OncoImmunology 3, e28288; February 2014; © 2014 Landes Bioscience

The transmembrane channel-like protein family and human papillomaviruses

Insights into epidermodysplasia verruciformis and progression to squamous cell carcinoma

Jaime S Horton[1,2] and Alexander J Stokes[1,2,3,*]

[1]Laboratory of Experimental Medicine; John A. Burns School of Medicine; Honolulu, HI USA;
[2]Department of Cell and Molecular Biology; John A. Burns School of Medicine; Honolulu, HI USA; [3]Chaminade University; Honolulu, HI USA

Keywords: Epidermodysplasia verruciformis, TMC8, TMC6, EVER1, EVER2, papillomavirus, cutaneous squamous cell carcinoma

Abbreviations: AEV, acquired epidermodysplasia verruciformis; CMI, cell-mediated immunity; CSCC, cutaneous squamous cell carcinoma; ERCC3, excision repair cross-complementing rodent repair deficiency, complementation group 3; ER, endoplasmic reticulum; EV, epidermodysplasia verruciformis; FADD, Fas-associated protein with death domain; FasL, Fas ligand; HPV, human papillomavirus; MST1, macrophage stimulating 1; MT, mechanotransducer; MTF-1, metal-responsive transcription factor 1; NF-κB, nuclear factor kappa B; NK, natural killer; NMSC, non-melanoma skin cancer; ORF, open reading frame; pRb: retinoblastoma protein; PBMC, peripheral blood mononuclear cell; RHOH, ras homolog gene family member H; RIPK1, receptor-interacting serine/threonine protein kinase 1; STK4, serine/threonine kinase 4; TGFβ, transforming growth factor beta; TMC6/EVER1, transmembrane channel-like 6; TMC8/EVER2, transmembrane channel-like 8;TNFα, tumor necrosis factor alpha; TRADD, TNF receptor type 1-associated death domain; TRAIL, TNF-related apoptosis-inducing ligand; UVR, ultraviolet radiation; XPB, xeroderma pigmentosum type B; XRCC1, x-ray repair cross-complementing protein 1; YAP, yes-associated protein; Zn2+, zinc; ZnT-1: zinc transporter 1

Epidermodysplasia verruciformis (EV) is a rare genodermatosis characterized by increased sensitivity to infection by the β-subtype of human papillomaviruses (β-HPVs), causing persistent, tinea versicolor-like dermal lesions. In a majority of affected individuals, these macular lesions progress to invasive cutaneous squamous cell carcinoma (CSCC) in sun-exposed areas. While mutations in transmembrane channel-like 6 (*TMC6 / EVER1*) and 8 (*TMC8 / EVER2*) have been causally linked to EV, their molecular functions are unclear. It is likely that their protective effects involve regulation of the β-HPV life cycle, host keratinocyte apoptosis vs. survival balance and/or T-cell interaction with infected host cells.

Introduction

TMC6 and TMC8 proteins have emerged as the causal link between *epidermodysplasia verruciformis* (EV), the β-genus of human papillomaviruses (β-HPVs) and squamous cell carcinoma (SCC). These proteins, as yet without a well-defined molecular function, appear to critically control the interplay between viral homeostasis, humoral immune response and UV damage. This review summarizes what is known to date of these key components and explores our current understanding of this complex disease process.

Human Papillomaviruses

Papillomaviruses are small, non-enveloped DNA viruses with tropism for cutaneous or mucosal stratified squamous epithelium. Human papillomaviruses have 5 phylogenetic genera encompassing at least 120 genotypes.[1] While genital HPV genotypes are grouped exclusively in the α-papillomavirus (α-HPV) genus, cutaneous HPVs demonstrate greater heterogeneity, belonging to alpha, beta, gamma, mu, and nu generas.[2] HPV genotypes associated with *epidermodysplasia verruciformis* (EV-HPVs) constitute the β genus that is further distributed

*Correspondence to: Alexander James Stokes; Email: astokes@hawaii.edu
Submitted: 12/12/2013; Revised: 02/13/2014; Accepted: 02/19/2014; Published Online: 04/15/2014
Citation: Horton J, Stokes AJ. The transmembrane channel-like protein family and human papillomaviruses. Insights into epidermodysplasia verruciformis and progression to squamous cell carcinoma. OncoImmunology 2014; 3:e28288; http://dx.doi.org/10.4161/onci.28288

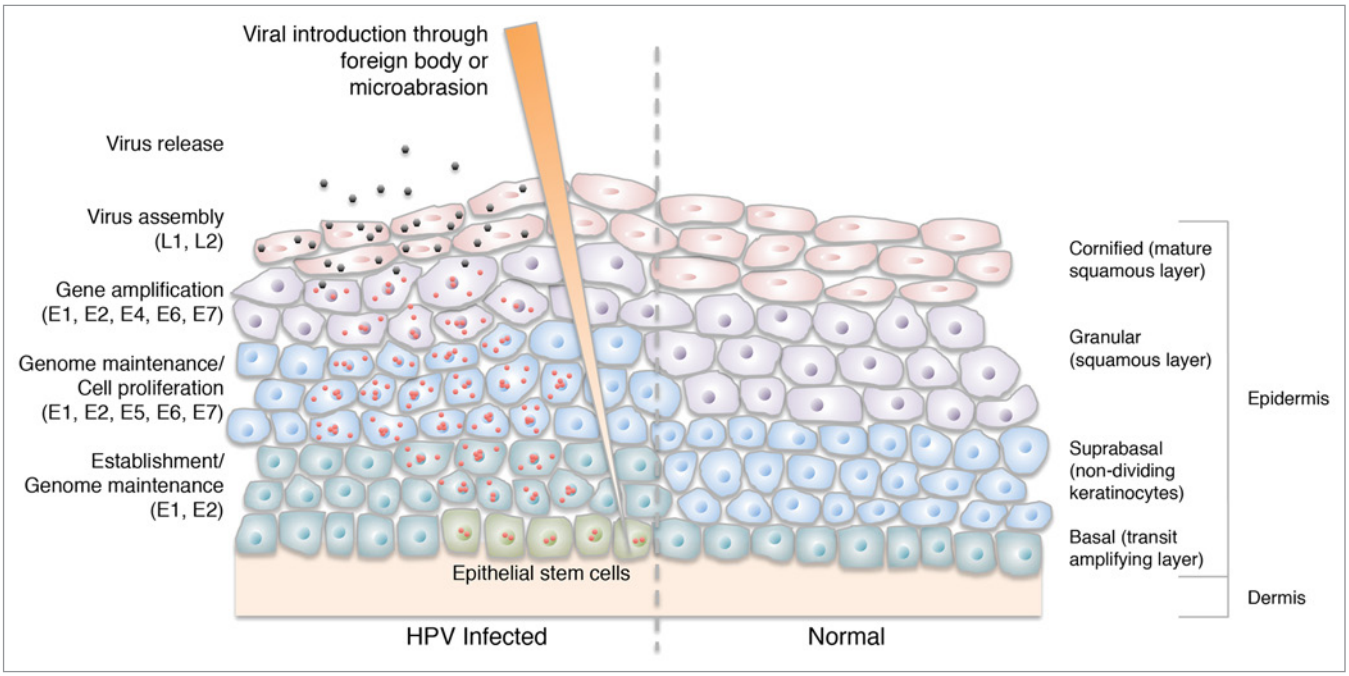

Figure 1. A schematic view of the human papillomavirus life cycle. Daughter cells of epithelial stem cells divide along the basement membrane and then mature vertically through the epithelium without further division (right side, normal epithelium). Stem cells in the basal layer of the epithelium become exposed to human papilloma virus (HPV) as a result of microabrasion or foreign body introduction (left side, infected epithelium). Expression of HPV genes is tightly regulated and linked to epithelial differentiation. Upon infection, viral genomes are established in the nucleus as episomes. Early viral proteins deregulate cell cycle control, allowing viral genome amplification in cells that normally would have exited the cell cycle. The late phase proteins encapsidate newly synthesized viral genomes and mature virions are shed from the most superficial layers of the epithelium. Adapted from Frazer, 2004.[109]

into 5 species, of which genotypes associated with *epidermodysplasia verruciformis* (EV-HPVs) mainly belong to the β1 (genotypes 5, 8, 14, 20, and 47) and β2 (genotype 17) species.[3] HPV5 and HPV8 are the genotypes most frequently associated with the development of non-melanoma skin cancer (NMSC) and account for more than 90% of EV-associated cancers.[4,5]

HPV Life Cycle

HPV genomes constitute less than 8000 base pairs, and can be separated into 2 coding regions, early (E) and late (L), as well as a non-coding regulatory region, also referred to as long control region or upstream regulatory region.[6] The L1-protein is the major capsid protein and is relatively well conserved between HPV types. This ubiquitous HPV protein is the basis of prophylactic vaccines and is used for classification and phylogenetic analysis.[1]

HPVs infect cells in the basal layer of stratified squamous epithelia, exposed during microabrasions or wounding. These basal cells are the only proliferating cells in normal epithelia, as differentiated cells in the suprabasal layers have exited the cell cycle. It is likely that cutaneous HPVs target the stem cells residing in the epidermal basal layer and the bulge of hair follicles.[7,8] HPV genomes are amplified with replication of host cells, followed by capsid protein synthesis, virion assembly, and release.[9] Infection results in the transient proliferation of keratinocytes that normally regress as a consequence of poorly understood cell-mediated innate and acquired immune responses.[10] HPV DNA can persist after lesion regression, with viral latency attributed to low-titer virus infection.[11] A summary of the viral life cycle is shown in **Figure 1**.

