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Evolution of Human Papillomavirus Carcinogenicity

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Abstract

Members of the *Alphapapillomavirus* genus are the causative agent for virtually all cases of cervical cancer. However, strains (commonly referred to as types) within this genus span the entire range of pathogenicity from highly carcinogenic (e.g., HPV16, odds ratio = 281.9, responsible for 50% of all cervical cancers), moderately carcinogenic (e.g., HPV31) to not carcinogenic (e.g., HPV71). The persistent expression of the viral oncoproteins (E6 and E7) from HPV16 has been shown to be necessary and sufficient to transform primary human keratinocytes *in vitro*. A plethora of functions have been described for both oncoproteins, and through functional comparisons between HPV16 and HPV6, a subset of these functions have been suggested to be oncogenic. However, extrapolating functional differences from these comparisons is unlikely to tease apart the fine details. In this review, we argue that a thorough understanding of the molecular mechanisms differentiating oncogenic from nononcogenic types should be obtained by performing functional assays in an evolutionary and epidemiological framework. We continue by interpreting some recent results using this paradigm and end by suggesting directions for future inquiries.

I. PAPILLOMAVIRUS CLASSIFICATION

In 2004, de Villiers and colleagues proposed quantitative thresholds for classification of PVs based on sequence identity, topology of phylogenetic trees, and biological characteristics (de Villiers *et al.*, 2004). Papillomaviruses sharing more than 60% nucleotide sequence identity across the major structural gene, L1, were classified within the same genus. These genera were named according to the Greek alphabet (de Villiers *et al.*, 2004). This was the first viral classification system based on sequence similarity recognized by the ICTV (Fauquet *et al.*, 2005). Within a specific genus, distinct PV strains, commonly referred to as “types”, were described as a virus for which the entire genome was cloned, and the L1 ORF shared less than 90% sequence similarity with any other previously defined type.

Additional research since 2004 has resulted in the identification of PVs from a large number of different host species ranging from turtles, birds, and mammals. A recent publication updating papillomavirus nomenclature assigned the 189 known PVs to 29 genera (Bernard *et al.*, 2010). Figure 1 shows the phylogenetic relationships between these types based on L1 the ORF.

II. CLINICAL IMPLICATIONS OF HUMAN PAPILLOMAVIRUS INFECTIONS

The phylogenetic tree shown in Fig. 1, clusters most HPVs into three main clades (α , β , and γ), roughly corresponding to preferred tissue tropism. In the remainder of this review, we will focus on the α -PV genus, but for completeness, we provide a quick overview of the other two main HPV genera.

The types within the β -PV genus present as cutaneous infections that are mostly latent in the general population (de Villiers *et al.*, 2004); however, in a subset of immunocompromised patients, these viral types can cause epidermodysplasia verruciformis (EV) (Dell'Oste *et al.*, 2009). These patients often develop cutaneous squamous-cell carcinoma. In 90% of the cases, these lesions can be shown to be positive for HPV-5 or -8 (Orth, 1986). However, at present, there is insufficient evidence to consider HPV-5 or -8 as independent human carcinogens (Bouvard *et al.*, 2010). The members of the γ -PV genus present as mainly commensal infections of the skin (de Villiers *et al.*, 2004). Interestingly, whereas most β - and γ -PVs have been isolated from the skin, recent evidence also suggests high prevalence of these viral types in the oral cavity (Burk, manuscript in preparation).

The α -PV genus contains viruses infecting cutaneous and mucosal keratinocytes. Typical cutaneous lesions present as verruca plantaris, verruca plana, or “butchers warts” depending on the infecting HPV type. Infections with HPV6 and 11 are mainly associated with condylomata accuminata; however, they are also the main culprits in the etiology of laryngeal papillomas and recurrent respiratory papillomatosis.

