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HUMAN PAPILLOMAVIRUS-ASSOCIATED CANCERS

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INTRODUCTION—HUMAN PAPILLOMAVIRUS (HPV) TYPES AND HPV LIFE CYCLE

HPV is a small, nonenvelope, double-stranded DNA virus of the Papillomaviridae family. Different HPVs are defined by their genotypes, with more than 100 described to date. HPV genotypes are clustered into genera (de Villiers et al., 2004) based on genetic relatedness. Within each genus, HPVs are categorized into species based on distinct genotypes; members of a species have similar biological properties or phenotypes. Differences of 10% or more in the viral capsid gene (L1) are noted as different HPV types, differences of 2%–10% as subtypes, and differences of less than 2% are variants (Fig. 1.1) (de Villiers et al., 2004; Doorbar, 2006).

The two main genera of HPV are the alpha and beta papillomaviruses, with nearly 90% of typed HPVs falling within these groups (Fig. 1.1). Genus alpha papillomaviruses are associated with genital and mucosal infections, although a few infect cutaneous epithelium. Genus alpha HPVs are further defined as high-risk or low-risk based on their association with anogenital cancers or benign genital warts, respectively. The genus beta papillomaviruses are associated with benign skin infections; however, in immunocompromised patients or patients with epidermodysplasia verruciformis (EV),

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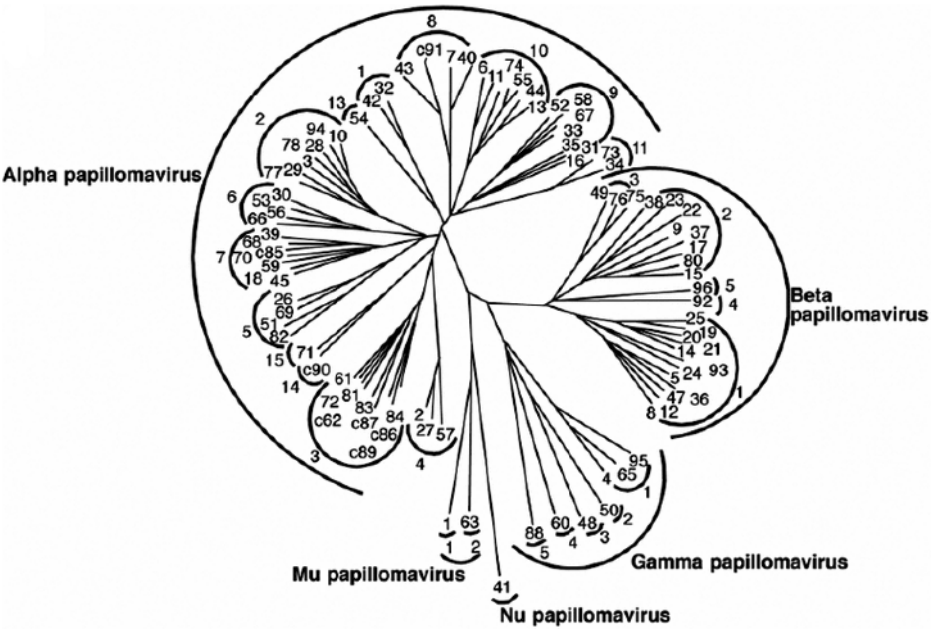

Figure 1.1. HPV cladogram. HPV types are categorized based on their genetic similarities. The genus alpha papillomaviruses primarily infect mucosal epithelium, and the genus beta papillomaviruses primarily infect the skin (figure from Doorbar, 2006).

a rare genetic disorder caused by mutations in the *EVER1* or *EVER2* genes (Ramoz et al., 2002; Keresztes et al., 2003; Kurima et al., 2003), squamous cell carcinomas (SCC) can develop.

HPV requires actively dividing and differentiating epithelium, typically the basal layer of stratified squamous epithelium, for its DNA replication, gene expression, and protein coat production. HPV reaches basal cells through epithelial microabrasions or at sites where the epithelium transitions from a monolayer to stratified squamous cells, such as at the cervical transformation zone or the anal verge (Fig. 1.2). Although these areas are more accessible to the virus for initial infection, they are poorer sites for viral production. This makes these anatomical transition areas sites where abnormal and inadequate viral gene expression, and ultimately HPV genome integration, are likely to occur. With HPV genome integration, regulatory viral genes may be lost, and epithelial cells are driven to immortalization, which can progress to cancer.