HPV Malignant Transformation and Early Gene Involvement

Failure of the immune system to clear persistent HPV infections can lead to the development of cancer over time. The transforming abilities of the high-risk cervical cancer genotypes HPV16 and 18 have been extensively studied.[9] In both α- and β-HPV, the E6 and E7 oncoproteins are crucial for transformation, maintaining proliferative ability, establishing genomic instability and preventing the apoptosis of infected cells undergoing HPV-mediated transformation. E6 binds and mediates the degradation of the tumor suppressor gene p53, while E7 activates telomerase and inactivates retinoblastoma protein (pRb), preventing cell cycle inhibition. The culmination of these effects is cell immortalization, prevention of apoptosis and continuous replication of viral DNA.[9,12]

Although the E6 and E7 of β-HPVs do not share the overall transforming potential of the high-risk a types, they still display oncogenic activities. HPV5 viral DNA does not integrate into host DNA, nor does its E6 degrade the p53 tumor suppressor

gene.[13] Instead, E6 from HPV5 and HPV8 are capable of reducing steady-state levels of several p53-modifying enzymes.[14] β-HPV E6 proteins also interfere with keratinocyte differentiation by preventing apoptotic caspase 14 (CASP14) activation[15] and disrupting cell cycle regulation through the inhibition of transforming growth factor β (TGFβ) via SMAD3 degradation.[16] Viral disruption of the skin's immune defenses is demonstrated by HPV8 E7 suppression of CCAAT/enhancer binding protein β (C/EBPβ), a critical regulator of the Langerhans cell chemoattractant protein CCL20.[17] Similarly, HPV-associated warts are protected from host immune responses and apoptotic signals by decreased Langerhans cell numbers.[18] Furthermore, inhibition of the transcriptional regulator NF-κB by E7 proteins from HPV20, 37, 38, 92, 93, and 96 may additionally contribute to host cell immortalization and dysregulation of the immune response.[19] Taken together, β-HPVs and their early gene products have multiple ways of dysregulating the host cell cycle, apoptosis and immune surveillance to establish infection and oncogenicity.

β-PV Mouse Models

Several β-PV transgenic mouse models have been developed, demonstrating the interaction between HPV, skin hyperproliferation and squamous cell carcinoma (SCC). Mice transgenic for the HPV8 early region under control of the keratin 14 promoter spontaneously develop invasive SCC-like lesions[20] and develop papilloma growth within 3 wk of a single UV (UV) A/B dose.[21,22] Hyperproliferation and increased sensitivity to chemical carcinogens are demonstrated in mice expressing E6 and E7 from HPV20, 27 or 38.[23,24] Recently, a humanized mouse model was created using artificial human skin with primary keratinocytes engineered to express the HPV5 E7 protein engrafted onto nude mice in which a diminished capacity of E7 to reduce pRb levels was observed relative to results in vitro studies.[25] Such findings demonstrate that β-PV mouse models will continue to be instrumental to further our understanding of cutaneous HPV tumorigenesis.

Epidermodysplasia Verruciformis

Epidermodysplasia verruciformis (EV) is a rare, autosomal recessive genodermatosis, first described by Lewandowsky and Lutz in 1922.[26] EV is characterized by the increased susceptibility to specific HPV genotypes that are referred to as the EV-HPVs, including HPV3, 5, 8, 9, 10, 12, 14, 15, 17, 19–25, 28, 29, 36, 46, 47, 49, and 50.[27] Viral infection in EV leads to tinea versicolor-like macules on the trunk, neck, arms, and face during early childhood. These benign forms are persistent, refractory and disseminated skin lesions resembling flat warts or presenting as macules of various colors. The HPV genotype determines the morphology of EV lesions and the risk of malignant conversion to cutaneous squamous cell carcinoma (CSCC).[28] EV patients are typically infected with more than 1, and up to 10, EV-HPV genotypes, with HPV5 and HPV8 classified as the high risk genotypes most frequently associated with skin carcinomas.[3]

EV-HPVs are prevalent in the normal skin of healthy adults. Considered as commensal viruses of human skin, β-HPVs are ubiquitous viruses causing asymptomatic cutaneous infections in the general population.[7,29,30] However, the quantity of β-HPV DNA found spread over the body is very low; even in SCC, viral load seldom reaches the level of 1 viral copy per cell, indicating that β-HPVs are not involved in the maintenance of the transformed state of tumor cells, unlike genital HPVs.[31] Although antibodies to the L1 capsid protein of HPV5 and other EV-HPV genotypes is low in the general population,[32] they are detected in most EV patients.[33] Approximately 3 quarters of EV patients with CSCC were found to have developed antibodies to HPV5 E5 and/or E7 oncoproteins,[3] suggesting that there is a defect in viral clearance prior to adaptive immune response in these patients.

Genetic Mutations Associated with EV

The familial occurrence of the disease led to the search for a putative EV gene. Homozygosity mapping led to the identification of a major susceptibility locus located in chromosomal region 17q25.3.[34] Genetic analysis of the region revealed 2 novel adjacent genes, *EVER1 (TMC6)* and *EVER2 (TMC8)*, encoding cytoplasmic proteins that colocalize with calnexin, an integral membrane protein in the endoplasmic reticulum (ER).[35] Homozygous, invalidating mutations in either gene are found in roughly 75% of EV patients[3] and confer susceptibility to EV.[34,35] A second locus was subsequently identified in chromosomal region 2p21-p24,[36] suggesting the nonallelic heterogeneity of the disease.

Several other EV-associated mutations have been identified and can be generally grouped into (1) mutations affecting the adaptive immune system, and (2) those that affect the host cell, specifically DNA repair/ excision systems. A variant of the *xeroderma pigmentosum type B (XPB)* gene (*ERCC3*) on 2q21 was found in an EV patient without mutations in *TMC6* or *TMC8*.[37] This gene encodes the excision repair cross-complementing rodent repair deficiency, complementation group 3 protein (ERCC3), a DNA helicase in nucleotide excision repair that is also a subunit of a class II transcription factor.[38] MST1 (macrophage stimulating 1/hepatocyte growth factor-like, or STK4, serine/threonine kinase 4) deficiency was also found to underlie susceptibility to EV-HPV infections.[37] Located on 20q11.2-q13.2, mutations in this gene lead to T-cell deficiencies, particularly naïve $CD4^+$ and $CD8^+$ T-cell lymphopenia.[39] MST1 also negatively regulates proliferation and promotes differentiation through inactivation of the yes-associated protein (YAP), which normally inhibits the antiproliferative Notch transcriptional pathway[40] that is negatively regulated by HPV8 and HPV17 E6.[41] Another mutation in 4p13, encoding the ras homolog gene family member H (*RHOH*), was also recently shown to cause T cell defects and susceptibility to EV-HPV infections.[42] *RHOH* encodes an atypical Rho GTPase expressed predominantly in

hematopoietic cells.[43] A final case in an EV patient lacking *TMC6* or *TMC8* mutations identified defective apoptotic Fas function and a perforin (*PRF1*) missense mutation, which may contribute to decreased viral clearance through impaired T cell-mediated cytotoxicity.[44] It is yet to be revealed whether TMC6 and TMC8 are specifically involved immunologically and/or oncogenically in the progression of EV and malignant transformation.

Immunological Aspects of EV

Although EV patients display abnormal susceptibility to a subset of HPV genotypes considered mostly harmless to the general population, they are not abnormally prone to additional bacterial, fungal, or viral infections, and humoral immunity appears to be preserved.[3] This suggests a functional deficiency in the immune response specific to EV-HPVs, and/or to keratinocytes infected with these viruses. In support of this, EV has been classified as a primary immunodeficiency with specific defects in innate immunity.[45] However, the specific cell-mediated immune responses toward EV HPVs or keratinocytes infected with these viruses remain obscure. Reduced peripheral blood mononuclear cell (PBMC)-mediated host cell lysis and attenuated natural cell-mediated cytotoxicity against HPV5-infected keratinocytes have been previously reported to occur in these patients.[46] T lymphocytes have also been shown to be unresponsive to autologous HPV-infected keratinocytes.[47] In general, reports of impaired cell-mediated immunity (CMI) in several EV patients include reduced T-cell responsiveness to mitogens,[48] and anergy to common skin antigens.[49,50] Findings in regards to T-cell counts have been mixed, with some reporting decreased T-lymphocyte counts and CD4/CD8 ratios,[51,52] while others find normal $CD4^+$ and $CD8^+$ counts and proliferative capacity in response to anti-CD3 stimulation.[53] Mild T-cell abnormalities have been observed in TMC8-deficient EV, including an increase in memory, effector memory and skin-homing T-cell subsets, and a bias of TCR Vαβ and Vγδ repertoires.[53] Controversy also exists over Langerhans cells, the major antigen-presenting cells in the epidermal layer, with several groups finding normal numbers and function,[47,54] while others have shown a drastic reduction in EV patients.[17] It is difficult, however, to determine whether these functional abnormalities are a primary effect in the pathogenesis of the disease, or secondary to chronic HPV infection.

The Development of EV in Immunosuppressed Patients

While the majority of medical literature concerns congenital EV occurring as a primary hereditary disease associated with HPV, in a minority of cases, EV is found in association with a state of impaired CMI. The term "acquired epidermodysplasia verruciformis" (AEV) has been introduced by Rogers and colleagues to describe this group.[55] EV has been reported multiple times in the HIV-infected population,[56,57] with lesions appearing clinically and histologically similar to that occurring in EV patients without generalized immunodeficiency and HPV detectable by polymerase chain reaction (PCR) testing.[56] Compared with primary EV, however, EV-HIV patients appear to have lower rates of malignant transformation, possibly due to the rarity of EV in this population. EV and EV-like syndromes have also been described in organ transplant recipients[58,59] and in systemic lupus erythematosus.[60] Of note, immunosuppression per se is insufficient for the initiation and/or maintenance of EV-HPV pathogenesis or oncogenesis, as there is a lack of persistent cutaneous lesions with high levels of EV-HPV DNA replication among patients with genetic, acquired, or iatrogenic depression of CMI.

