CUTANEOUS MALIGNANCY

The Viral Etiology of Skin Cancer

Jordan M. Meyers[1] and Karl Munger[1,2]

[1]Division of Infectious Diseases, Brigham and Women's Hospital, Department of Medicine and Program in Virology, Harvard Medical School, Boston, Massachusetts, USA

Correspondence: Karl Munger, E-mail: karl.munger@tufts.edu

[2]Present Address: Department of Developmental, Molecular and Chemical Biology, Tufts University School of Medicine, Boston, Massachusetts, USA

doi:10.1038/skinbio.2014.6

The concept that viruses may be etiological agents of cancers is as old as the discovery of viruses themselves. In 1908, 3 years before Peyton Rous was passaging what later became to be known as Rous Sarcoma Virus in chickens, two Danish scientists, Ellerman and Bang, were characterizing a transmissible filtrate that reproducibly caused leukemia in chickens. These findings were received with harsh skepticism, and the scientific community did not universally accept the concept that tumors could be caused by transmissible agents. Richard Shope, a colleague of Peyton Rous at the Rockefeller Institute, identified an infectious agent that infected cottontail rabbits. It caused cutaneous papillomas that could grow to be quite large and which may be the basis of sightings of the mystical and ravenous ''Jackelope'' of southwestern American lore. Shope later collaborated with Rous to demonstrate that exposure of these papillomas to coal tar or infection of a host that does not support viral replication caused malignant progression to skin cancers. This infectious agent, the cottontail rabbit papillomavirus or *Sylvilagus floridanus* Papillomavirus 1, was the first virus linked to a cancer in a mammalian host (Javier and Butel, 2008; Moore and Chang, 2010) (Figure 1).

PAPILLOMAVIRUSES

Papillomaviruses are small, non-enveloped viruses with double-stranded circular DNA genomes of approximately 8,000 bp in size. Transcription is unidirectional, i.e., only one of the two strands is known to encode genetic information. Papillomavirus genomes consist of three major regions: an early region that encodes five to seven non-structural, regulatory ''E'' open reading frames, the late region encoding the major and minor capsid proteins, L1 and L2, respectively, and a non-coding region referred to as the ''long control region'', which contains sequences that regulate viral gene transcription and genome replication. Papillomaviruses have been detected throughout the animal kingdom. They are highly species specific and infect squamous epithelia. Papillomaviruses have been classified based on the degree of sequence identity and are referred to as genotypes. More than 170 human papillomavirus types have been characterized and most of them fall within the alpha, beta, gamma, and, mu genera (Bernard *et al.*, 2010).

BETA HUMAN PAPILLOMAVIRUSES AND NON-MELANOMA SKIN CANCERS

Around the same time that Shope and Rous discovered that cottontail rabbit papillomavirus caused skin cancers in rabbits, Felix Lewandowsky and William Lutz described a rare skin disorder that would be known as epidermodysplasia verruciformis (EV; Lewandowski and Lutz, 1922). EV patients develop widespread wart-like lesions that can cover entire portions of their skin and frequently develop malignant skin tumors, particularly at sun-exposed areas. Seminal work by Stefania Jablonska and Gerard Orth linked human papillomavirus (HPV) infections with skin lesions and cancers in EV patients (Orth *et al.*, 1978). This work predates Harald zur Hausen's discovery of the mucosal-specific alpha-type HPVs, HPV16, and HPV18, as etiological agents of cervical carcinoma. EV patients suffer from a deficiency that prevents effective clearance of beta HPV infections. Interestingly, however, EV patients do not seem to be at a higher risk for bacterial or other viral infections, including alpha HPV infections (Gewirtzman *et al.*, 2008).

The genetic basis of EV was discovered in 2002 when Favre and colleagues discovered that EV patients harbored mutations in either one of two adjacent genes, *TMC6* or *TMC8*, on chromosome 17 (Ramoz *et al.*, 2002). These genes encode the transmembrane proteins, EVER1 and EVER2, which localize to endoplasmic reticulum membranes and may be involved in intracellular zinc transport. How this relates to susceptibility to persistent cutaneous HPV infections remains to be fully delineated.