Within the α -PV genus, most research has focused on cervical carcinogenesis (see Section III); however, infection with HPV16 is responsible for the preponderance of HPV-induced tumors at sites other than the cervix. HPV16 has been found to be responsible for the majority of vaginal, vulvar, penile, and anal cancers (Munoz *et al.*, 2006). In addition, increasing evidence is linking α -HPV infection to the development of head and neck squamous-cell carcinoma (McKaig *et al.*, 1998), and conjunctival cancers (Nakamura *et al.*, 1997).

III. HPV AND CERVICAL CARCINOGENESIS

Yearly, the worldwide incidence of cervical cancer has been about 510,000 new cases, with nearly 300,000 patients dying (WHO: http://www.who.int/vaccine_research/diseases/hpv/en/). Most cases of cervix cancer (80%) occur in developing countries, particularly Asia. Increasing epidemiological evidence has identified persistent infection with oncogenic human α -papillomaviruses as the causative agents of cervical carcinoma. However, of the over 120 identified HPVs, only a limited subset of viruses (about 20 so-called oncogenic types (OTs)) have carcinogenic potential (Bouvard *et al.*, 2010), with HPV types 16, 18, 31, 33, 45, 52, and 58 showing the highest association with cervical malignancies (Smith *et al.*, 2007). Infections are mainly transmitted through direct skin or mucosal contact, with a substantial probability of transmission with every sexual contact (Burchell *et al.*, 2006). There appears to be a step-wise progression from infection through progressively severe intraepithelial neoplasia (from CIN stage 1 to CIN3) to invasive cancer. However, at each stage there is the possibility for viral clearance and/or regression of the lesion (Schiffman and Kjaer, 2003; Fig. 2). So, even though approximately 45% of women will acquire an infection within the first 36 months of sexual activity (Ho *et al.*, 1998), most infections will be cleared within 2 years. This is consistent with the data that 90% of new infections with carcinogenic HPV types will not be of clinical significance (Rodriguez *et al.*, 2010). Nevertheless, even though persistent OT HPV infection and malignant progression are highly associated (Ho *et al.*, 1995; Nobbenhuis *et al.*, 1999; Schlecht *et al.*, 2001), it is the potential to progress to malignant lesions that separates the OT viruses from equally well-

persisting NOT viruses (Burk *et al.*, 2009; Schiffman *et al.*, 2005). This observation suggests a functional difference between carcinogenesis independent of immune evasion and persistence.

IV. HPV LIFE CYCLE

HPV is a circular double-stranded DNA virus with a genome of about 7900 bp. The genome codes for eight proteins on the same sense strand and carries one upstream regulatory region (URR), also known as the noncoding region (Fig. 3). The genes are named based on their expression during epithelial differentiation (early (E) or late (L)). The viral life cycle is tightly linked to the differentiation state of the host cell it infects (Fig. 4). Following establishment of viral infection in the basal layer of the epithelium, the viral genome is maintained at about 100 copies per cell (Watts *et al.*, 1983). The expression of the E1 and E2 proteins is thought to be essential for viral episomal maintenance. The E2 protein binds to a palindromic motif [AACCg(N₄)cGGTT] in the URR (Blakaj *et al.*, 2009), which allows for the recruitment of the E1 helicase (consensus motif AACNAT), which in turn recruits host replication factors to the viral origin of replication (Yang *et al.*, 1991). The requirement of host factors (e.g., DNA polymerase α /primase (Bonne-Andrea *et al.*, 1995; Conger *et al.*, 1999; Masterson *et al.*, 1998) and the single-stranded-DNA-binding protein RPA (Han *et al.*, 1999)) for viral DNA replication implies that the terminally differentiated host cells need to reenter the cell cycle. The expression of two viral proteins, E6 and E7, has been shown to play a key role in this process (see Section VI).