Cutaneous Squamous Cell carcinoma and EV

In the fourth or fifth decade of life, roughly half of affected EV patients will develop SCC, primarily Bowen's type carcinoma in situ or invasive SCC, occurring mainly on sun-exposed areas.[61] SCC is 1 of the 2 major types of NMSC, which is more common than lung, breast, prostate, and colon cancers combined, with a rate reported to be increasing by 4–8% yearly.[62] In comparison to benign lesions, malignant lesions can be more closely described as verruca-like papillomatous lesions or seborrheic keratosis-like lesions.[63] β-HPV types are more common than other HPV types in NMSC,[64,65] but only a subset of β-HPVs is typically associated with malignant conversion, namely, HPV5, as well as occasionally HPV8, 14, 17, 20, or 47.[3]

UV Exposure as a Co-factor in the Development of EV-SCC

UV radiation (UVR), an environmental carcinogen, plays an important role in the induction of SCC in EV patients. Indeed, lymphocytes from EV patients challenged with UVR, but not gamma rays, showed more chromosomal aberrations than controls.[66] Solar UVR, consisting of UVC (190–280 nm), UVB (280–320 nm), and UVA (320–400 nm), is the major risk factor for the development of NMSC in the general population, acting through both immunosuppressive and mutagenic effects.[67]

UVB-specific mutations in p53 are seen in the majority of SCCs,[68] with signature UV-induced C→T mutations of the p53 gene and abnormal p53 protein expression also associated with EV tumor progression.[69] Purdie and colleagues first demonstrated UV-induced HPV77 promoter activation in cutaneous lesions from organ transplant recipients,[70] and Akgul et al. confirmed the UVB modulation of β-HPV promoter activity for several virus types and p53-response elements.[71] In addition to damaging keratinocyte DNA, UVB also suppresses the skin's immune system by inhibiting dendritic cells of the skin via the immunosuppressive cytokine interleukin-10 (IL-10).[72] UVR also inhibits apoptosis of UV-damaged cells by downregulating Fas ligand (FasL, CD95L) and TNF-related apoptosis inducing ligand (TRAIL) expression in keratinocytes.[73,74]

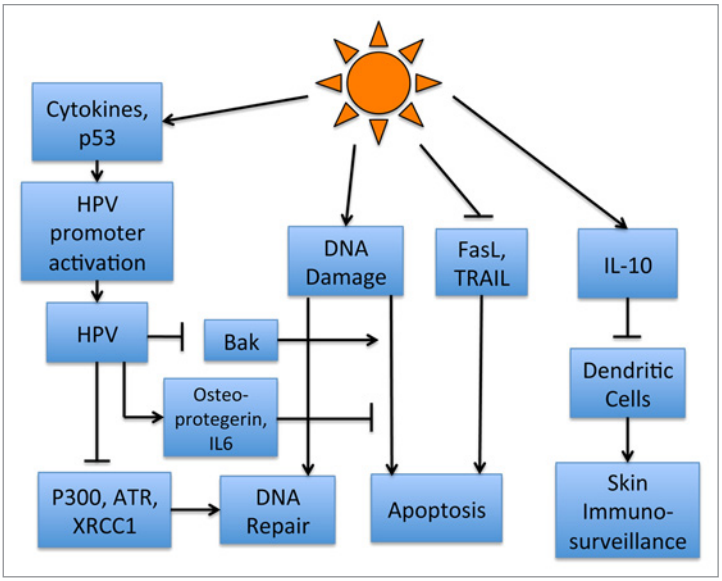

Figure 2. UV radiation (UVR) mutates keratinocyte DNA and inhibits apoptosis of UV-damaged cells through downregulation of FasL and TRAIL. It also induces immunosuppressive IL-10 expression, which inhibits dendritic cells in the skin, suppressing the skin's immune system. UV-induced proinflammatory cytokines and p53 expression modulate the promoter activity of a number of HPV types. HPV further prevents DNA damage repair by promoting degradation of p300, inhibition of XRCC1 and a reduction in ATR. HPVs also limit apoptosis through proteolytic degradation of pro-apoptotic Bak and upregulation of osteoprotegerin and IL-6. Over time, these interactions result in the accumulation and propagation of somatic mutations, and ultimately, tumorigenesis. FasL: Fas ligand/CD95 ligand, TRAIL: tumor necrosis factor (TNF)-related apoptosis inducing ligand, IL-10: interleukin 10, XRCC1: X-ray repair cross-complementing protein 1, ATR: ataxia telangiectasia-mutated (ATM) and Rad3-related kinase.

HPV oncoproteins are also capable of preventing the apoptosis of UV-damaged cells in SCCs[75] through proteolysis of the pro-apoptotic regulator BAK,[76] inhibition of apoptosis inducing factor (AIF) release,[77] and homeodomain-interacting protein kinase 2 (HIPK2)-mediated p53 phosphorylation.[78] Furthermore, HPV proteins block UV-induced apoptosis by upregulating the TNF-superfamily member osteoprotegerin (OPG) and IL-6, both of which inhibit the extrinsic and intrinsic apoptotic pathways, respectively.[79] Others have found no inhibition of UVB-induced apoptosis in keratinocytes transduced with E6 and E7 of HPV5, HPV8, HPV14, HPV24, HPV36, HPV38, and HPV49, even after p53 reactivation.[80] Thus E6 and E7 are likely to exert differing effects on UV-induced apoptosis according to the β-HPV type and the cellular context.

In addition to inadequate removal of cells by apoptosis, failure to repair damaged DNA can lead to the propagation of somatic mutations and ultimately, to carcinogenesis.[81] E6 derived from either HPV5 or HPV8 enhances the carcinogenic potential of UVB exposure by disrupting p300 DNA damage repair and interfering with cell-cycle checkpoint signaling.[82] Furthermore, HPV1 and HPV8 E6 directly bind to X-ray repair cross-complementing protein 1 (XRCC1), interfering with single-strand break repair.[83] The E6 protein of HPV5 has also been shown to compromise the repair of UV-induced thymine dimers.[84] Disruption of the DNA repair process may be a critical element in the progression of HPV-induced oncogenesis. Overall, UV exposure in EV patients not only damages keratinocyte DNA, but also promotes viral transcription and replication, resulting in HPV impairment of DNA damage repair coincident with attenuated apoptotic cell death, and ultimately, tumorigenesis (**Fig. 2**).

Transmembrane Channel-like (TMC) Family

The mammalian transmembrane channel-like (*TMC*) gene family is comprised of 8 genes, *TMC1* to *8*, with *TMC6* equivalent to the locus referred to as *EVER1* and *TMC8* also known as *EVER2*. Although none of the mammalian *TMC* genes share significant sequence similarity to any other genes or motifs, they each encode a conserved 120 amino acid domain termed the TMC domain.[85] The TMC family is grouped into 3 subfamilies, A, B, and C, distinguished on the basis of sequence homology and structural similarities between their respective genes.[86] Subfamily A consists of *TMC1*, *TMC2,* and *TMC3*, subfamily B consists of *TMC5* and *TMC6*, and subfamily C consists of *TMC4*, *TMC7,* and *TMC8*. The *TMC* genes map to 6 chromosomal locations in both human and mouse,[86] and have also been found in other vertebrates and invertebrates, including zebra fish, nematode, pufferfish, *C. elegans*, mosquito, and *D. melanogaster*.[85,86] So far, no evidence for TMC gene expression has been found among fungi or plants.

The *TMC* gene family was discovered through positional cloning of the gene underlying both dominant and recessive nonsyndromic sensorineural hearing loss at the DFNA36 and DFNB7/B11 loci, respectively, in chromosomal region 9q13-q21.[87] These allelic disorders are caused by mutation of *TMC1*, the mouse ortholog also exhibiting dominant and recessive mutant alleles that cause hearing loss in the Beethoven (*Bth*) and deafness (*dn*) mutant mouse strains, respectively.[88] *Tmc4* thru *7* are expressed in most murine organs, while *Tmc1* and *3* mRNAs are detectable in most neuronal organs, as well as some non-neuronal organs. *Tmc2* transcripts are detected in testis, and *Tmc8* mRNA is detected in thymus, spleen and lung.[86]

The *TMC* genes are predicted to encode proteins with at least 6 conserved transmembrane domains, raising the possibility that TMC proteins may function as ion channels, pumps, or transporters.[87] Recently, TMC proteins were suggested to be evolutionarily related to the anoctamin (ANO) family of calcium-activated chloride channels, sharing conserved amino acid residues in several transmembrane spanning regions.[89] The cochlear electrophysiologic phenotype of *dn* mutant mice is consistent with an ion channel defect,[90] and *tmc1* has been shown to encode a sodium-sensitive channel required for salt chemosensation in *C. elegans*.[91] Furthermore, Tmc1 and Tmc2 were recently suggested to be essential for targeting and interacting with, but not constituting, mechanotransducer (MT) channels in cochlear hair cells. They are thought to serve as protein partners in a transduction complex necessary for MT conductance and calcium permeability.[92]

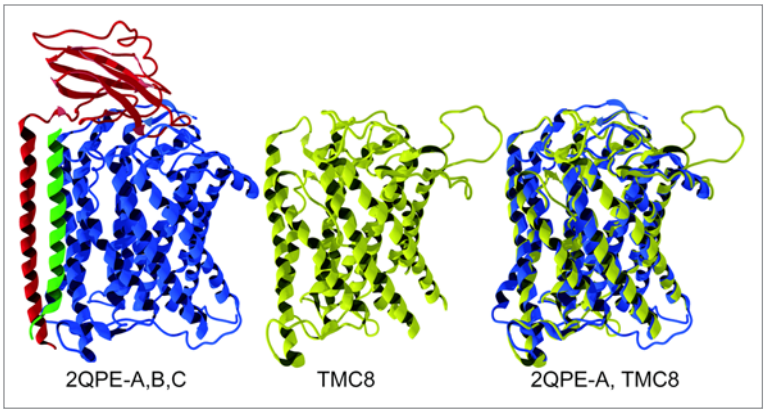

Figure 3. Unbiased protein homology modeling of TMC8 reveals structural similarities with subunit 1 of Cytochrome Ba3 Oxidase. Crystal structure representation of Cytochrome BA3 Oxidase (2QPE) from *Thermus thermophiles* (left); 2QPE Chain A (blue), 2QPE Chain B (red), and 2QPE Chain C (green). TMC8 reverse homology modeled using CPHmodels-3.2[93] (middle, yellow), and overlaid with 2PQE Chain A (right).