EPIDEMIOLOGY OF HPV INFECTIONS AND CANCER

Genus Alpha HPV Infection in Women

HPV is the most common sexually transmitted infection (STI). Seventy-five percent of men and women in the United States have evidence of a current or prior genus alpha

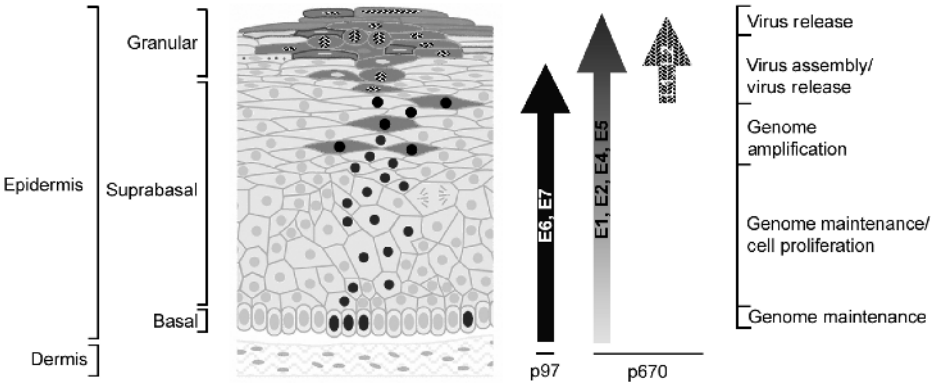

Figure 1.2. HPV life cycle. HPV infects the basal layer of stratified squamous epithelium. E6 and E7 drive continued proliferation as cells differentiate in the suprabasal layers and other early genes increase HPV gene expression and genome amplification. In the epithelial granular layers, the late L1 and L2 proteins are expressed, forming infectious virions that are released through desquamation (figure adapted from Doorbar, 2006).

TABLE 1.1. HPV-Associated Cancers

Cancer Type	HPV+ (%)
Cervical squamous cell carcinoma[1–3]	89.3–99.7
Cervical adenocarcinoma[1]	81.8
Vulvar[1]	89
Vaginal[1]	90.7
Anal[1,4]	84–93.8
Penile[1,5]	40–81.8
Head and neck[6]	25
Oropharynx/tonsil[7,8]	43.6–72

Note: The majority of anogenital cancers are associated with alpha genus HPV infections, and a significant subset of head and neck cancers are also associated with HPV.
[1]Carter et al. (2001).
[2]Munoz et al. (2003).
[3]Walboomers et al. (1999).
[4]Frisch et al. (1997).
[5]Gross and Pfister (2004).
[6]Gillison et al. (2000).
[7]Schwartz et al. (1998).
[8]D'Souza et al. (2007).

infection by serology, HPV DNA or RNA testing, cervical dysplasia, or genital warts (Koutsky and Kiviat, 1999). High-risk HPV infection has been identified as the key to cervical intraepithelial neoplasia and cancer (Table 1.1) (Durst et al., 1983; Koutsky et al., 1992; Munoz et al., 1992, 2003; Walboomers et al., 1999); however, most

high-risk infections do not become cancerous, so high-risk HPV is required but not sufficient for cancer progression.

Several natural history studies have been conducted to document the frequency and length of HPV infection in sexually active female adolescents and adults (Burk et al., 1996; Ho et al., 1998; Moscicki et al., 2001; Woodman et al., 2001; Richardson et al., 2003; Winer et al., 2003, 2005). A prospective longitudinal study of female university students found that with each new lifetime partner, the risk of HPV acquisition rose: 3% for virginal women, 7% for women with one lifetime partner, 33% for women with two to four partners, and 53% for women with five or more partners (Burk et al., 1996). Moscicki et al.'s (2001) longitudinal study of female adolescents found that their risk of HPV infection was also directly associated with the number of new partners as well as evidence of other STIs on exam. Winer et al. (2003) found that cumulative incident HPV infections in university women was 32.3% by 24 months, which is consistent with other studies (Ho et al., 1998; Richardson et al., 2003; Winer et al., 2003). Woodman et al. (2001) found a cumulative incidence of HPV infection in 15- to 19-year-olds at 44% by 36 months and 60% at 5 years.

In university women, new partners and smoking were risk factors for HPV acquisition (Winer et al., 2003), and the younger a female is at coitarche, the more likely she is to become infected with HPV (Kahn et al., 2002). Several studies have found that males' behaviors impact HPV infection in their female partners. The more lifetime partners a male has can increase the risk of HPV infection in a female partner; the briefer the relationship between a male and female can also increase the risk (Burk et al., 1996; Ho et al., 1998; Winer et al., 2003). So, the behavior of college-aged women and adolescents, as well as their partners, is an important risk factor for HPV infection. Young age at sexual debut, multiple partners, short relationships, smoking, and presence of other STIs all put teenagers and young women at risk for HPV.