In order to achieve a productive infection, the viral genomic copy number needs to be amplified prior to being packaged into viral capsids. The event triggering productive infection is not well understood, but is related to the differentiation state of the host cell. It has been shown that a viral differentiation-dependent promoter element is key to the increased expression of the E1, E2, E4, and E5 proteins (Bodily and Meyers, 2005; Spink and Laimins, 2005). Following the beginning of genomic amplification, L1 and L2 expression is upregulated both at the RNA and protein levels (Schwartz, 2000). It is interesting that the L1 and L2 levels appear to also be controlled through the use of suboptimal codons (Zhou *et al.*, 1999). Although the expression of L1 is sufficient to achieve viral packaging, the presence of a L2 protein substantially increases the efficiency (Zhao *et al.*, 1998). In addition, it has been suggested that the E2 protein, through its specific binding to the viral genome, also plays a role in packaging (Zhao *et al.*, 2000). Finally, expression of the E4 protein disrupts the integrity of the cornified cell layer assisting in efficient escape from the epithelial cell surface (Doorbar *et al.*, 1991).

V. HPV VIRAL CARCINOGENICITY IS CORRELATED WITH THE EARLY VIRAL REGION

Taxonomic classification of PVs places most HPVs in three main genera, roughly corresponding to body sites of infection. Essentially, all known HPVs infecting the cervicovaginal epithelium are contained within the α -PV genus (Fig. 1; Bernard *et al.*, 2010; de Villiers *et al.*, 2004). Consistent with this observation, the α -PV genus contains all the viruses for which there is sufficient epidemiological evidence to be considered oncogenic (Bouvard *et al.*, 2010; Schiffman *et al.*, 2005). The α -PV genus consists of three main clades, the high-risk (HR), which contains all the OTs, and two low-risk (LR1 and LR2) clades (Schiffman *et al.*, 2005). However, previous work identified significant phylogenetic incongruence at the putative high-risk (i.e., cancer-associated, HR) node (Fig. 5; Narechania *et al.*, 2005). A common oncogenic ancestor shared homology across the early gene ORFs, but not the structural (late) genes, that is, in the late gene trees, the HR- and LR-clades are mixed, whereas these clades are monophyletic and separate when considering the early

genes. In addition, Fig. 5 shows two phylogenetic trees based on the E6 and E7 oncogenes; when comparing these trees, it can be noticed that both LR-clades form a monophyletic group, independent from the HR-clade. These data support the notion that HPV species groups followed distinct evolutionary paths, with differences noted for the early and late genes (Narechania *et al.*, 2005). This might help explain how specific HPVs evolved to infect different cell types while preserving oncogenic capabilities (e.g., HPV16 and -18 are disproportionately associated with squamous and adenosquamous carcinoma, respectively; Smith *et al.*, 2007).

The observation that phylogenetic approaches indicate an association of the early region with oncogenic potential appears to suggest an evolutionary relatedness and origin of viral carcinogenicity. Based on this hypothesis, the comparison of phylogenetically related types, with distinct epidemiological risks, should allow researchers to tease apart what separates oncogenic HPV types (OTs) from nononcogenic HPV types (NOTs) within the HR-clade.

VI. THE E6 AND E7 VIRAL ONCOGENES

As introduced in Section IV, a PV infection requires the host cell machinery to drive its replication. In order to utilize the DNA replication machinery, the virus needs to subvert the cells' terminal differentiation process. This will result in an increased number of infected cells, and eventually the production of infectious virions. Both the E6 and E7 proteins have been implicated in the stimulation of cell cycle progression. The following is not meant to be an exhaustive listing of E6 and E7 functions, since several recent reviews have been dedicated to both these proteins. We will, however, briefly examine some of these functions (Table I).

The ability to degrade p53, thus interfering with the cellular stress response is probably the best-characterized function of the viral E6 protein (Band *et al.*, 1991). HPV16 E6 forms a complex with p53 and the 100 kDa E6AP, an E3 ubiquitin ligase (Scheffner *et al.*, 1993). This interaction results in the ubiquitination of p53 and its subsequent degradation through the proteasomal pathway. However, HPV-induced immortalization of primary keratinocytes does not appear to depend on p53 degradation (Kiyono *et al.*, 1998). However, it is clear that E6-induced degradation of p53 as well as Bak (a proapoptotic protein; Thomas and Banks, 1999) represents a strong antiapoptotic signal. In addition, the increased mitotic activity through E6 interaction with Blk (a SRC family protein kinase) further facilitates acquired mutagenic effects (Oda *et al.*, 1999).