TMC Protein Structure

TMC proteins are predicted to contain 6 to 10 membrane-spanning domains, without N-terminal signal peptide sequences or trafficking signals. All of the proteins are predicted to reside in the plasma membrane, with the C-termini of all but TMC6 predicted to be cytoplasmic.[85] Numerous posttranslational modification sites have been predicted in each human and mouse amino acid sequence for amidation, glycosylation, and myristoylation, as well as phosphorylation.[85] There is a high degree of conservation between human and murine TMC proteins (75–96%).[86]

Although predicted to be an ion channel protein, TMC8 is predicted to share structural similarities with subunit 1 of Cytochrome Ba3 Oxidase from *Thermus thermophilus* (2QPE) (**Fig. 3**) when using unbiased protein homology modeling.[93] 2QPE belongs to the heme-copper oxidase I superfamily of transmembrane protein complexes located in the respiratory chains of prokaryotes and mitochondria that catalyze the reduction of O_2 and simultaneously pump protons across the membrane.[94] This homology may suggest a similar bimetallic core and/or redox function for TMC8. In support of the latter, TMC8 possesses a CXXC motif, a well-characterized redox-active disulfide sequence that may serve as a reduction state molecular switch.[95]

TMC6 and TMC8 Origins and Expression

TMC6 and *TMC8* are adjacent genes oriented in a head-to-head configuration on chromosome 17q25.3 in the human,[85] with approximately 1.6 kb overlap. In the mouse, *Tmc6* and *Tmc8* are separated by 2 kb in a head-to-head configuration on chromosome 11.[85] Sharing only 28.4% homology, their least conserved regions are their amino and carboxyl termini.[3] All EV-associated TMC6 and TMC8 mutations identified so far eliminate the conserved TMC domain, which is theorized to mediate molecular properties such as cellular ion homeostasis, signal transduction, or homotypic or heterotypic assembly into multimeric complexes.[36,85,96,97] Previously published data indicate that *TMC6* and *TMC8* are highly expressed in various types of hematopoietic cells, including $CD4^+$ and $CD8^+$ T-lymphocytes, B-lymphocytes, and NK cells.[98] *TMC6* was shown to be significantly transcribed in endothelial cells, bone marrow $CD33^+$ myeloid cells, as well as dendritic cells.[98] Transient protein expression of TMC6 and TMC8 has also been localized to the ER of transfected HaCaT keratinocytes,[35] although this may not reflect their subcellular distribution in situ.[85] We find the highest concentrations of TMC8 protein in lysates prepared from skin and breast tissues, with less expression apparent in testes, uterus, skeletal muscle, and thymus, and little TMC8 expressed in lung or ovary (**Fig. 4**). TMC8 expression was not detected in peripheral blood leukocytes, lymph node, or spleen (**Fig. 4**). Staining of human skin also indicates high expression of TMC8 (**Fig. 4**, green) in the stratum basale, adjacent to the basement membrane (laminin, **Fig. 4**, purple), It is diminished in the stratum spinosum, and is absent in the stratum corneum (**Fig. 4**). These data suggest that the primary function of TMC8 is unlikely to be generally immunocyte-related, but rather, that TMC8 is most important in skin and breast epithelia.

TMC6 and TMC8 Protection from EV-HPV Infection or Oncogenesis

Although the link between TMC6 and TMC8 mutations and EV has now been well established, the molecular role(s) of TMCs in controlling the progression of the disease remains unclear. Nonetheless, we hypothesize that these TMC proteins work to protect healthy individuals from EV-HPV infection and/or oncogenesis via one or more of the following mechanisms: (1) disruption of the HPV life cycle through host cell cycle regulation, (2) regulation of T-cell-mediated viral clearance, or (3) control of the apoptosis/survival balance in host keratinocytes, especially during UV-induced DNA damage.

TMC6 and 8 Modulation of the HPV Life Cycle

Cellular proliferation is an important part of HPV maintenance, with evidence that viral oncoproteins manipulate host cellular proteins to maintain cell replication and/or for quiescent cells to re-enter the cell cycle. TMC8 may influence cell cycle homeostasis, as keratinocytes with a mutated TMC8 grow at a greater rate than wild-type keratinocytes.[99] TMC6 and TMC8 proteins have been shown to form complexes and interact with zinc transporter 1 (ZnT-1),[99] suppressing the activation of the AP-1, Elk-1, and Fos transcription factors.[99] This may have a pivotal effect on the HPV life cycle, as there are multiple AP-1 binding sites within the LCR of the HPV genome that enhance E6/E7 expression.[100,101] In keratinocytes, the TMC/ZnT-1 complex also modulated intracellular zinc (Zn^{2+})

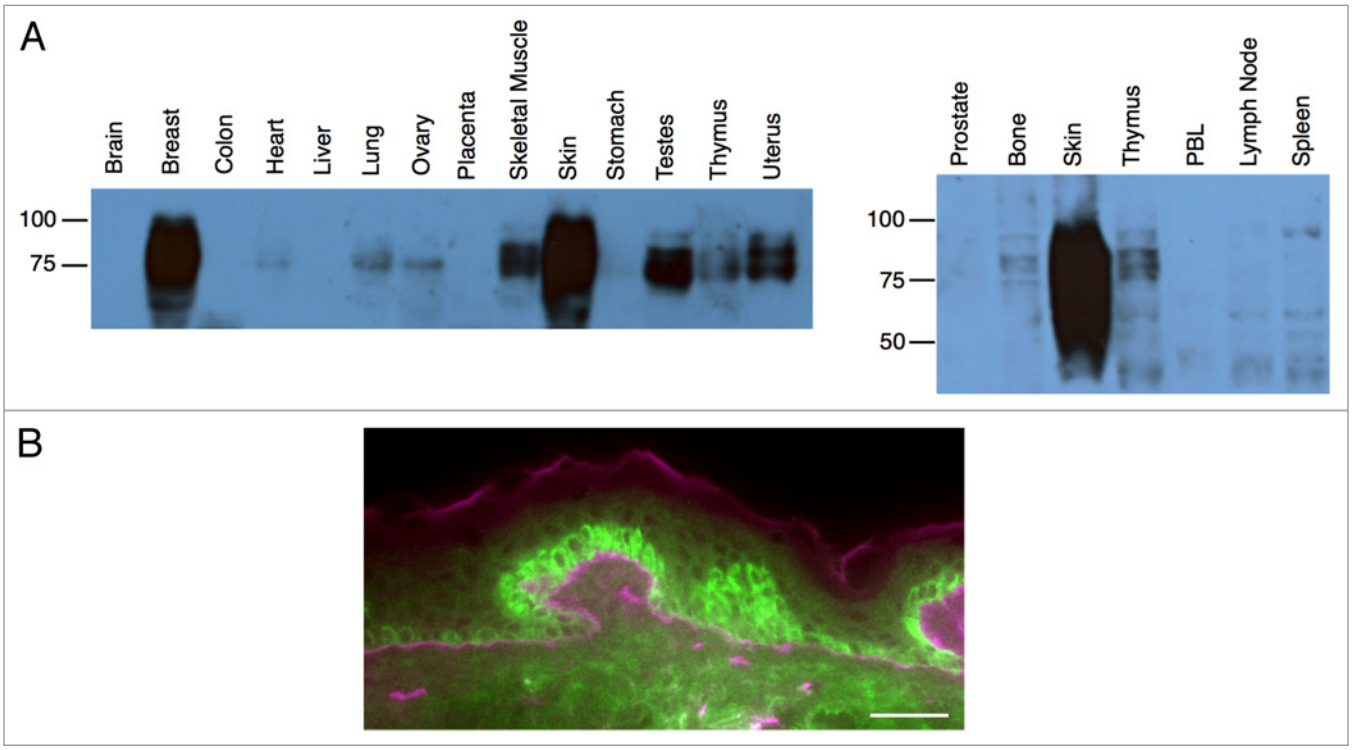

Figure 4. TMC8 protein expression. Expression analysis of transmembrane channel-like 8 (TMC8) by western blot (**A**) and immunofluorescence staining (**B**). (**A**) Human tissue lysates (15 μg, BioChain) were boiled in reducing sample buffer, resolved by 10% SDS-PAGE, transferred to PVDF membrane and probed with an anti-TMC8 antibody (Bethyl Labs). (**B**) Frozen human skin section (Biochain, CA) stained with antibodies against TMC8 (Bethyl Labs, green) and basal lamina marker laminin (US Biological, purple). Scale bar: 200 μm.