Beta HPV genomes can readily be detected in tumor cells of EV patients and also are likely etiologic agents of non-melanoma skin cancers (NMSCs) that arise in chronically immune-suppressed patients (Majewski and Jablonska, 2002; Proby *et al.*, 2011; Iannacone *et al.*, 2013; Neale *et al.*, 2013). Whether or not beta HPV infections also contribute to NMSCs in other patients has been a matter of debate, mostly because subclinical beta HPV infections are very widespread and not every tumor cell is HPV positive in these patients (Arron *et al.*, 2011). As detailed below, this does not rule out, however, that infections with some beta HPVs may be drivers of NMSC initiation in the general population.

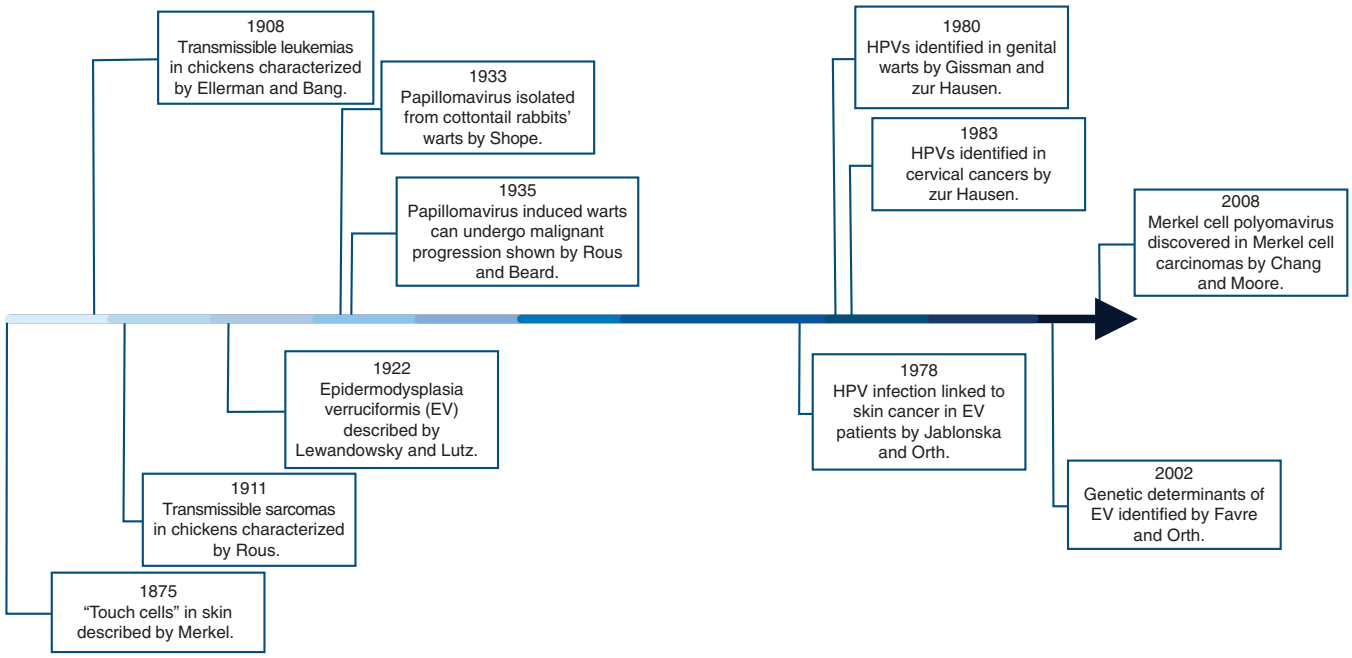

Figure 1. Milestones in the viral etiology of skin cancer. Major discoveries in the field are indicated on a time scale. See text for details and references.

MECHANISTIC CONTRIBUTIONS OF BETA HPVS TO NMSC DEVELOPMENT

Most of the fundamental concepts of how HPVs contribute to human cancer formation have been established by studies with alpha HPVs, which preferentially infect mucosal epithelia. These HPVs have been studied extensively and they fall into "high-risk" and "low-risk" groups based on their propensities to cause lesions that can undergo malignant progression. Notably, high-risk alpha HPV infections cause almost all cases of cervical carcinomas, a significant fraction of other anogenital tract tumors as well as oropharyngeal cancers. Overall, approximately, 5% of all human cancers are caused by high-risk alpha HPV infections. These cancers regularly maintain viral gene expression; every tumor cell generally contains and expresses HPV sequences, and they remain "addicted" to expression of the E6 and E7 oncogenes. The high-risk alpha HPV E6 and E7 proteins target and functionally compromise the p53 and retinoblastoma (pRB) tumor suppressors, respectively, which are frequently mutated in non-HPV-associated cancers.