Recently, the importance of cell polarity in the development of cancer has started to be appreciated (reviewed in Wodarz and Nathke, 2007). It is likely that the interactions of the E6 protein with cell polarity factors such as the PDZ domain-containing proteins (Thomas *et al.*, 2008) and paxillin (Tong and Howley, 1997; Tong *et al.*, 1997) provide an important mechanism for progression to malignancy. In addition, the degradation of E6TP1, a novel GAP protein, also appears to play an important role in HPV carcinogenesis (Gao *et al.*, 1999). Interestingly, amongst the different functions of E6, it appears that activation of the hTERT promoter (reviewed in Galloway *et al.*, 2005) in combination with the combined perturbation of the p16/pRb pathway is sufficient to immortalize primary HFKs (Kiyono *et al.*, 1998). Nevertheless, additional factors are probably needed for induction of malignancy in infected humans.

HPV16 E7 has been shown to preferentially bind to the hypophosphorylated (cell cycle inhibiting) pRb. This results in a decreased interaction of pRb with E2F and progression through the cell cycle (Chellappan *et al.*, 1992). It appears that the complex formed by pRb, E7, and the ubiquitin ligase cullin 2 is responsible for the E7-induced degradation of pRb (Huh *et al.*, 2007). In addition, E7 also interacts with two other members of the pocket

protein family, p107 and p130, which also negatively regulate E2F activity (Dyson *et al.*, 1989; Genovese *et al.*, 2008; Helt and Galloway, 2003; Morris *et al.*, 1993). The direct interactions of the E7 protein with the histone H1 kinase (Davies *et al.*, 1993), cyclin A (Arroyo *et al.*, 1993), cyclin E (McIntyre *et al.*, 1996), the histone deacetylase complex Mi2B (Brehm *et al.*, 1999), and several transcription factors (c-jun, AP1 (Antinore *et al.*, 1996) and TATA binding protein (Massimi *et al.*, 1997)) further allow the virus to abrogate the normal cell cycle control pathways and reenter the DNA synthesis phase.

Finally, it has been shown that both pRb and p21 need to be inactivated through E7 expression to circumvent cell cycle arrest (Helt *et al.*, 2002). Interestingly, the expression of E7 stabilizes p21 and p53 levels (Jones *et al.*, 1997, 1999), so expression of E6 proteins in turn needs to reduce the levels of p53 induced by E7 expression.

In addition to modulating cell cycle control, the E7 protein has been implicated in the immune response. The E7 protein downregulates the levels of human toll-like receptor hTLR9 (Hasan *et al.*, 2007) and the ER protein TAP-1 (Vambutas *et al.*, 2001). It was recently shown that variations within the TAP-1 gene were associated with increased risk for high-grade cervical neoplasia (Einstein *et al.*, 2009), suggesting that changing the expression levels of these proteins might play a role in viral immune evasion.

Nonetheless, it is important to note that all PVs need to “kick start” the cell cycle, to complete their life cycle. This has to be achieved irrespective of carcinogenic potential. So, it appears unlikely that the tweaking of basic cell cycle regulators is what separates oncogenic (OT) from nononcogenic (NOT) types. We argue that the subtleties of viral oncogenesis are best understood through the comparison of phylogenetically related types with different biological behaviors. We suggest that a thorough understanding of the evolutionary history, as well as the epidemiology of these viruses, should guide the interpretation of biochemical assays.