distribution,[99] possibly affecting several physiological processes including the host-virus interaction,[102] but none specifically demonstrated. The E5 oncoprotein of HPV16, a high-risk cervical HPV type, has been shown to bind the TMC/ZnT-1 complex and prevent its inhibition of AP-1 transcriptional activity,[99] which may explain how genital HPVs such as HPV16 overcome the protective effects of TMC6 and 8. However, b-, g-, and m-HPVs are reported to lack the E5 ORF that is present in the genomes of genital HPVs,[103] and may account for the asymptomatic natures and limited replication of these HPV types in healthy individuals. It is proposed that in EV patients infected with E5-lacking β-HPVs, mutations in TMC6 or TMC8 prevent ZnT-1 complex formation, making AP-1 and Zn^{2+} levels sufficient for viral transcription. However, it is unclear why EV patients with TMC6 or 8 mutations are not more prone to infection with genital HPV types.[104,105]

TMC6 and 8 Regulation of Immune Function

The skin is the largest organ in area, containing multiple immunocompetent cells that contribute to cutaneous immunity against environmental microbes and other foreign materials. TMC8 protein expression was found to be much greater in human skin compared with other tissues tested (**Fig. 4**), suggesting an important yet undefined role in cutaneous immune function. Clinical observations indirectly suggest that the protective role of TMC6 or 8 may not be limited to the epidermis, but may also involve the adaptive immune system. It has been hypothesized that mutations in *TMC6* or *8* downregulate CMI by decreasing the ability of cells to present EV-HPV antigen-derived peptides to T-lymphocytes triggering the clearance of HPV-infected keratinocytes.[106] *TMC6* and *8* genes are abundantly expressed in murine and human T cells, and activation of $CD4^+$ and $CD8^+$ T cells via the T-cell receptor (TCR) was found to trigger a rapid decrease in *TMC6* and *TMC8* expression, accompanied by an accumulation of free zinc ions.[107] Interestingly, lymphoblastoid and primary T cells from TMC8-deficient patients show similarly elevated Zn^{2+} levels.[107] The same study also showed that an excess of Zn^{2+} blocked T-cell activation and proliferation. However, a more recent study showed that TMC8-deficiency does not seem to significantly impair T-cell development or function, suggesting that the maintenance of Zn^{2+} homeostasis by TMC proteins is not essential for their normal development or activation.[53] Thus, while TMC6 and 8 may be involved in the regulation of cellular zinc ion homeostasis in lymphocytes, this role is not essential for normal T-cell function.

TMC6 and 8 control of Host Keratinocyte Apoptosis/Survival Balance

TMC8 was shown to sensitize cells to tumor necrosis factor α (TNFα)-induced apoptosis, through nuclear factor kappa B

(NF-κB) activation and formation of the pro-apoptotic complex II, consisting of receptor-interacting serine/threonine protein kinase 1 (RIPK1), Fas-associated protein with death domain (FADD) and caspase-8.[108] All such effects were independent of Fas ligand (FasL) stimulation. Importantly, TNFα-induced cell death was mediated by the interaction of the TMC domain of TMC8 with TRADD.[108] Furthermore, the authors showed that a specific TMC8 polymorphism associated with persistence of β-HPV, EV, and SCC was less effective in triggering TNFα-induced apoptosis. Taken together, these studies point to a role for TMC8 in overcoming the persistence of HPV-EV lesions and their progression to tumors via apoptosis induction.

Conclusions

SCC development in EV-HPV patients is a multi-step process, requiring the coordinated targeting of multiple cellular signaling pathways by HPV oncoproteins to maintain infected cells in a proliferative state thereby facilitating viral replication and persistence. It is clear that TMC6 and TMC8 proteins serve as fundamental regulators of EV-HPV persistence and oncogenicity, yet their specific molecular functions in EV and SCC remain unclear. The recent discoveries of several non-*TMC* mutations associated with EV have revealed functional deficiencies in base excision repair, T cells, keratinocyte proliferation/differentiation, and apoptosis. It is tempting to speculate that TMC proteins may share some overlapping functionalities or phenotypes since mutations in TMC6 and 8 also result in EV pathology. In addition to investigating these possibilities, characterization of a TMC8 transgenic mouse model, functional analyses of TMC binding partners and electrophysiological analysis should begin to shed further light on the means by which these proteins protect against HPV-EV. Such approaches may facilitate a paradigm shift in our understanding of this primary immunodeficiency and the function of these proteins in response to viral infection.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by grants from the National Institutes of Health, NIMHD P20MD006084, the Hawaii Community Foundation, Leahi Fund 13ADVC-60228 and the Air Force Research Laboratories Minority leadership Program FA8650–13-C-5800. Clearance number, 88ABW-2014-0827. Support for core facilities was provided by NIMHD G12 MD007601; and NIGMS P20GM103466. Thanks to Jason C Barnhill for the TMC8 multi-tissue western blot, and Kimber Cochrane for the TMC8 staining of human skin.

References

1. Bernard HU, Burk RD, Chen Z, van Doorslaer K, zur Hausen H, de Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology 2010; 401:70-9; PMID:20206957; http://dx.doi.org/10.1016/j.virol.2010.02.002
2. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. Virology 2004; 324:17-27; PMID:15183049; http://dx.doi.org/10.1016/j.virol.2004.03.033
3. Orth G. Genetics of epidermodysplasia verruciformis: Insights into host defense against papillomaviruses. Semin Immunol 2006; 18:362-74; PMID:17011789; http://dx.doi.org/10.1016/j.smim.2006.07.008
4. Majewski S, Jablonska S. Do epidermodysplasia verruciformis human papillomaviruses contribute to malignant and benign epidermal proliferations? Arch Dermatol 2002; 138:649-54; PMID:12020228; http://dx.doi.org/10.1001/archderm.138.5.649
5. Lane JE, Bowman PH, Cohen DJ. Epidermodysplasia verruciformis. South Med J 2003; 96:613-5; PMID:12938792; http://dx.doi.org/10.1097/01.SMJ.0000054544.47233.7C
6. McLaughlin-Drubin ME, Münger K. Oncogenic activities of human papillomaviruses. Virus Res 2009; 143:195-208; PMID:19540281; http://dx.doi.org/10.1016/j.virusres.2009.06.008
7. Boxman IL, Berkhout RJ, Mulder LH, Wolkers MC, Bouwes Bavinck JN, Vermeer BJ, ter Schegget J. Detection of human papillomavirus DNA in plucked hairs from renal transplant recipients and healthy volunteers. J Invest Dermatol 1997; 108:712-5; PMID:9129220; http://dx.doi.org/10.1111/1523-1747.ep12292090
8. Hufbauer M, Biddle A, Borgogna C, Gariglio M, Doorbar J, Storey A, Pfister H, Mackenzie I, Akgül B. Expression of betapapillomavirus oncogenes increases the number of keratinocytes with stem cell-like properties. J Virol 2013; 87:12158-65; PMID:24006432; http://dx.doi.org/10.1128/JVI.01510-13
9. Moody CA, Laimins LA. Human papillomavirus oncoproteins: pathways to transformation. Nat Rev Cancer 2010; 10:550-60; PMID:20592731; http://dx.doi.org/10.1038/nrc2886
10. Stanley M. Immune responses to human papillomavirus. Vaccine 2006; 24(Suppl 1):S16-22; PMID:16219398; http://dx.doi.org/10.1016/j.vaccine.2005.09.002
11. Doorbar J. Latent papillomavirus infections and their regulation. Curr Opin Virol 2013; 3:416-21; PMID:23816390; http://dx.doi.org/10.1016/j.coviro.2013.06.003
12. Ishiji T. Molecular mechanism of carcinogenesis by human papillomavirus-16. J Dermatol 2000; 27:73-86; PMID:10721654
13. Favre M, Ramoz N, Orth G. Human papillomaviruses: general features. Clin Dermatol 1997; 15:181-98; PMID:9167903; http://dx.doi.org/10.1016/S0738-081X(97)00008-4
14. Wallace NA, Gasior SL, Faber ZJ, Howie HL, Deininger PL, Galloway DA. HPV 5 and 8 E6 expression reduces ATM protein levels and attenuates LINE-1 retrotransposition. Virology 2013; 443:69-79; PMID:23706308; http://dx.doi.org/10.1016/j.virol.2013.04.022
15. Kazem S, van der Meijden E, Struijk L, de Gruijl FR, Feltkamp MC. Human papillomavirus 8 E6 disrupts terminal skin differentiation and prevents pro-Caspase-14 cleavage. Virus Res 2012; 163:609-16; PMID:22230316; http://dx.doi.org/10.1016/j.virusres.2011.12.015
16. Mendoza JA, Jacob Y, Cassonnet P, Favre M. Human papillomavirus type 5 E6 oncoprotein represses the transforming growth factor beta signaling pathway by binding to SMAD3. J Virol 2006; 80:12420-4; PMID:17020941; http://dx.doi.org/10.1128/JVI.02576-05
17. Sperling T, Ołdak M, Walch-Rückheim B, Wickenhauser C, Doorbar J, Pfister H, Malejczyk M, Majewski S, Keates AC, Smola S. Human papillomavirus type 8 interferes with a novel C/EBPβ-mediated mechanism of keratinocyte CCL20 chemokine expression and Langerhans cell migration. PLoS Pathog 2012; 8:e1002833; PMID:22911498; http://dx.doi.org/10.1371/journal.ppat.1002833
18. Iwatsuki K, Nakayama Y, Hamada T, Nakanishi G, Shirafuji Y, Morizane S. Immunological and structural remodeling in human papillomavirus-induced warts and Bowen disease. Eur J Dermatol 2013;(Forthcoming) PMID:23644544
19. Byg LM, Vidlund J, Vasiljevic N, Clausen D, Forslund O, Norrild B. NF-κB signalling is attenuated by the E7 protein from cutaneous human papillomaviruses. Virus Res 2012; 169:48-53; PMID:22776252; http://dx.doi.org/10.1016/j.virusres.2012.06.028
20. Schaper ID, Marcuzzi GP, Weissenborn SJ, Kasper HU, Dries V, Smyth N, Fuchs P, Pfister H. Development of skin tumors in mice transgenic for early genes of human papillomavirus type 8. Cancer Res 2005; 65:1394-400; PMID:15735026; http://dx.doi.org/10.1158/0008-5472.CAN-04-3263
21. Lazić D, Alborzi F, Marcuzzi GP, Angel P, Hess J, Pfister H, Akgül B. Enhanced StefinA and Sprr2 expression during papilloma formation in HPV8 transgenic mice. J Dermatol Sci 2011; 62:84-90; PMID:21458245; http://dx.doi.org/10.1016/j.jdermsci.2011.02.006
22. Hufbauer M, Lazić D, Reinartz M, Akgül B, Pfister H, Weissenborn SJ. Skin tumor formation in human papillomavirus 8 transgenic mice is associated with a deregulation of oncogenic miRNAs and their tumor suppressive targets. J Dermatol Sci 2011; 64:7-15; PMID:21763111; http://dx.doi.org/10.1016/j.jdermsci.2011.06.008