The transition from a documented HPV infection to a low-grade squamous intraepithelial lesion (LGSIL) Pap smear is directly associated with a persistent infection as well as daily cigarette smoking in women (Moscicki et al., 2001). In a study of university women who had an HPV infection, nearly half of them developed cervical squamous intraepithelial lesions (SILs) and more than a quarter developed vaginal SILs within 36 months of their infection (Winer et al., 2005). These SILs regressed within 4.7–5.5 months on average (Winer et al., 2005).

LGSIL in adolescents also typically regress: 91% within 3 years (Moscicki et al., 2004). Women typically require a referral for colposcopy with the diagnosis of LGSIL; however, as adolescents and young women up to 21 years old have a greater likelihood of clearance of their HPV infection and regression of clinical disease, management of atypical cells of undetermined significance (ASCUS) and LGSIL Pap smears has been modified accordingly (http://www.asccp.org/consensus/cytological.shtml) (Wright et al., 2007). In contrast, older women are less able to clear infections and are more likely to have abnormal Pap smears, including high-grade squamous intraepithelial lesions (HGSIL) (Herrero et al., 2000; Castle et al., 2005), perhaps due to their relatively poor immune response to HPV infections (Garcia-Pineres et al., 2006).

Since the implementation of Pap smears for all sexually active women in the United States, rates of cervical cancer have fallen three-quarters since the 1950s. Within the

past 30 years, cervical cancer incidence has decreased from 14.2 per 100,000 in 1973 to nearly 3 per 100,000 in 1998, with a goal of 2 per 100,000 in Healthy People 2010 (http://www.ahrq.gov/clinic/3rduspstf/cervcan/cervcanrr.htm). However in the United States, women are still diagnosed with cervical cancer and die from this disease. In 2003, 11,820 women were diagnosed with cervical cancer and 3919 women died from it (U.S. Cancer Statistics Working Group, 2006). This makes understanding the infection and progression of disease critical to continued diagnosis and treatment.

More than 95% of cervical cancers contain high-risk HPV DNA, and HPV is also present in the majority of vulvar and vaginal cancers (Table 1.1) (Walboomers et al., 1999; Carter et al., 2001; Munoz et al., 2003). As the anal verge is similar to the cervix in transitioning from stratified squamous epithelium to columnar cells, HPV can also infect these cells and is associated with anal cancer in women. The rate of female anal cancer is increasing, likely representing a change in women's sexual behaviors (Frisch et al., 1993, 1999).

Genus Alpha HPV Infection in Men

HPV infection in men has been less well studied. HPV has been detected in 42%–80% of penile cancers (Table 1.1) (Gross and Pfister, 2004; Daling et al., 2005), the rate of which may reflect a greater difficulty of sampling for HPV from the penis, or the overlap of two distinct cancer types, one due to high-risk HPV infection and one not (Bleeker et al., 2006; Micali et al., 2006; Partridge and Koutsky, 2006). When compared with women, men have a less good antibody response to infection, which may be due to lower viral load or faster clearance of infection (Partridge and Koutsky, 2006). It has been shown that circumcision is protective against HPV acquisition in men (Hernandez et al., 2008), as well as cervical cancer in their wives (Castellsague et al., 2002); when circumcised, the amount of columnar epithelium on the glans of the penis is reduced, making it more difficult for HPV to infect.

Like in women, anal cancer in men is associated with HPV infection and is increasing in incidence (Table 1.1) (Frisch et al., 1993, 1997, 1999; Daling et al., 2004). Unlike women, men who have sex with men have a high (over 50%) and persistent HPV infection rate regardless of age (Chin-Hong et al., 2004), as well as a high and persistent level of abnormal anal cytology (Chin-Hong et al., 2005). The high level of infection and abnormal cytology put men who have sex with men at greater risk for anal cancer when compared with women and cervical HPV infections.

Patients who are human immunodeficiency virus (HIV) positive or have acquired immune deficiency syndrome (AIDS) are at increased risk for abnormal cytology on anal cytology (Palefsky et al., 1998; Frisch et al., 2000; Sobhani et al., 2004). Although regular screening for men who have sex with men has not been universally recommended, studies have shown screening programs for anal cytology similar to cervical Pap smears to be reliable and potentially helpful (Cranston et al., 2004; Mathews et al., 2004).