VII. EVOLUTIONARY HISTORY OF THE E6 AND E7 ONCOPROTEINS

The E6 protein is a small basic protein of about 150 amino acids. Every E6 protein contains four CxxC domains, which are involved in the formation of N- and C-terminal zinc-binding domains (Grossman and Laimins, 1989; Fig. 6). The presence of these domains has been shown to be essential for the proteins' functions. A solution structure of the E6 C-terminal domain has been proposed (Nomine *et al.*, 2003), and was used to suggest that a single-domain protein possessing the same fold might have once existed. It was recently shown that a short protein reminiscent of a single domain E6 (Nomine *et al.*, 2006) was present before reptiles and mammals diverged about 310 MYA (Herbst *et al.*, 2009; Van Doorslaer *et al.*, 2009). The discovery of these short E6 proteins allowed for the experimental confirmation of a hypothesis stipulated over 20 years ago (Cole and Danos, 1987). It appears that the E6 ORF gained access into the viral genome after which an (internal) duplication gave rise to the typical E6 protein we observe today in the majority of extant PVs (Van Doorslaer *et al.*, 2009). The absence of an E6 ORF in certain PVs (Chen *et al.*, 2007; Van Doorslaer *et al.*, 2006), suggests a recent loss of this ORF from these viruses.

At 100 aa, the E7 protein is shorter than the E6 protein, and it also contains a single CxxC Zn-finger domain (Fig. 6). Sequence analysis of the E6N, E6C, and E7 Zn-binding domains suggests E7 is a duplication of the ancestral single E6 domain (Van Doorslaer, unpublished). The recently solved E7 structures show a N-terminal region, which includes the conserved pRb-binding motif (consensus LXCXE), followed by an obligate homodimeric 50-residue zinc-binding domain (Liu *et al.*, 2006; Ohlenschlager *et al.*, 2006).

Two groups independently showed that the E6 genes mutated at a higher rate compared to the rest of the viral genome (Garcia-Vallve *et al.*, 2005; Rector *et al.*, 2007). Based on the analysis of feline PVs, Rector and colleagues quantified the different mutation rates to be $\sim 2.4 \times 10^{-8}$ versus 1.95×10^{-8} nucleotide substitutions/site/year, respectively (Rector *et al.*, 2007). Interestingly, this means that the E6 mutates at basically the same rate as the URR (2.69×10^{-8}). Since the URR is non-coding, one would expect this region to have a high mutational rate and be highly variable (Chen *et al.*, 2005). One of the potential explanations suggested for the elevated E6 mutation rate is that the E6 ORF gained access into the viral genome after the basic genome structure was formed (Garcia-Vallve *et al.*, 2005). The suggested duplication event might also aid in explaining this phenomenon (Van Doorslaer *et al.*, 2009). Alternatively, the E6 ORF might be under less stringent purifying selection due to selective functions necessary for the PV life cycle. The observed incongruencies between the different viral regions (Fig. 5; Garcia-Vallve *et al.*, 2005; Narechania *et al.*, 2005) argue that the PV genome consists of three independently evolving units (E6–E7, E1–E2, and L1–L2), with oncogenicity most closely tracking the evolution of the E6 and E7 ORFs.

VIII. TOWARD A BIOCHEMICAL UNDERSTANDING OF ALPHAPAPILLOMAVIRUS ONCOGENICITY

The correlation between the early region phylogeny and viral oncogenicity implies that evolutionary changes conveying carcinogenicity to the most recent common ancestor (MRCA) of the current HR-clade are maintained within today's extant OT HPVs.

As mentioned earlier, previous studies have shown that OT HPV types, HPV16 and HPV18, degraded p53, whereas NOT types HPV6 and -11 were unable to do so (Lechner and Laimins, 1994; Scheffner *et al.*, 1990). Based on these observations, it has long been assumed that p53 degrading ability was strongly associated with the oncogenicity by OT HPVs. In addition, it has been argued that the ability to interact with PDZ domain-containing cellular proteins was a unique feature of OT HPVs (Kiyono *et al.*, 1997; Lee *et al.*, 1997). The importance of degrading PDZ domain-containing proteins for viral oncogenesis was supported by the observation that the E7 protein might complement this function in RhPV1-induced oncogenesis (Tomaic *et al.*, 2009). However, with HPV6 and -11 causing condyloma accuminata and laryngeal papillomas, while HPV16 and -18 primarily infecting the cervix, it is possible that the observed differences in activity are due to tissue tropism differences. This is supported by data showing that skin types associated with non-melanoma skin cancer do not affect p53 levels (Steger and Pfister, 1992). We need to be careful that the comparison of types that are distantly related does not result in overinterpretation of biochemical data.