It has been proposed that beta HPVs may be similarly classified into "high-risk" and "low-risk" groups. HPV5 and the phylogenetically related HPV8 have been originally isolated from NMSCs arising in EV patients (Fuchs *et al.*, 1986; Zachow *et al.*, 1987). Hence, these viruses may be considered "high-risk" for NMSC development in EV patients. Experiments with transgenic mice are consistent with this model. Expression of the early coding region of HPV8 from the basal keratinocyte-specific keratin 14 promoter causes spontaneous development of malignant skin tumors in transgenic mice (Schaper *et al.*, 2005). Additional studies revealed that HPV8 E6 and, surprisingly, E2, scored as the major transforming proteins in this model (Pfefferle *et al.*, 2008; Marcuzzi *et al.*, 2009). While these tumors will arise spontaneously, UV irradiation dramatically accelerates carcinogenesis, thereby recapitulating a key risk factor of EV-associated cancers.

Unlike what has been reported for high-risk alpha HPVs, the HPV5 and HPV8 E7 proteins only weakly associate with and do not destabilize pRB, and similarly the E6 proteins do directly inhibit p53 activity (Caldeira *et al.*, 2003; Rozenblatt-Rosen *et al.*, 2012; White *et al.*, 2012a, b). HPV5 and HPV8 E6 proteins, however, have been reported to inhibit proapoptotic factors activated during UV damage and impair DNA damage response pathways. Several groups have reported that beta HPV E6 proteins can trigger the degradation of the proapoptotic BCL2 family member BAK through a proteasome-dependent pathway (Jackson *et al.*, 2000; Underbrink *et al.*, 2008). BAK is normally retained in the mitochondria but is released and induces apoptosis following UV exposure. BAK degradation in beta HPV-infected cells may, therefore, blunt the apoptotic response to UV irradiation and allow survival of cells that have suffered extensive DNA damage and possibly acquired oncogenic mutations.

There is evidence that the repair of UV-induced DNA damage is inhibited in HPV8 E6 expressing cells (Simmonds and Storey, 2008; Underbrink *et al.*, 2008). HPV5 and HPV8 E6 proteins also inhibit double-strand DNA break repair by associating with and destabilizing the histone acetyl transferase, p300 (Howie *et al.*, 2011; Wallace *et al.*, 2012), which can regulate activity of the ATM/ATR kinases by acetylation. Similar to subverting

apoptosis signaling though BAK degradation, blunting DNA break repair may allow for accumulation of mutations in beta HPV-infected cells, thereby facilitating malignant progression. According to such a model, beta HPV infections contribute to cancer initiation in non-EV patients through a ''hit-and-run'' mechanism, and as viral gene expression may not be necessary for the maintenance of the transformed state, it might explain why the viral genome is not detected in all tumor cells (Arron *et al.*, 2011).

HPV5 or HPV8 E6 expression in transgenic mice or in organotypic tissue culture models of skin dramatically inhibits epithelial differentiation (Akgul *et al.*, 2007; Marcuzzi *et al.*, 2009). This ability of E6 to uncouple the processes of epithelial differentiation and proliferation may be relevant to the viral life cycle, as viral genome synthesis and progeny formation is restricted to terminally differentiated cells that have normally withdrawn from the proliferative pool. As HPVs require cellular DNA synthesis for the replication of their genomes, it is essential that cell cycle proficiency be maintained during differentiation. One of the critical regulators of epithelial differentiation is NOTCH signaling. Several recent studies have shown that the HPV5 and HPV8 E6 proteins inhibit NOTCH signaling by interacting with MAML proteins, critical co-activators of the NOTCH transcription complex (Brimer *et al.*, 2012; Rozenblatt-Rosen *et al.*, 2012; Tan *et al.*, 2012; Meyers *et al.*, 2013). NOTCH has tumor suppressor activities in epithelia, and inactivating NOTCH pathway mutations are highly prevalent in SCCs (Agrawal *et al.*, 2011; Stransky *et al.*, 2011).

HPV5 E6 has also been shown to inhibit TGF (transforming growth factor)-beta signaling in keratinocytes through destabilization of the SMAD3/4 transcriptional complex (Mendoza *et al.*, 2006). Similar to NOTCH, TGF-beta signaling can be oncogenic or tumor suppressive in different tissues and/or at different stages of carcinogenesis. Future work will help to unravel how disruption of NOTCH and/or TGF-beta signaling may contribute to the life cycle of beta HPV and/or contribute to NMSC formation.