23. Dong W, Kloz U, Accardi R, Caldeira S, Tong WM, Wang ZQ, Jansen L, Dürst M, Sylla BS, Gissmann L, et al. Skin hyperproliferation and susceptibility to chemical carcinogenesis in transgenic mice expressing E6 and E7 of human papillomavirus type 38. J Virol 2005; 79:14899-908; PMID:16282489; http://dx.doi.org/10.1128/JVI.79.23.14899-14908.2005
24. Michel A, Kopp-Schneider A, Zentgraf H, Gruber AD, de Villiers EM. E6/E7 expression of human papillomavirus type 20 (HPV-20) and HPV-27 influences proliferation and differentiation of the skin in UV-irradiated SKH-hr1 transgenic mice. J Virol 2006; 80:11153-64; PMID:16971438; http://dx.doi.org/10.1128/JVI.00954-06
25. Buitrago-Pérez Á, Hachimi M, Dueñas M, Lloveras B, Santos A, Holguín A, Duarte B, Santiago JL, Akgül B, Rodríguez-Peralto JL, et al. A humanized mouse model of HPV-associated pathology driven by E7 expression. PLoS One 2012; 7:e41743; PMID:22911850; http://dx.doi.org/10.1371/journal.pone.0041743
26. Lewandowsky F, Lutz W. Ein Fall einer bisher nicht beschriebenen Hauterkrankung (Epidermodysplasia verruciformis). Arch Derm Syphilol 1922; 141:193; http://dx.doi.org/10.1007/BF01938833
27. Lutzner MA, Blanchet-Bardon C, Orth G. Clinical observations, virologic studies, and treatment trials in patients with epidermodysplasia verruciformis, a disease induced by specific human papillomaviruses. J Invest Dermatol 1984; 83(Suppl):18s-25s; PMID:6330217; http://dx.doi.org/10.1111/1523-1747.ep12281128
28. Orth G, Jablonska S, Jarzabek-Chorzelska M, Obalek S, Rzesa G, Favre M, Croissant O. Characteristics of the lesions and risk of malignant conversion associated with the type of human papillomavirus involved in epidermodysplasia verruciformis. Cancer Res 1979; 39:1074-82; PMID:218721
29. Astori G, Lavergne D, Benton C, Höckmayr B, Egawa K, Garbe C, de Villiers EM. Human papillomaviruses are commonly found in normal skin of immunocompetent hosts. J Invest Dermatol 1998; 110:752-5; PMID:9579540; http://dx.doi.org/10.1046/j.1523-1747.1998.00191.x
30. Antonsson A, Karanfilovska S, Lindqvist PG, Hansson BG. General acquisition of human papillomavirus infections of skin occurs in early infancy. J Clin Microbiol 2003; 41:2509-14; PMID:12791874; http://dx.doi.org/10.1128/JCM.41.6.2509-2514.2003
31. Weissenborn SJ, Nindl I, Purdie K, Harwood C, Proby C, Breuer J, Majewski S, Pfister H, Wieland U. Human papillomavirus-DNA loads in actinic keratoses exceed those in non-melanoma skin cancers. J Invest Dermatol 2005; 125:93-7; PMID:15982308; http://dx.doi.org/10.1111/j.0022-202X.2005.23733.x
32. Stark S, Petridis AK, Ghim SJ, Jenson AB, Bouwes Bavinck JN, Gross G, Stockfleth E, Fuchs PG, Pfister H. Prevalence of antibodies against virus-like particles of Epidermodysplasia verruciformis-associated HPV8 in patients at risk of skin cancer. J Invest Dermatol 1998; 111:696-701; PMID:9764856; http://dx.doi.org/10.1046/j.1523-1747.1998.00360.x
33. Favre M, Orth G, Majewski S, Baloul S, Pura A, Jablonska S. Psoriasis: A possible reservoir for human papillomavirus type 5, the virus associated with skin carcinomas of epidermodysplasia verruciformis. J Invest Dermatol 1998; 110:311-7; PMID:9540967; http://dx.doi.org/10.1046/j.1523-1747.1998.00164.x
34. Ramoz N, Rueda LA, Bouadjar B, Favre M, Orth G. A susceptibility locus for epidermodysplasia verruciformis, an abnormal predisposition to infection with the oncogenic human papillomavirus type 5, maps to chromosome 17qter in a region containing a psoriasis locus. J Invest Dermatol 1999; 112:259-63; PMID:10084299; http://dx.doi.org/10.1046/j.1523-1747.1999.00536.x
35. Ramoz N, Rueda LA, Bouadjar B, Montoya LS, Orth G, Favre M. Mutations in two adjacent novel genes are associated with epidermodysplasia verruciformis. Nat Genet 2002; 32:579-81; PMID:12426567; http://dx.doi.org/10.1038/ng1044
36. Ramoz N, Taïeb A, Rueda LA, Montoya LS, Bouadjar B, Favre M, Orth G. Evidence for a nonallelic heterogeneity of epidermodysplasia verruciformis with two susceptibility loci mapped to chromosome regions 2p21-p24 and 17q25. J Invest Dermatol 2000; 114:1148-53; PMID:10844558; http://dx.doi.org/10.1046/j.1523-1747.2000.00996.x
37. Crequer A, Picard C, Patin E, D'Amico A, Abhyankar A, Munzer M, Debré M, Zhang SY, de Saint-Basile G, Fischer A, et al. Inherited MST1 deficiency underlies susceptibility to EV-HPV infections. PLoS One 2012; 7:e44010; PMID:22952854; http://dx.doi.org/10.1371/journal.pone.0044010
38. Oh KS, Khan SG, Jaspers NG, Raams A, Ueda T, Lehmann A, Friedmann PS, Emmert S, Gratchev A, Lachlan K, et al. Phenotypic heterogeneity in the XPB DNA helicase gene (ERCC3): xeroderma pigmentosum without and with Cockayne syndrome. Hum Mutat 2006; 27:1092-103; PMID:16947863; http://dx.doi.org/10.1002/humu.20392
39. Nehme NT, Pachlopnik Schmid J, Debeurme F, André-Schmutz I, Lim A, Nitschke P, Rieux-Laucat F, Lutz P, Picard C, Mahlaoui N, et al. MST1 mutations in autosomal recessive primary immunodeficiency characterized by defective naive T-cell survival. Blood 2012; 119:3458-68; PMID:22174160; http://dx.doi.org/10.1182/blood-2011-09-378364
40. Avruch J, Zhou D, Fitamant J, Bardeesy N. Mst1/2 signalling to Yap: gatekeeper for liver size and tumour development. Br J Cancer 2011; 104:24-32; PMID:21102585; http://dx.doi.org/10.1038/sj.bjc.6606011
41. Tan MJ, White EA, Sowa ME, Harper JW, Aster JC, Howley PM. Cutaneous β-human papillomavirus E6 proteins bind Mastermind-like coactivators and repress Notch signaling. Proc Natl Acad Sci U S A 2012; 109:E1473-80; PMID:22547818; http://dx.doi.org/10.1073/pnas.1205991109
42. Crequer A, Troeger A, Patin E, Ma CS, Picard C, Pedergnana V, Fieschi C, Lim A, Abhyankar A, Gineau L, et al. Human RHOH deficiency causes T cell defects and susceptibility to EV-HPV infections. J Clin Invest 2012; 122:3239-47; PMID:22850876; http://dx.doi.org/10.1172/JCI62949
43. Tybulewicz VL, Henderson RB. Rho family GTPases and their regulators in lymphocytes. Nat Rev Immunol 2009; 9:630-44; PMID:19696767; http://dx.doi.org/10.1038/nri2606
44. Zavattaro E, Azzimonti B, Mondini M, De Andrea M, Borgogna C, Dell'Oste V, Ferretti M, Nicola S, Cappellano G, Carando A, et al. Identification of defective Fas function and variation of the perforin gene in an epidermodysplasia verruciformis patient lacking EVER1 and EVER2 mutations. J Invest Dermatol 2008; 128:732-5; PMID:17960179; http://dx.doi.org/10.1038/sj.jid.5701124
45. Al-Herz W, Bousfiha A, Casanova JL, Chapel H, Conley ME, Cunningham-Rundles C, Etzioni A, Fischer A, Franco JL, Geha RS, et al. Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. Front Immunol 2011; 2:54; PMID:22566844
46. Majewski S, Malejczyk J, Jablonska S, Misiewicz J, Rudnicka L, Obalek S, Orth G. Natural cell-mediated cytotoxicity against various target cells in patients with epidermodysplasia verruciformis. J Am Acad Dermatol 1990; 22:423-7; PMID:2155952; http://dx.doi.org/10.1016/0190-9622(90)70058-P
47. Cooper KD, Androphy EJ, Lowy D, Katz SI. Antigen presentation and T-cell activation in epidermodysplasia verruciformis. J Invest Dermatol 1990; 94:769-76; PMID:2162366; http://dx.doi.org/10.1111/1523-1747.ep12874631
48. Prawer SE, Pass F, Vance JC, Greenberg LJ, Yunis EJ, Zelickson AS. Depressed immune function in epidermodysplasia verruciformis. Arch Dermatol 1977; 113:495-9; PMID:848981; http://dx.doi.org/10.1001/archderm.1977.01640040103018
49. Gliński W, Jablonska S, Langner A, Obalek S, Haftek M, Proniewska M. Cell-mediated immunity in epidermodysplasia verruciformis. Dermatologica 1976; 153:218-27; PMID:1017532; http://dx.doi.org/10.1159/000251060
50. Pereira de Oliveira WR, Carrasco S, Neto CF, Rady P, Tyring SK. Nonspecific cell-mediated immunity in patients with epidermodysplasia verruciformis. J Dermatol 2003; 30:203-9; PMID:12692356
51. Gliński W, Obałek S, Jabłońska S, Orth G. T cell defect in patients with epidermodysplasia verruciformis due to human papillomavirus type 3 and 5. Dermatologica 1981; 162:141-7; PMID:6265297; http://dx.doi.org/10.1159/000250262
52. Majewski S, Skopinska-Rozewska E, Jabłonska S, Wasik M, Misiewicz J, Orth G. Partial defects of cell-mediated immunity in patients with epidermodysplasia verruciformis. J Am Acad Dermatol 1986; 15:966-73; PMID:3491095; http://dx.doi.org/10.1016/S0190-9622(86)70258-2
53. Crequer A, Picard C, Pedergnana V, Lim A, Zhang SY, Abel L, Majewski S, Casanova JL, Jablonska S, Orth G, et al. EVER2 deficiency is associated with mild T-cell abnormalities. J Clin Immunol 2013; 33:14-21; PMID:22903682; http://dx.doi.org/10.1007/s10875-012-9749-1
54. Majewski S, Jablonska S. Epidermodysplasia verruciformis as a model of human papillomavirus-induced genetic cancers: the role of local immunosurveillance. Am J Med Sci 1992; 304:174-9; PMID:1335684; http://dx.doi.org/10.1097/00000441-199209000-00006
55. Rogers HD, Macgregor JL, Nord KM, Tyring S, Rady P, Engler DE, Grossman ME. Acquired epidermodysplasia verruciformis. J Am Acad Dermatol 2009; 60:315-20; PMID:19150275; http://dx.doi.org/10.1016/j.jaad.2008.08.035
56. Kaushal A, Silver S, Kasper K, Severini A, Hamza S, Keynan Y. Epidermodysplasia verruciformis in an HIV-infected man: a case report and review of the literature. Top Antivir Med 2012; 20:173-9; PMID:23363696
57. Jacobelli S, Laude H, Carlotti A, Rozenberg F, Deleuze J, Morini JP, Franck N, Gorin I, Avril MF, Dupin N. Epidermodysplasia verruciformis in human immunodeficiency virus-infected patients: a marker of human papillomavirus-related disorders not affected by antiretroviral therapy. Arch Dermatol 2011; 147:590-6; PMID:21242344; http://dx.doi.org/10.1001/archdermatol.2010.399
58. Morrison C, Eliezri Y, Magro C, Nuovo GJ. The histologic spectrum of epidermodysplasia verruciformis in transplant and AIDS patients. J Cutan Pathol 2002; 29:480-9; PMID:12207742; http://dx.doi.org/10.1034/j.1600-0560.2002.290806.x
59. Kunishige JH, Hymes SR, Madkan V, Wyatt AJ, Uptmore D, Lazar AJ, Giralt S, Rady P, Tyring S. Epidermodysplasia verruciformis in the setting of graft-versus-host disease. J Am Acad Dermatol 2007; 57(Suppl):S78-80; PMID:17938030; http://dx.doi.org/10.1016/j.jaad.2006.04.035
60. Holmes C, Chong AH, Tabrizi SN, Downes N, Nindl I. Epidermodysplasia verruciformis-like syndrome in association with systemic lupus erythematosus. Australas J Dermatol 2009; 50:44-7; PMID:19178492; http://dx.doi.org/10.1111/j.1440-0960.2008.00502.x
61. de Oliveira WR, Festa Neto C, Rady PL, Tyring SK. Clinical aspects of epidermodysplasia verruciformis. J Eur Acad Dermatol Venereol 2003; 17:394-8; PMID:12834447; http://dx.doi.org/10.1046/j.1468-3083.2003.00703.x