Figure 7 shows a summary of recent data on how the E6 proteins of different types affect the steady-state levels of p53 and Magi1c and hDLG (PDZ-containing proteins; Hiller *et al.*, 2006; Hiller *et al.*, 2008; Muench *et al.*, 2009). This tree, based on the nucleotide sequence of the E6 proteins, clusters all the oncogenic types (Bouvard *et al.*, 2010) in a single (red) HR-clade; however, not all members of this clade actually cause disease. The data clearly show that p53 degradation by OT types is not highly correlated with oncogenicity. On the contrary, it appears that this trait was inherited from the common ancestor of this clade. The most parsimonious explanation would be that the types no longer able to degrade p53 (e.g., HPV40, 6, 11, 44, 54, etc.) lost this ability after the HR MCRA diverged. This suggests that additional functions separate the NOTs from the OTs. Based on Fig. 7, the PDZ-proteins might be interesting candidates. Degradation of Magi1c appears to recapitulate a significant portion of the epidemiological classification of OT HPVs. However, the use of the mouse homolog of Magi1c (Dobrosotskaya *et al.*, 1997) in an *in vitro* assay (Muench *et al.*, 2009) might obscure the interpretation of the data, especially when compared to the human hDLG

used in the same study. However, the data do warrant a detailed investigation into the effects of phylogenetically related viral types on PDZ-containing proteins.

The goal of these comparative genomic studies should be to identify the biochemical functions that separate the OT from the NOTs. Since both E6 and E7 are necessary to successfully transform primary keratinocytes (Munger *et al.*, 1989), it is possible that the OT-defining functionality lies within the E7 protein and/or an interaction between the E6 and E7 proteins, introducing a level of complexity into the biological system. Of interest is the observation that two natural variants of HPV16 E6 (from the European prototype (E(p) and Asian American (AA) (nonEuropean) lineages (Chen *et al.*, 2005)) affect *in vitro* transformation differently in a manner corresponding to their differences in epidemiological risk classification (Richard *et al.*, 2010). The authors used prototype E7 in conjunction with E(p) E6 or AA E6 (Q14H/H78Y/L83V) in an array of functional studies. They observed no differences in p53 inactivation or telomerase activation. However, only the AA variant was able to successfully transform the primary HFKs. In addition, proteomic analysis showed markedly different signatures between both sets of cell lines (Richard *et al.*, 2010). Not only did this study suggest an important role for the E6 protein, but it also illustrated beautifully how an understanding of the epidemiology and evolutionary path of these viruses can guide *in vitro* experiments.

IX. CONCLUSIONS AND PERSPECTIVES

In the present review, we argue that the identification of functions associated with HPV oncogenic potential should be done carefully and with a consideration of viral epidemiology and evolutionary history. More detailed investigations have suggested that the differences between OT and NOT α -papillomavirus types are more complex than originally anticipated.

Phylogenetic analysis emerged from the field of systematics, where it added an extra dimension to anatomical trait analysis for species classification. However, phylogeny has gained access into all fields of biology, having been applied to understand virulence (Hon *et al.*, 2006), drug resistance (Seret *et al.*, 2009), the origin of epidemics (Smith *et al.*, 2009), and forensic investigations (Arens, 1999). We argue that the wealth of HPV sequence and epidemiological data now opens the door for HPV VWAS (viral genome wide association studies). The goal of human GWAS studies is to identify SNPs associated with a specific phenotype or disease. The 8 kb size of the HPV viral genome, coupled with excellent epidemiological data and the extreme oncogenic risk of some HPV types but not others, make HPV genome analyses an excellent model system to dissect the genetic contribution of viral nucleotides to disease. We also point out that the extant human genital HPVs show a strong correlation between the epidemiological classification and the evolutionary history of their oncogenic proteins. Building on these arguments, we believe that evolutionary analyses will allow researchers to identify those evolutionary changes potentially conferring carcinogenic potential to the OTs. These identified SNPs can next be manipulated *in vitro* and *in vivo* to qualitatively test the differences between these proteins.