The E6 and E7 proteins of other beta HPVs, including HPV types 20, 27, and 38, also exhibit carcinogenic activities in transgenic mouse models, although, and in contrast to the HPV8 model, tumor formation was strictly dependent on UV exposure (Dong *et al.*, 2005; Michel *et al.*, 2006; Viarisio *et al.*, 2011). HPV38 has been studied in some detail, and in contrast to many other beta HPVs, HPV38 can immortalize primary human epithelial cells and has transforming activities *in vitro*. Unlike HPV5 and HPV8, HPV38 E6 has been reported to cause p53 inactivation, and HPV38 E7 has been shown to efficiently associate with pRB and trigger its degradation (Caldeira *et al.*, 2003; Accardi *et al.*, 2006). These activities of the HPV38 E6 and E7 proteins are somewhat reminiscent of cervical cancer associated, high-risk alpha HPVs. Whether humans infected with HPV38 are at a particularly high risk for NMSC development remains to be determined.

MERKEL CELL CARCINOMA

Merkel cell carcinoma (MCC) is a highly metastatic, aggressive skin cancer, whose occurrence is on the rise. Merkel cells were first described over a 100 years ago by Friederich Sigmund Merkel, and they are involved in fine touch sensing and are detected throughout the epithelium in cutaneous skin. Although originally thought to be derived from the neural crest, Merkel cells express specific cytokeratin markers and may be of an epithelial lineage (Bardot *et al.*, 2013). An infectious etiology has been suggested for MCCs as they are more prevalent in immunosuppressed patients, and sequences corresponding to a previously unknown human polyomavirus, Merkel cell polyomavirus (MCPyV) were isolated from MCCs in 2008 (Feng *et al.*, 2008). It is now generally accepted that the vast majority of MCCs harbor MCPyV sequences (Rodig *et al.*, 2012).

Polyomaviruses, particularly the simian vacuolating virus 40, have been studied extensively. Polyomaviruses are similar to papillomaviruses in that they contain small double-stranded DNA genomes, but they have a distinct genomic organization. In contrast to papillomaviruses, polyomavirus early and late genes are encoded on different strands of the genome, and the MCPyV early region encodes three major proteins through alternative splicing: small and large tumor antigens (T antigen) as well as a more recently identified splice variant that has been referred to as ALTO (Carter *et al.*, 2013). MCPyV sequences are commonly found integrated in MCCs, resulting in C-terminal truncation of large T antigen as well as ALTO (DeCaprio and Garcea, 2013). Both small T antigen and the truncated large T antigen proteins are thought to contribute to the tumorigenicity of MCPyV, though there is still debate on whether small T antigen is required for tumor maintenance and the potential role of ALTO remains to be determined (Angermeyer *et al.*, 2013; Shuda *et al.*, 2013). MCPyV large T antigen shares biological activities with simian vacuolating virus 40 large T antigen and binds pRB, but the growth promoting activity for MCPyV large T antigen is only seen with the tumor-associated truncation mutants (Cheng *et al.*, 2013). MCPyV small T antigen is a potent oncogene, as it can induce anchorage- and contact-independent growth of rodent fibroblasts and decrease the serum requirement of human cells (Shuda *et al.*, 2011). Current research is focused on identifying the mechanistic basis of the unique carcinogenic activity of MCPyV. Serology studies suggest that similar to beta HPVs, MCPyV infections appear to be frequent and occur in early childhood (DeCaprio and Garcea, 2013), but it is unknown whether MCPyV can establish a low-level life-long persistent infection and, if so, whether the initial infection and/or the persistently infected reservoir involve Merkel cells. In summary, MCPyV-associated MCCs similar to beta HPV-associated NMSCs represent very rare and atypical outcomes of very frequent infections.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGEMENTS

The research in the authors' laboratory is supported by Public Health Service grants CA081135, CA066980, and CA141583 (KM). JMM is a Ryan Fellow.

TO CITE THIS ARTICLE

Meyers JM, Munger K (2014) The viral etiology of skin cancer. *J Invest Dermatol* 134: E29–E32.

REFERENCES

Accardi R, Dong W, Smet A *et al.* (2006) Skin human papillomavirus type 38 alters p53 functions by accumulation of deltaNp73. *EMBO Rep* 7:334–40.