Genus Alpha HPVs and Head and Neck Cancers

Head and neck cancers are associated with genus alpha HPV infections (Table 1.1), although this is a weaker correlation than with anogenital cancers (Fakhry and Gillison,

2006). Also, although tobacco and alcohol use are risk factors for non-HPV-associated head and neck cancers, this is not true in HPV-associated head and neck cancers (Fakhry and Gillison, 2006; D'Souza et al., 2007; Gillison et al., 2008). HPV appears to be important, specifically in oropharyngeal cancers, such as the tonsils and the base of the tongue. The tonsils mimic the cervix and the anal verge as sites of metaplastic epithelium, making them an appropriate site for HPV infection. Studies have shown patients with anogenital cancers have a two-and-a-half- to fourfold increased risk of tonsillar cancer, and their partners are at increased risk of tonsillar cancer or cancer of the tongue (Frisch and Biggar, 1999; Hemminki et al., 2000).

A case-control study of patients with oropharyngeal cancer showed sexual behavior as a primary risk factor; a high lifetime number of vaginal-sex partners or oral-sex partners was associated with cancer (3.1 and 3.4 odds ratio, respectively) (D'Souza et al., 2007). Oral infection with high-risk HPV types were strongly associated with oropharyngeal cancer (12.3 odds ratio) (D'Souza et al., 2007). Again, understanding sexual behaviors is critical for predicting changes in oral cancer epidemiology, just as seen in anal cancer incidence.

Genus Beta HPVs and Skin Cancer

Nonmelanoma skin cancer is the most common cancer in the United States, with more than 1 million cases diagnosed annually (http://www.cancer.gov/cancertopics/types/skin). Nonmelanoma SCC accounts for 20%–30% of skin cancers, and SCC has been associated with genus beta HPV infection on sun-exposed skin. This cancer rate is even higher among two clusters of patients: organ transplant patients who are on immunosuppressive therapy and EV patients. Patients with EV develop lifelong warty lesions, 25% of which convert to SCC in sun-exposed areas (Orth et al., 1978, 1979; Ostrow et al., 1982; Orth, 2006). Genus beta HPVs are ubiquitously found in skin and hair follicle samples (Orth, 2006) and typically lead to benign lesions. However, the increasing number of transplant patients makes this infection a concern for future patients. The mechanism of genus beta HPVs leading to cancer is different than genus alpha HPVs and cancers, and it is the focus of ongoing research.

HPV EARLY AND LATE GENE EXPRESSION

The mechanism by which HPV binds to and enters basal epithelial cells is not known entirely, although both binding to laminin 5 and glycosaminoglycans are likely important (Joyce et al., 1999; Selinka et al., 2002; Culp et al., 2006). Once the virus infects cells, the HPV coat is removed and the HPV genome enters the nucleus. All HPV genomes have at least two promoters named early and late for their timed expression during the viral life cycle (Fig. 1.3). The E6 and E7 genes (designated E for early) are expressed from the early promoter (p97), and the E1, E2, E4, and E5 genes are expressed from the late promoter (p670) (Fig. 1.2) but utilize the early polyadenylation site. The early open reading frames drive viral DNA replication and expression, as well as dysregulate the normal epithelial cell cycle for the benefit of HPV viral production.

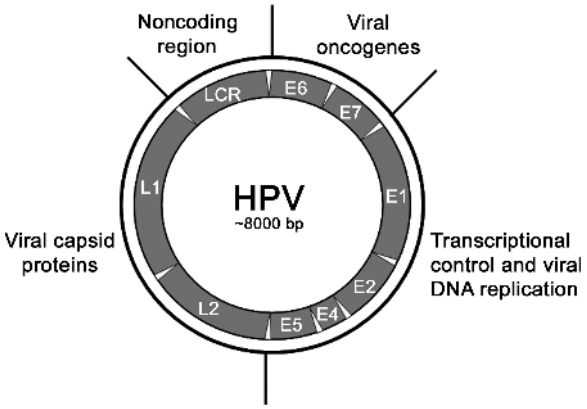

Figure 1.3. HPV genome. HPV is a double-stranded DNA virus with early (E) and late (genes) designated for their expression during the HPV life cycle. LCR=long control region.