Although an effective HPV vaccine for HPV types 6, 11, 16, and 18 is currently available, the world population of women will be at risk for cervix cancer for generations to come. This is due to the high cost of the vaccine, the lack of therapeutic activity in women currently infected, and the low penetration of vaccine in the most at-risk population in the developing world. Moreover, cervix cancer is a solid tumor that has a complex biology, given the average 20–40 years of development from the time of infection. Further studies are warranted not only for the public health benefits, but also for the potential to investigate the mechanisms of HPV pathogenicity and viral behavior.

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FIGURE 1.
Bayesian phylogenetic tree based on the L1 nucleotide sequences of 189 papillomaviruses.
Adapted from Bernard and colleagues (Bernard *et al.*, 2010).

FIGURE 2.

Model of HPV infection of the cervix natural history and progression to cancer. Most infections are quickly cleared through the innate immune system. Even following establishment, the host immune response can still clear the viral infection. The persistently infected epithelium (1–10 years) will support clonal progression, leading to CIN3. In a final, not well-understood step, the lesion progresses into invasive carcinoma.

FIGURE 3.

The structure and organization of the HPV genome. The reference HPV16 genome is shown. The PV genome consists of a circular double-stranded DNA genome. The genome is divided into three regions. We indicated the upstream regulatory region (URR, gray box). The viral genome carries all ORFs on a single sense strand, with the E4 ORF overlapping the E2 ORF. The early genes (E, solid arrows) and late (L, dashed arrows) structural genes are indicated.

FIGURE 4.

Epithelial infection by papillomaviruses. The key events that occur following infection are tightly linked to the differentiation state of the infected cell. The arrows next to the figure indicate the temporal pattern of the indicated proteins' expression, while the color represents the amounts expressed. On the far right, the effects of timed expression are summarized. Cells with red nuclei are progressing through the cell cycle. Since cells above the basal layer are terminally differentiated, this implies that the viral oncogenes, E6 and E7, are subverting cell cycle control mechanisms. Epithelial differentiation activates the differentiation-dependent promoters, resulting in increased expression of the viral replication proteins (E1, E2, E4, and E5) and genome replication (green cells with red nuclei). While continuing on their differentiating path, cells containing amplified viral DNA (yellow) begin expressing the structural L1 and L2 genes. The green/yellow cells contain infectious particles, which are shed at the epithelial surface. It appears that increased expression of the E4 protein near the epithelial surface is involved in viral shedding. Figure adapted from Doorbar (2006).

FIGURE 5.

Phylogenetic trees based on the six main viral ORFs show the incongruence between each viral region. Maximum Likelihood phylogenetic trees were built on the nucleotide sequences of all-known HPVs within the α -PV genus. The optimal tree was selected from 50 independent ML searches under the GTRGAMMA model, as implemented in RAXML (Stamatakis *et al.*, 2005). For improved readability, the branches were collapsed according to the accepted HPV classification (Bernard *et al.*, 2010; de Villiers *et al.*, 2004). Numbers next to the collapsed clade correspond to the species within the α -PV genus. HPV54 is the sole representative of the alpha-13 species. Branches are colored to illustrate viral risk classification; HR (red), LR1 (blue), and LR2 (green) (Burk *et al.*, 2009). To test for incongruency between the different trees, the Shimodaira–Hasegawa test for topology (Shimodaira and Hasegawa, 1999) was run within RAXML. The test indicates that the topologies of all six trees are significantly different, suggesting different evolutionary histories for all six viral regions. In the trees based on the early regions (E1–E7), the HR-clade (red) forms a monophyletic clade, separate from the LR-clades, whereas in the late region trees, the HR and LR-clades are mixed (Narechania *et al.*, 2005). This suggests that oncogenicity traces along the early region. This is further supported by the observation that the HR and LR-clades are monophyletic (and thus separate) in the oncogene (E6 and E7) trees.