Agrawal N, Frederick MJ, Pickering CR *et al.* (2011) Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. *Science* 333:1154–7.

Akgul B, Ghali L, Davies D *et al.* (2007) HPV8 early genes modulate differentiation and cell cycle of primary human adult keratinocytes. *Exp Dermatol* 16:590–9.

Angermeyer S, Hesbacher S, Becker JC *et al.* (2013) Merkel cell polyomavirus-positive Merkel cell carcinoma cells do not require expression of the viral small T antigen. *J Invest Dermatol* 133:2059–64.

Arron ST, Ruby JG, Dybbro E *et al.* (2011) Transcriptome sequencing demonstrates that human papillomavirus is not active in cutaneous squamous cell carcinoma. *J Invest Dermatol* 131:1745–53.

Bardot ES, Valdes VJ, Zhang J *et al.* (2013) Polycomb subunits Ezh1 and Ezh2 regulate the Merkel cell differentiation program in skin stem cells. *EMBO J* 32:1990–2000.

Bernard HU, Burk RD, Chen Z *et al.* (2010) Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology* 401:70–9.

Brimer N, Lyons C, Wallberg AE *et al.* (2012) Cutaneous papillomavirus E6 oncoproteins associate with MAML1 to repress transactivation and NOTCH signaling. *Oncogene* 31:4639–46.

Caldeira S, Zehbe I, Accardi R *et al.* (2003) The E6 and E7 proteins of the cutaneous human papillomavirus type 38 display transforming properties. *J Virol* 77:2195–206.

Carter JJ, Daugherty MD, Qi X *et al.* (2013) Identification of an overprinting gene in Merkel cell polyomavirus provides evolutionary insight into the birth of viral genes. *Proc Natl Acad Sci USA* 110:12744–9.

Cheng J, Rozenblatt-Rosen O, Paulson KG *et al.* (2013) Merkel cell polyomavirus large T antigen has growth-promoting and inhibitory activities. *J Virol* 87:6118–26.

DeCaprio JA, Garcea RL (2013) A cornucopia of human polyomaviruses. *Nat Rev Microbiol* 11:264–76.

Dong W, Kloz U, Accardi R *et al.* (2005) Skin hyperproliferation and susceptibility to chemical carcinogenesis in transgenic mice expressing E6 and E7 of human papillomavirus type 38. *J Virol* 79:14899–908.

Feng H, Shuda M, Chang Y *et al.* (2008) Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* 319:1096–100.

Fuchs PG, Iftner T, Weninger J *et al.* (1986) Epidermodysplasia verruciformis-associated human papillomavirus 8: genomic sequence and comparative analysis. *J Virol* 58:626–34.

Gewirtzman A, Bartlett B, Tyring S (2008) Epidermodysplasia verruciformis and human papilloma virus. *Curr Opin Infect Dis* 21:141–6.

Howie HL, Koop JI, Weese J *et al.* (2011) Beta-HPV 5 and 8 E6 promote p300 degradation by blocking AKT/p300 association. *PLoS Pathog* 7:e1002211.

Iannacone MR, Gheit T, Pfister H *et al.* (2013) Case-control study of genus-beta human papillomaviruses in plucked eyebrow hairs and cutaneous squamous cell carcinoma. *Int J Cancer* 134:2231–44.

Jackson S, Harwood C, Thomas M *et al.* (2000) Role of Bak in UV-induced apoptosis in skin cancer and abrogation by HPV E6 proteins. *Genes Dev* 14:3065–73.

Javier RT, Butel JS (2008) The history of tumor virology. *Cancer Res* 68:7693–706.

Lewandowski F, Lutz W (1922) Ein Fall einer bisher noch nicht beschriebenen Hauterkrankung (Epidermodysplasia verruciformis). *Arch Derm Syph* 141:193–203.

Majewski S, Jablonska S (2002) Do epidermodysplasia verruciformis human papillomaviruses contribute to malignant and benign epidermal proliferations? *Arch Dermatol* 138:649–54.

Marcuzzi GP, Hufbauer M, Kasper HU *et al.* (2009) Spontaneous tumour development in human papillomavirus type 8 E6 transgenic mice and rapid induction by UV-light exposure and wounding. *J Gen Virol* 90:2855–64.

Mendoza JA, Jacob Y, Cassonnet P *et al.* (2006) Human papillomavirus type 5 E6 oncoprotein represses the transforming growth factor beta signaling pathway by binding to SMAD3. *J Virol* 80:12420–4.