The E2 protein has several critical roles in HPV genome expression and replication. It is expressed early in the viral life cycle and is found in basal and suprabasal layers of stratified squamous epithelium infected by HPV. E2 binds as a dimer to DNA and recognizes the palindromic motif AACCg(N_4)cGGTT in the noncoding region of the HPV genome 5′ of the early promoter (Hines et al., 1998; Masterson et al., 1998; Stubenrauch et al., 1998; Dell et al., 2003). E2 recruits the HPV viral helicase E1 to the viral origin and increases the DNA-binding affinity to the noncoding region (Masterson et al., 1998; Sun et al., 1998; Conger et al., 1999; Titolo et al., 2003). Both E1 and E2 together utilize cellular machinery for DNA replication and transcription (Masterson et al., 1998; Conger et al., 1999; Muller et al., 2002; Clower et al., 2006a,b). Although E2 is a transcriptional activator at low concentrations, high levels of E2 repress expression of E6 and E7 from the late promoter (Steger and Corbach, 1997; Francis et al., 2000). Finally, E2 also functions to segregate the HPV genome as cells divide by tethering the genome to cellular chromosomes during mitosis (You et al., 2004; McPhillips et al., 2005, 2006; Oliveira et al., 2006).

The E4 protein is found in the suprabasal and granular layers of stratified squamous epithelium. Without a functional E4 protein, HPV episomal DNA cannot amplify from their initial 50–100 copies per cell to the several thousands normally seen (Wilson et al., 2005).

E5 protein has effects on both cellular transformation and viral genome amplification. Although HPV E5 has little effect on monolayer undifferentiated keratinocytes *in vitro*, E5 does increase the number of suprabasal cells dividing in organotypic cultures grown to mimic stratified squamous epithelium (Genther et al., 2003). Additionally, in differentiated keratinocytes, E5 induces HPV genome amplification. HPV 16E5 can cause epithelial hyperplasia, abnormal cellular differentiation, and skin tumors when expressed in mice. This effect occurs through the epidermal growth factor receptor, although this may not be consistent across all HPV types (Fehrmann et al., 2003; Genther et al., 2005).

The viral coat proteins L1 and L2 are expressed from the late promoter after a change in splicing patterns and a transition to the late polyadenylation site. Three hundred sixty L1 proteins organize into 72 capsomers (Modis et al., 2002), with one L2 protein associated with each pentavalent capsomer (Trus et al., 1997). The capsomers self-assemble without HPV DNA *in vitro* as viruslike particles (VLPs) and in the cellular nucleus *in vivo* to encapsulate the HPV genome into infectious virus particles.

GENUS ALPHA HPV ONCOGENES

E6 and E7 expression is normally regulated by E2. In persistent HPV infections, HPV viral DNA can integrate into cellular DNA, and through this integration, the E2 gene is typically lost (Jeon et al., 1995). Unchecked, E6 and E7 mRNA and protein levels increase (Jeon and Lambert, 1995; Jeon et al., 1995), and this drives cell cycle dysregulation, cellular transformation (Munger et al., 1989), and immortalization (Hawley-Nelson et al., 1989). Cells with integrated high-risk HPV DNA grow faster, and for more populations doublings in culture, than do cells with extrachromosomal HPV DNA (Jeon et al., 1995). In transgenic mice expressing the early genes of HPV 16 that are exposed to estrogen, reproductive tract tumors develop (Arbeit et al., 1996; Elson et al., 2000; Riley et al., 2003). When high-risk E6 and E7 are coexpressed in epithelial cells in culture, they become immortalized (Kiyono et al., 1998), and when E6 and E7 expression is reduced, cervical cancer cell line's growth is arrested and tumor formation in nude mice is blunted (von Knebel et al., 1992; Francis et al., 2000). Both the E6 and E7 genes push the oncogenic potential of epithelial cells through their dysregulation of cell cycle progression, senescence, and apoptosis.

Genus Alpha HPV E6 Oncogene

The E6 protein is critical to the HPV life cycle (Fig. 1.4). Mouse models have been used to document the oncogenic potential of the genus alpha E6 gene. Transgenic mice expressing HPV 16E6 in their epithelium have both a promotion and a progression of skin tumors when compared with nontransgenic mice (Simonson et al., 2005). When mice expressing HPV 16E6 are exposed to estrogen, they develop cervical dysplasias, and with prolonged exposure to estrogen, they can develop cervical cancers (Shai et al., 2007a). This is strong evidence that the high-risk HPV E6 is an oncogene.

E6 binds an endogenous E3 ubiquitin ligase, E6-associated protein (E6-AP), found in epithelial cells (Huibregtse et al., 1991, 1993). E6 and E6-AP bind the critical cell cycle protein p53 and target it for degradation by the 26S proteasomal pathway (Scheffner et al., 1993), and there is evidence that HPV 16E6 itself can blunt the effect of p53 without E6-AP (Shai et al., 2007b). When p53 is lost, epithelial cells no longer recognize senescence or apoptosis signals and continue to divide throughout the stratified squamous epithelial layers. This is key to HPV production, but moreover, this is critical to the immortalization of epithelial cells and the stochastic collection of genetic and epigenetic mutations that lead to cancer.