FIGURE 6.

E6 and E7 protein structures. (A) The positions of the CxxC-N₂₉-CxxC domains in both proteins are indicated. The CxxC regions highlighted in blue and red correspond to the regions highlighted in (B) and (C), respectively. In E6 amino acid F47 is highlighted, a position implicated in the interaction with E6AP and degradation of p53 (Nomine *et al.*, 2006). Y81 is the most N-terminal residue solved in the structure shown in (B). The region involved in binding to PDZ proteins is indicated in gray and is not shown in the structure in (B). In the E7 model, the pRb binding domain (LxCxE) and the starting point of the structure shown in (C), A55 are indicated. (B) Shows the NMR structure of the C-terminal domain of HPV16 E6 (Nomine *et al.*, 2006). The file was downloaded from the pdb (www.rcsb.org/pdb) with accession number 2FK4 and visualized in MacPymol (<http://www.pymol.org>). Both CxxC regions are highlighted. (C) Shows the NMR structure of the C-terminal domain of HPV45 E7 (Ohlenschlager *et al.*, 2006). The file was downloaded from the pdb (www.rcsb.org/pdb) with accession number 2EWL and visualized in MacPymol (<http://www.pymol.org>). Both CxxC regions are highlighted.

FIGURE 7.

Evolutionary and epidemiologically correlated analysis of p53 and PDZ degradation activities. This figure shows the E6 tree depicted in Fig. 5. Branches were culled when no biochemical data was available. The α -PV species are named according to de Villiers *et al.* (de Villiers *et al.*, 2004). The epi. column shows the epidemiological classification (oncogenic, OT; nonOT, NOT, insufficient data, N/A) according to Bouvard *et al.* (2010). The following columns summarize the data presented in p53 (1) (Hiller *et al.*, 2006), p53 (2) (Hiller *et al.*, 2008), p53 (3) (Fu *et al.*, manuscript submitted), Mag1c (4), and hDLG (4) (Muench *et al.*, 2009). In these columns, “yes” indicates degradation of the target, “no” means no degrading potential, and “~” signifies intermediate potential. Branches are colored according to Fig. 5, with the red, HR-clade clustering both the OT and NOT types.

TABLE I

Summary of the interacting partners of the HPV oncoproteins

Partner	Function	Reference
E6		
Bak	Bcl2 family member	Thomas and Banks (1999)
Blk	src family tyrosin protein kinase	Oda <i>et al.</i> (1999)
E6AP	E3 Ubiquitin ligase	Rolfe <i>et al.</i> (1995)
E6TP1	Putative GAP protein	Gao <i>et al.</i> (1999)
hTERT	Maintains telomere length	Veldman <i>et al.</i> (2001)
p53	Tumor suppressor	Werness, <i>et al.</i> (1990)
Paxillin	Focal adhesion protein	Tong and Howley (1997)
PDZ-containing proteins	Cellular polarity	Thomas <i>et al.</i> (2008)
E7		
AP1	Transcription factor	Antinore <i>et al.</i> (1996)
c-jun	Transcription factor	Antinore <i>et al.</i> (1996)
Cullin 2	E3 Ubiquitin ligase	Huh <i>et al.</i> (2007)
Cyclin A	Cell cycle control	Arroyo <i>et al.</i> (1993)
Cyclin E	Cell cycle control	McIntyre <i>et al.</i> (1996)
Histone H1 kinase	Cell cycle control	Davies <i>et al.</i> (1993)
hTLR9	Innate immunity	Hasan <i>et al.</i> (2007)
Mi2B	Histone deacetylase complex	Brehm <i>et al.</i> (1999)
p21	Cell cycle control	Helt <i>et al.</i> (2002)
Pocket protein family	Cell cycle control	Helt and Galloway (2003)
TAP-1	Antigen processing	Vambutas <i>et al.</i> (2001)
TBP	Transcription factor	Massimi, <i>et al.</i> (1997)