Meyers JM, Spangle JM, Munger K (2013) The human papillomavirus type 8 E6 protein interferes with NOTCH activation during keratinocyte differentiation. *J Virol* 87:4762–7.

Michel A, Kopp-Schneider A, Zentgraf H *et al.* (2006) 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* 80:11153–64.

Moore PS, Chang Y (2010) Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nat Rev Cancer* 10:878–89.

Neale RE, Weissenborn S, Abeni D *et al.* (2013) Human papillomavirus load in eyebrow hair follicles and risk of cutaneous squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev* 22:719–27.

Orth G, Jablonska S, Favre M *et al.* (1978) Characterization of two types of human papillomaviruses in lesions of epidermodysplasia verruciformis. *Proc Natl Acad Sci USA* 75:1537–41.

Pfefferle R, Marcuzzi GP, Akgul B *et al.* (2008) The human papillomavirus type 8 E2 protein induces skin tumors in transgenic mice. *J Invest Dermatol* 128:2310–5.

Proby CM, Harwood CA, Neale RE *et al.* (2011) A case-control study of betapapillomavirus infection and cutaneous squamous cell carcinoma in organ transplant recipients. *Am J Transplant* 11:1498–508.

Ramoz N, Rueda LA, Bouadjar B *et al.* (2002) Mutations in two adjacent novel genes are associated with epidermodysplasia verruciformis. *Nat Genet* 32:579–81.

Rodig SJ, Cheng J, Wardzala J *et al.* (2012) Improved detection suggests all Merkel cell carcinomas harbor Merkel polyomavirus. *J Clin Invest* 122:4645–53.

Rozenblatt-Rosen O, Deo RC, Padi M *et al.* (2012) Interpreting cancer genomes using systematic host network perturbations by tumour virus proteins. *Nature* 487:491–5.

Schaper ID, Marcuzzi GP, Weissenborn SJ *et al.* (2005) Development of skin tumors in mice transgenic for early genes of human papillomavirus type 8. *Cancer Res* 65:1394–400.

Shuda M, Chang Y, Moore PS (2013) Merkel cell polyomavirus positive Merkel cell carcinoma requires viral small T antigen for cell proliferation. *J Invest Dermatol* 134:1479–81.

Shuda M, Kwun HJ, Feng H *et al.* (2011) Human Merkel cell polyomavirus small T antigen is an oncoprotein targeting the 4E-BP1 translation regulator. *J Clin Invest* 121:3623–34.

Simmonds M, Storey A (2008) Identification of the regions of the HPV 5 E6 protein involved in Bak degradation and inhibition of apoptosis. *Int J Cancer* 123:2260–6.

Stransky N, Egloff AM, Tward AD *et al.* (2011) The mutational landscape of head and neck squamous cell carcinoma. *Science* 333:1157–60.

Tan MJ, White EA, Sowa ME *et al.* (2012) Cutaneous beta-human papillomavirus E6 proteins bind Mastermind-like coactivators and repress Notch signaling. *Proc Natl Acad Sci USA* 109:E1473–80.

Underbrink MP, Howie HL, Bedard KM *et al.* (2008) E6 proteins from multiple human betapapillomavirus types degrade Bak and protect keratinocytes from apoptosis after UVB irradiation. *J Virol* 82:10408–17.

Viarisio D, Mueller-Decker K, Kloz U *et al.* (2011) E6 and E7 from beta HPV38 cooperate with ultraviolet light in the development of actinic keratosis-like lesions and squamous cell carcinoma in mice. *PLoS Pathog* 7:e1002125.

Wallace NA, Robinson K, Howie HL *et al.* (2012) HPV 5 and 8 E6 abrogate ATR activity resulting in increased persistence of UVB induced DNA damage. *PLoS Pathog* 8:e1002807.

White EA, Kramer RE, Tan MJ *et al.* (2012a) Comprehensive analysis of host cellular interactions with human papillomavirus E6 proteins identifies new E6 binding partners and reflects viral diversity. *J Virol* 86:13174–86.

White EA, Sowa ME, Tan MJ *et al.* (2012b) Systematic identification of interactions between host cell proteins and E7 oncoproteins from diverse human papillomaviruses. *Proc Natl Acad Sci USA* 109:E260–7.

Zachow KR, Ostrow RS, Faras AJ (1987) Nucleotide sequence and genome organization of human papillomavirus type 5. *Virology* 158:251–4.