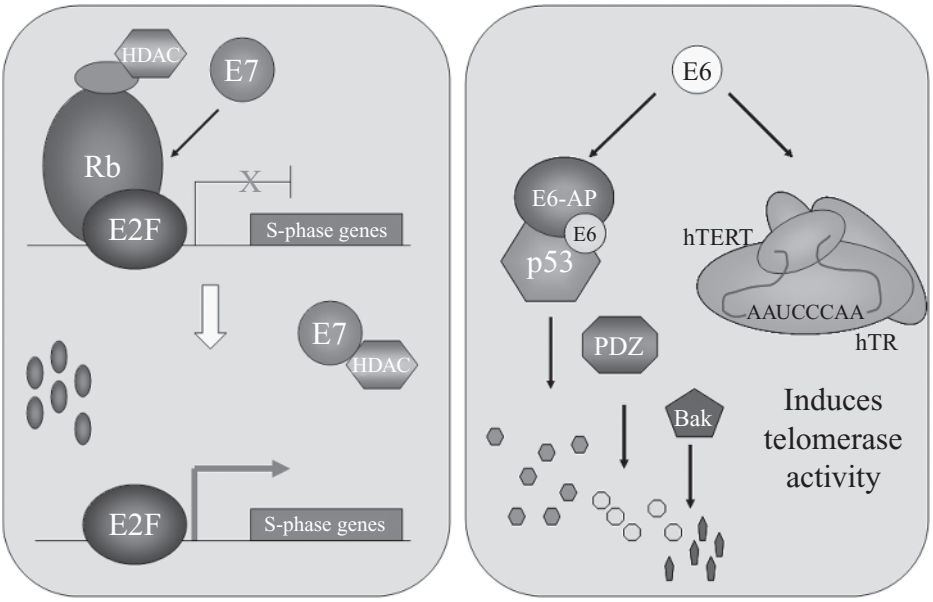

Figure 1.4. High-risk E6 and E7 roles in epithelial cells. E7 targets retinoblastoma protein, and other pocket proteins, for ubiquitin-mediated proteasomal degradation, allowing E2F to transactivate S-phase genes. E6 targets p53, PDZ domain-containing proteins, and Bak for degradation as well as activates the expression of hTERT, the catalytic subunit of telomerase. HDAC=histone deacetylase, a transcriptional repressor.

A second key role of high-risk HPV E6 in cell cycle dysregulation is the activation of telomerase (Fig. 1.4). Telomerase is a ribonucleoprotein that includes its catalytic subunit hTERT, its RNA component hTR, and dyskerin (Kilian et al., 1997; Meyerson et al., 1997; Nakamura et al., 1997; Counter et al., 1998; Cohen et al., 2007). Telomerase extends the repetitive telomeric DNA that caps the ends of chromosomes and protects genes within the chromosome from serial erosion with each round of DNA replication. Telomerase is normally expressed in stem cells, and telomerase activity is proportionate to the expression level of hTERT (Counter et al., 1998). In differentiated cells, hTERT expression is repressed. As these differentiated cells divide, their telomeres shorten; the age of a cell is therefore reflected in the inverse length of its telomeres. When the telomeres of a cell become critically shortened, it signals for that cell to go through senescence or apoptosis. In most cancers, telomerase is activated, so its expression is critical to the immortalization of cells and to oncogenic progression (Shay and Bacchetti, 1997).

E6 and E6-AP activate expression of hTERT in epithelial cells through transcriptional activation at the promoter (Gewin and Galloway, 2001; Gewin et al., 2004; Galloway et al., 2005; Liu et al., 2005). Additional regulators of hTERT that interact with E6 and E6-AP have been identified, such as c-myc, Mad, Max, NFX1-91, and NFX1-123 (Wang et al., 1998; Takakura et al., 1999; Wu et al., 1999; Oh et al., 1999,

2001; Veldman et al., 2001, 2003; McMurray and McCance, 2003; Gewin et al., 2004; Katzenellenbogen et al., 2007). Each of these endogenous protein partners is important in hTERT activation during HPV infection and HPV genome integration.

The loss of p53 and activation of telomerase are the two critical events driven by E6. These allow differentiated epithelial cells to continue to divide when they otherwise would not. They no longer recognize signals from DNA damage through the p53 pathway to go through apoptosis or senescence, and they no longer recognize their age as telomerase extends the ends of chromosomes. Loss of p53 and activation of telomerase are required in most cancers, so the role of E6 in oncogenic progression of HPV cancers is intuitive.

