnPV 1 has a broader tissue specificity than other PV investigated so far, and viral persistence and viral load has been correlated with the development of skin tumors [152]. Thus, *Mastomys coucha* is an excellent animal model for the study of naturally PV-induced skin carcinogenesis.

4.2.4. Transgenic mice

HPV 1 causes hyperproliferation and alterations of skin differentiation in a transgenic mouse model examining benign warts of the animals [153]. The expression of the early genomic region, under the control of the keratin-6 promoter, shows a similar phenotype like human virus-induced warts. Pfister and colleagues have established the first transgenic mouse model for studies on beta-HPV types using the complete early region of HPV 8 under the control of the keratin-14 promoter [154]. The mice have spontaneously developed cutaneous SCC without additional carcinogens, and benign skin tumours have been observed in 91% of the animals. The same research group has generated HPV 8-transgenic mice that affect exclusively the gene(s) E2, E6, E7, or E6/E7. Spontaneously developed skin tumours have been observed in E2-, E6-, and E6/E7-transgenic mice, whereas the exclusive expression of HPV 8 E7 is not sufficient to induce tumour growth (H. Pfister, personal communication). Tommasino and co-workers [155] have established transgenic mice expressing E6/E7 of HPV 38 under the control of the human keratin-10 promoter. The animals have developed skin tumours after a two-stage carcinogen treatment. Cellular proliferation, hyperplasia, and dysplasia in the epidermis have been spontaneously induced by HPV 38 E6/E7. Transforming properties of HPV 8 and HPV 38 have been thus shown in the system of a transgenic mice model.

5. Conclusions

The skin of most humans is infected with an individual spectrum of different cutaneous HPV types, and hair follicles are probably their reservoir. During host-linked evolution, cutaneous HPV types may have established different ecological niches in human skin tissues including a mutualistic cooperation between HPV and their host cells. Specific triggers such as UV-induced DNA-damage in sun-exposed cells, immunosuppression (e.g., locally UV-induced, systemically in OTR), and/or inactivation of host controlled viral life cycle (e.g., in EV patients) lead to increased HPV replication and subsequently to a higher virus load within the cells. The anti-apoptotic effect of cutaneous HPV types in UV-damaged keratinocytes probably results in persistent viral infections and hence the accumulation of further DNA mutations, putatively leading to immortalised cells (Fig. 2). Thus, cutaneous HPV types seem to be involved as a co-factor in the early onset of cutaneous SCC.

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