62. Mudigonda T, Pearce DJ, Yentzer BA, Williford P, Feldman SR. The economic impact of non-melanoma skin cancer: a review. J Natl Compr Canc Netw 2010; 8:888-96; PMID:20870635
63. Patel T, Morrison LK, Rady P, Tyring S. Epidermodysplasia verruciformis and susceptibility to HPV. Dis Markers 2010; 29:199-206; PMID:21178278; http://dx.doi.org/10.1155/2010/345436
64. Iftner A, Klug SJ, Garbe C, Blum A, Stancu A, Wilczynski SP, Iftner T. The prevalence of human papillomavirus genotypes in nonmelanoma skin cancers of nonimmunosuppressed individuals identifies high-risk genital types as possible risk factors. Cancer Res 2003; 63:7515-9; PMID:14612553
65. Forslund O, Iftner T, Andersson K, Lindelof B, Hradil E, Nordin P, Stenquist B, Kirnbauer R, Dillner J, de Villiers EM; Viraskin Study Group. Cutaneous human papillomaviruses found in sun-exposed skin: Beta-papillomavirus species 2 predominates in squamous cell carcinoma. J Infect Dis 2007; 196:876-83; PMID:17703418; http://dx.doi.org/10.1086/521031
66. Au WW, Wilkinson GS, Tyring SK, Legator MS, el Zein R, Hallberg L, Heo MY. Monitoring populations for DNA repair deficiency and for cancer susceptibility. Environ Health Perspect 1996; 104(Suppl 3):579-84; PMID:8781386
67. Brash DE. Sunlight and the onset of skin cancer. Trends Genet 1997; 13:410-4; PMID:9351343; http://dx.doi.org/10.1016/S0168-9525(97)01246-8
68. Brash DE, Pontén J. Skin precancer. Cancer Surv 1998; 32:69-113; PMID:10489624
69. Padlewska K, Ramoz N, Cassonnet P, Riou G, Barrois M, Majewski S, Croissant O, Jablonska S, Orth G. Mutation and abnormal expression of the p53 gene in the viral skin carcinogenesis of epidermodysplasia verruciformis. J Invest Dermatol 2001; 117:935-42; PMID:11676835; http://dx.doi.org/10.1046/j.0022-202x.2001.01515.x
70. Purdie KJ, Pennington J, Proby CM, Khalaf S, de Villiers EM, Leigh IM, Storey A. The promoter of a novel human papillomavirus (HPV77) associated with skin cancer displays UV responsiveness, which is mediated through a consensus p53 binding sequence. EMBO J 1999; 18:5359-69; PMID:10508168; http://dx.doi.org/10.1093/emboj/18.19.5359
71. Akgül B, Lemme W, García-Escudero R, Storey A, Pfister HJ. UV-B irradiation stimulates the promoter activity of the high-risk, cutaneous human papillomavirus 5 and 8 in primary keratinocytes. Arch Virol 2005; 150:145-51; PMID:15654507; http://dx.doi.org/10.1007/s00705-004-0398-4
72. Shurin MR, Yurkovetsky ZR, Tourkova IL, Balkir L, Shurin GV. Inhibition of CD40 expression and CD40-mediated dendritic cell function by tumor-derived IL-10. Int J Cancer 2002; 101:61-8; PMID:12209589; http://dx.doi.org/10.1002/ijc.10576
73. Bachmann F, Buechner SA, Wernli M, Strebel S, Erb P. Ultraviolet light downregulates CD95 ligand and TRAIL receptor expression facilitating actinic keratosis and squamous cell carcinoma formation. J Invest Dermatol 2001; 117:59-66; PMID:11442750; http://dx.doi.org/10.1046/j.0022-202x.2001.01380.x
74. Erb P, Ji J, Kump E, Mielgo A, Wernli M. Apoptosis and pathogenesis of melanoma and nonmelanoma skin cancer. Adv Exp Med Biol 2008; 624:283-95; PMID:18348464; http://dx.doi.org/10.1007/978-0-387-77574-6_22
75. Jackson S, Ghali L, Harwood C, Storey A. Reduced apoptotic levels in squamous but not basal cell carcinomas correlates with detection of cutaneous human papillomavirus. Br J Cancer 2002; 87:319-23; PMID:12177802; http://dx.doi.org/10.1038/sj.bjc.6600431
76. Jackson S, Harwood C, Thomas M, Banks L, Storey A. Role of Bak in UV-induced apoptosis in skin cancer and abrogation by HPV E6 proteins. Genes Dev 2000; 14:3065-73; PMID:11114894; http://dx.doi.org/10.1101/gad.182100
77. Leverrier S, Bergamaschi D, Ghali L, Ola A, Warnes G, Akgül B, Blight K, García-Escudero R, Penna A, Eddaoudi A, et al. Role of HPV E6 proteins in preventing UVB-induced release of pro-apoptotic factors from the mitochondria. Apoptosis 2007; 12:549-60; PMID:17195958; http://dx.doi.org/10.1007/s10495-006-0004-1
78. Muschik D, Braspenning-Wesch I, Stockfleth E, Rösl F, Hofmann TG, Nindl I. Cutaneous HPV23 E6 prevents p53 phosphorylation through interaction with HIPK2. PLoS One 2011; 6:e27655; PMID:22110707; http://dx.doi.org/10.1371/journal.pone.0027655
79. Tomlins C, Storey A. Cutaneous HPV5 E6 causes increased expression of Osteoprotegerin and Interleukin 6 which contribute to evasion of UV-induced apoptosis. Carcinogenesis 2010; 31:2155-64; PMID:20884685; http://dx.doi.org/10.1093/carcin/bgq200
80. Guerrini JS, Bouvard V, Oswald E, Alonso A, Prétet JL, Tommasino M, Mougin C, Aubin F. E6 and E7 proteins from different beta-papillomaviruses types do not interfere in UVB-induced apoptosis of HaCaT keratinocytes. Exp Dermatol 2011; 20:71-3; PMID:21158941; http://dx.doi.org/10.1111/j.1600-0625.2010.01197.x
81. Ichihashi M, Ueda M, Budiyanto A, Bito T, Oka M, Fukunaga M, Tsuru K, Horikawa T. UV-induced skin damage. Toxicology 2003; 189:21-39; PMID:12821280; http://dx.doi.org/10.1016/S0300-483X(03)00150-1
82. Wallace NA, Robinson K, Howie HL, Galloway DA. HPV 5 and 8 E6 abrogate ATR activity resulting in increased persistence of UVB induced DNA damage. PLoS Pathog 2012; 8:e1002807; PMID:22807682; http://dx.doi.org/10.1371/journal.ppat.1002807
83. Iftner T, Elbel M, Schopp B, Hiller T, Loizou JI, Caldecott KW, Stubenrauch F. Interference of papillomavirus E6 protein with single-strand break repair by interaction with XRCC1. EMBO J 2002; 21:4741-8; PMID:12198176; http://dx.doi.org/10.1093/emboj/cdf443
84. Giampieri S, Storey A. Repair of UV-induced thymine dimers is compromised in cells expressing the E6 protein from human papillomaviruses types 5 and 18. Br J Cancer 2004; 90:2203-9; PMID:15150558
85. Kurima K, Yang Y, Sorber K, Griffith AJ. Characterization of the transmembrane channel-like (TMC) gene family: functional clues from hearing loss and epidermodysplasia verruciformis. Genomics 2003; 82:300-8; PMID:12906855; http://dx.doi.org/10.1016/S0888-7543(03)00154-X
86. Keresztes G, Mutai H, Heller S. TMC and EVER genes belong to a larger novel family, the TMC gene family encoding transmembrane proteins. BMC Genomics 2003; 4:24; PMID:12812529; http://dx.doi.org/10.1186/1471-2164-4-24
87. Kurima K, Peters LM, Yang Y, Riazuddin S, Ahmed ZM, Naz S, Arnaud D, Drury S, Mo J, Makishima T, et al. Dominant and recessive deafness caused by mutations of a novel gene, TMC1, required for cochlear hair-cell function. Nat Genet 2002; 30:277-84; PMID:11850618; http://dx.doi.org/10.1038/ng842
88. Vreugde S, Erven A, Kros CJ, Marcotti W, Fuchs H, Kurima K, Wilcox ER, Friedman TB, Griffith AJ, Balling R, et al. Beethoven, a mouse model for dominant, progressive hearing loss DFNA36. Nat Genet 2002; 30:257-8; PMID:11850623; http://dx.doi.org/10.1038/ng848
89. Hahn Y, Kim DS, Pastan IH, Lee B. Anoctamin and transmembrane channel-like proteins are evolutionarily related. Int J Mol Med 2009; 24:51-5; PMID:19513534; http://dx.doi.org/10.3892/ijmm_00000205
90. Steel KP, Bock GR. The nature of inherited deafness in deafness mice. Nature 1980; 288:159-61; PMID:7432512; http://dx.doi.org/10.1038/288159a0
91. Chatzigeorgiou M, Bang S, Hwang SW, Schafer WR. tmc-1 encodes a sodium-sensitive channel required for salt chemosensation in C. elegans. Nature 2013; 494:95-9; PMID:23364694; http://dx.doi.org/10.1038/nature11845
92. Kim KX, Beurg M, Hackney CM, Furness DN, Mahendrasingam S, Fettiplace R. The role of transmembrane channel-like proteins in the operation of hair cell mechanotransducer channels. J Gen Physiol 2013; 142:493-505; PMID:24127526; http://dx.doi.org/10.1085/jgp.201311068
93. Nielsen M, Lundegaard C, Lund O, Petersen TN. CPHmodels-3.0--remote homology modeling using structure-guided sequence profiles. Nucleic Acids Res 2010; 38:W576-81; PMID:20542909; http://dx.doi.org/10.1093/nar/gkq535
94. Chang HY, Choi SK, Vakkasoglu AS, Chen Y, Hemp J, Fee JA, Gennis RB. Exploring the proton pump and exit pathway for pumped protons in cytochrome ba3 from Thermus thermophilus. Proc Natl Acad Sci U S A 2012; 109:5259-64; PMID:22431640; http://dx.doi.org/10.1073/pnas.1107345109
95. Wouters MA, Fan SW, Haworth NL. Disulfides as redox switches: from molecular mechanisms to functional significance. Antioxid Redox Signal 2010; 12:53-91; PMID:19634988; http://dx.doi.org/10.1089/ars.2009.2510
96. Tate G, Suzuki T, Kishimoto K, Mitsuya T. Novel mutations of EVER1/TMC6 gene in a Japanese patient with epidermodysplasia verruciformis. J Hum Genet 2004; 49:223-5; PMID:15042430; http://dx.doi.org/10.1007/s10038-004-0135-6
97. Sun XK, Chen JF, Xu AE. A homozygous nonsense mutation in the EVER2 gene leads to epidermodysplasia verruciformis. Clin Exp Dermatol 2005; 30:573-4; PMID:16045695; http://dx.doi.org/10.1111/j.1365-2230.2005.01858.x
98. Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D, Zhang J, Soden R, Hayakawa M, Kreiman G, et al. A gene atlas of the mouse and human protein-encoding transcriptomes. Proc Natl Acad Sci U S A 2004; 101:6062-7; PMID:15075390; http://dx.doi.org/10.1073/pnas.0400782101
99. Lazarczyk M, Pons C, Mendoza JA, Cassonnet P, Jacob Y, Favre M. Regulation of cellular zinc balance as a potential mechanism of EVER-mediated protection against pathogenesis by cutaneous oncogenic human papillomaviruses. J Exp Med 2008; 205:35-42; PMID:18158319; http://dx.doi.org/10.1084/jem.20071311
100. Peto M, Tolle-Ersü I, Kreysch HG, Klock G. Epidermal growth factor induction of human papillomavirus type 16 E6/E7 MRNA in tumor cells involves two AP-1 binding sites in the viral enhancer. J Gen Virol 1995; 76:1945-58; PMID:7636475; http://dx.doi.org/10.1099/0022-1317-76-8-1945
101. Kyo S, Klumpp DJ, Inoue M, Kanaya T, Laimins LA. Expression of AP1 during cellular differentiation determines human papillomavirus E6/E7 expression in stratified epithelial cells. J Gen Virol 1997; 78:401-11; PMID:9018063
102. Lazarczyk M, Favre M. Role of Zn2+ ions in host-virus interactions. J Virol 2008; 82:11486-94; PMID:18787005; http://dx.doi.org/10.1128/JVI.01314-08