High-risk E6 proteins have roles beyond p53 degradation and telomerase activation (Fig. 1.4). High-risk E6 proteins have a four-amino-acid domain at their extreme C-terminus. This domain is required to bind proteins that contain a PDZ domain, such as *P*SD-95, *D*lg (disk large protein), and *Z*O-1 (hence the name PDZ), as well as human Scribble, MUPP-1, and MAGI-1, 2, and 3. These PDZ proteins are found primarily in the apicobasal region of epithelial cells, and their interaction with and degradation by E6 may be important in HPV-driven cell growth, metaplasia, malignant progression, and metastatic disease (Dobrosotskaya and James, 2000; Glaunsinger et al., 2000; Thomas et al., 2002; Watson et al., 2003; Lee and Laimins, 2004; Massimi et al., 2004). Transgenic mice expressing HPV 16E6 with the PDZ-binding domain deleted are unable to develop epithelial hyperplasia, induce DNA synthesis, or promote papillomas when compared with HPV 16E6 wild-type (WT) mice (Nguyen et al., 2003; Simonson et al., 2005). However, once tumors do develop, 16E6 WT and PDZ-binding-domain-deleted mice have equal numbers of carcinomas (Simonson et al., 2005). So, the PDZ-binding domain of high-risk E6 proteins may be critical to the initial steps of oncogenesis. Other PDZ-domain-containing proteins have been identified as targets for degradation by high-risk E6. E6-AP is required for the degradation of some PDZ-containing proteins, such as Scribble and Dlg4/SAP97 (Nakagawa and Huibregtse, 2000; Handa et al., 2007), but perhaps not others (Pim et al., 2000; Grm and Banks 2004; Grm et al. 2004), although there is conflicting data on this (Brimer et al., 2007; Kuballa et al., 2007). Finally, E6 and E6-AP can bind other cellular regulators such as Bak, a protein in the apoptosis cascade, and target it for degradation like p53 (Thomas and Banks, 1998). Therefore, the tumorigenic potential of high-risk E6 involves other cellular proteins beyond p53 and hTERT.

Genus Alpha HPV E7 Oncogene

Like E6, E7 expression is normally regulated by E2. Also, like E6, high-risk E7 is an oncogene (Fig. 1.4); keratinocytes expressing HPV 16E7 can become immortalized in culture, although coexpression of HPV 16E6 accelerates this process (Halbert et al., 1991). Transgenic mice that express the HPV 16E7 protein in their epithelium develop skin hyperplasia and, late in life, skin tumors (Herber et al., 1996). Those mice that express E7 at a high level can have stunted growth and early mortality from the severe epithelial hyperplasia of the esophagus causing dysphagia (Herber et al., 1996). When these same mice are exposed to estrogen, they quickly develop tumors

of the reproductive tract (Riley et al., 2003). All of these tissue culture and mouse model studies are strong evidence that high-risk E7 is an oncogene, driving epithelial cell proliferation and cancer.

E7 functions in tandem with E6 to drive differentiated epithelial cells to continue to divide. E7 is known to bind to many endogenous cell cycle proteins, including pocket proteins retinoblastoma protein (Rb), p107 and p130 (Dick and Dyson, 2002; Zhang et al., 2006), and cyclin-dependent kinase inhibitors p21 and p27 (Funk et al., 1997; Helt et al., 2002). The interaction of E7 and Rb has been the most studied of these interactions (Fig. 1.4). E7 degrades Rb through the ubiquitin-mediated proteasomal pathway (Boyer et al., 1996; Helt and Galloway, 2001; Dick and Dyson, 2002), and the degradation of Rb by E7 allows the transcription factor E2F to activate expression from S-phase promoters, driving DNA replication and cellular division. Transgenic mice expressing HPV 16E7 and a knocked-out Rb that cannot be bound by E7 have little skin hyperplasia, have a normal cell cycle arrest to DNA damage, and do not increase p21 protein levels (Balsitis et al., 2005). However, transgenic mice expressing HPV 16E7 and treated with estrogen develop cervical cancers whether or not Rb can be degraded (Balsitis et al., 2006). Therefore, the ability of high-risk E7 to degrade Rb is more important in skin tumorigenesis than in cervical cancer in mouse models. Other pocket proteins are bound by E7, and those interactions may be as important in the HPV life cycle as they are in oncogenic progression (Zhang et al., 2006). Finally, high-risk E7 can increase expression of DEK, a senescence inhibitory protein, which may be critical in HPV malignant progression (Wise-Draper et al., 2005).

High-risk E7 affects gene expression through its interaction with transcription factors and also chromatin remodeling proteins. HPV 16E7 can bind several factors from the AP1 family of transcription factors, which are likely important in driving the cell cycle (Antinore et al., 1996). E7 can bind to histone deacetylases (HDAC) (Brehm et al., 1999; Longworth et al., 2005), and this can allow continued cell growth (Brehm et al., 1999) and HPV DNA replication (Longworth et al., 2005) (Fig. 1.4). Combined, E7 can drive differentiated cells to continue to divide, activate genes normally quiescent in differentiated cells, and ignore DNA damage signals.

GENUS BETA HPV ONCOGENES

Like genus alpha HPV E6 and E7, genus beta HPV E6 and E7 have been increasingly identified as oncogenes. However, unlike genus alpha oncogenes in anogenital cancers, skin cancers do not universally have evidence of E6 or E7 DNA, and in an HPV-positive skin cancer, not all cells would have HPV DNA. So, a continued effect by genus beta HPV is not required in SCC development, and the cancer is not a clonal outgrowth from a genus beta HPV-positive cell, making their malignant progression fundamentally different from the classic high-risk HPV types. Despite these differences, genus beta HPV E6 and E7 are important in SCCs, especially in immunocompromised hosts, blocking apoptosis and activating telomerase. Their study is becoming increasingly important as the population at risk, including solid organ and bone marrow transplant patients, grows.

HPV 38 E6 and E7 expressed together in primary epithelial cells can drive DNA replication and the cell cycle, block senescence by Ras, and induce anchorage independence in fibroblast cultures, all of which point to transforming properties of beta HPV oncogenes (Caldeira et al., 2003). Organotypic epithelial cultures grown with several beta HPV E6 and E7 types show decreased differentiation of skin cells and basal cells within the suprabasal region (Boxman et al., 2001). Transgenic mice expressing the early genes of HPV 8 in the dermis develop skin tumors, and a subset develops SCC (Schaper et al., 2005), pointing to their importance in skin cancer progression.

Genus Beta HPV E6 Oncogene

Genus alpha HPV E6 binds E6-AP to degrade p53 and block apoptotic signals from DNA damage. Genus beta HPV E6 does not affect p53 protein levels, but instead, it targets proteins downstream of p53 for degradation. In the skin, the most common DNA damage seen is ultraviolet (UV) irradiation on sun-exposed skin; therefore, studies focus on this effect. In keratinocytes, p53 and p21 are induced after UV irradiation, as is the proapoptotic protein Bak. p53 activates the cascade of proapoptotic signals that lead to caspase release from mitochondria and cell death. In cultured and organotypic cells expressing genus beta HPV E6 types, p53 and p21 expression is increased after UV irradiation, but Bak is not induced. These cells do not undergo apoptosis but continue to divide despite double-stranded DNA damage (Jackson et al., 2000). Also, studies of HPV 77 E6 show disruption of proapoptotic gene activation by p53 after UV irradiation in epithelial cells (Giampieri et al., 2004). p53 levels may be important in E6 expression, as p53 can bind the HPV 8 E6 promoter, and it directly competes with HPV 8 E2 for binding and regulation of expression (Akgul et al., 2003). New evidence also points to a subset of genus beta E6 proteins that can activate telomerase and extend the life span of epithelial cells in culture (Bedard et al., 2008).

Genus Beta HPV E7 Oncogene

The genus beta E7 protein can function like genus alpha E7, triggering continued cellular proliferation while epithelial cells are terminally differentiating. Several genus beta E7 proteins have been reported to bind to Rb, and a subset of those can target Rb for degradation, although not all. However, in addition to the known cell cycle effects of E7, keratinocytes expression HPV 8 E7 protein can degrade the basement membrane, induce matrix metalloproteinase expression, and invade stromal tissues in organotypic cultures (Akgul et al., 2005; Smola-Hess et al., 2005). So, E7 alone may drive the oncogenic and metastatic potential of beta HPV-infected skin cells and push infected cells toward invasion even before collecting DNA damage from UV exposure.

FUTURE ISSUES

Although behavior modifications can decrease HPV infection rates, and consistent screening for and treatment of cytological abnormalities can decrease cancer rates and deaths, prophylactic vaccination against specific HPV types has the potential to

eliminate a significant burden of disease. The transition from HPV infection to anogenital cancers can take decades to occur, so the full impact of prophylactic vaccination will likely take a generation to be seen.

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