103. Bravo IG, Alonso A. Mucosal human papillomaviruses encode four different E5 proteins whose chemistry and phylogeny correlate with malignant or benign growth. J Virol 2004; 78:13613-26; PMID:15564472; http://dx.doi.org/10.1128/JVI.78.24.13613-13626.2004

104. Beskow AH, Rönnholm J, Magnusson PK, Gyllensten UB. Susceptibility locus for epidermodysplasia verruciformis not linked to cervical cancer in situ. Hereditas 2001; 135:61-3; PMID:12035615; http://dx.doi.org/10.1111/j.1601-5223.2001.00061.x

105. Castro FA, Ivansson EL, Schmitt M, Juko-Pecirep I, Kjellberg L, Hildesheim A, Gyllensten UB, Pawlita M. Contribution of TMC6 and TMC8 (EVER1 and EVER2) variants to cervical cancer susceptibility. Int J Cancer 2012; 130:349-55; PMID:21387292; http://dx.doi.org/10.1002/ijc.26016

106. Carré D, Dompmartin A, Verdon R, Comoz F, Le Brun E, Freymuth F, Leroy D. Epidermodysplasia verruciformis in a patient with HIV infection: no response to highly active antiretroviral therapy. Int J Dermatol 2003; 42:296-300; PMID:12694498; http://dx.doi.org/10.1046/j.1365-4362.2003.01707_2.x

107. Lazarczyk M, Dalard C, Hayder M, Dupre L, Pignolet B, Majewski S, Vuillier F, Favre M, Liblau RS. EVER proteins, key elements of the natural anti-human papillomavirus barrier, are regulated upon T-cell activation. PLoS One 2012; 7:e39995; PMID:22761942; http://dx.doi.org/10.1371/journal.pone.0039995

108. Gaud G, Guillemot D, Jacob Y, Favre M, Vuillier F. EVER2 protein binds TRADD to promote TNF-α-induced apoptosis. Cell Death Dis 2013; 4:e499; PMID:23429285; http://dx.doi.org/10.1038/cddis.2013.27

109. Frazer IH. Prevention of cervical cancer through papillomavirus vaccination. Nat Rev Immunol 2004; 4:46-54; PMID:14704767; http://dx.doi.org/10.1038/nri1260