Abstract

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer with an annual incidence of approximately 400,000 worldwide. Although the principal risk factors for head and neck cancer remain tobacco and alcohol use, human papillomavirus (HPV) has recently been found to be etiologically associated with 20 to 25% of HNSCC, mostly in the oropharynx. HPV causes human cancers by expressing two viral oncoproteins, E6 and E7. These oncoproteins degrade and destabilize two major tumor suppressor proteins, p53 and pRb, through ubiquitination. Additional studies have shown that E6 and E7 can directly bind to multiple host proteins other than p53 and pRb (e.g., Bak and p21\(^{CIP1}\)), further contributing to genetic instability. However, expression of E6 and E7 alone is not sufficient for cellular transformation, and the additional genetic alterations necessary for malignant progression in the setting of virus-induced genomic instability are unknown. In addition to the etiological differences, HPV-positive cancers are clinically distinct when compared with HPV-negative cancers with regard to treatment response and survival outcome, with tumor HPV-positivity being a favorable prognostic biomarker. Further understanding of carcinogenesis and clinical behavior of HPV-positive cancers will improve disease prevention, patient care, and surveillance strategies for HNSCC patients.

Background

Human papillomavirus (HPV) is a circular, double-stranded DNA virus. The viral genome, consisting of approximately 8,000 base pairs in size, encodes two regulatory proteins (from “early” genes E1 and E2), three oncoproteins (E5, E6, and E7), and two structural capsid proteins (from “late” genes L1 and L2; reviewed in ref. 1). More than 100 unique HPV types are known, but these different types are generally divided into those with a predilection to infect the skin versus mucosal surfaces (2). Mucosal HPV infections are well known to associate with a spectrum of human diseases from benign papillomas (or warts) to invasive carcinomas including cervical, vulvar, vaginal, anal, penile, and more recently head and neck squamous cell carcinoma (HNSCC; reviewed in refs. 1, 3, 4). Because carcinomas of the cervix and anogenital region have been extensively reviewed in the past, we will focus on the recent data in HNSCC in this review.

HNSCC is the sixth most common cancer with an annual incidence of approximately 400,000 worldwide (5). These cancers arise from five major anatomic sites: oral cavity, oropharynx, nasopharynx, hypopharynx, and larynx. Although Epstein-Barr virus is a long-established cause of nasopharyngeal cancer (6), a causal association between HPV and oropharyngeal cancer has only recently been established (7, 8). Compared with the association of HPV-negative tumors with heavy tobacco and alcohol use, HPV-positive tumors are strongly associated with sexual behavior, which is consistent with the known predominant means of HPV transmission via sexual contact (9). The incidence of HPV-related cancers has been increasing since the early 1990s in the United States and Western Europe, but the underlying reasons for this rapid increase are unclear (10, 11).

The HPV life cycle is complex. During early infection, viral DNA is present as a nuclear episome at low copy number in the basal cell layer of the stratified epithelium. HPV DNA is amplified and encapsidated to progeny virions only in terminally differentiated epithelial cells (reviewed in ref. 4). Recent data indicate that establishment of the initial infection is tightly linked to cell cycle progression through the mitotic phase (12). When human kidney cells (293T) or immortalized keratinocytes (HaCaT) are exposed to HPV in the presence of approximately 5,000 bioactive compounds via high-throughput screening, a subset of cell cycle inhibitors (including etoposide, aphidicolin, and 5-fluorouracil) blocks HPV infection. This effect is observed even at low concentrations that do not affect...
ubiquitination induces degradation of pRb resulting in uncon-
trolled G1/S phase of the cell cycle (26). In the absence of the pRb function, the E2F family of transcription factors is released and transcription of S-phase genes occurs, leading to cell prolif-
eration. When E6 and E7 proteins are silenced in HPV-positive oropharyngeal cancer cell lines using shRNAs, the expression of p53 and pRb restored, as are p53-dependent up-regulation of CDKN1A (p21Cip1) and FAS expression, and pRb-dependent down-regulation of DEK and B-MYB. Furthermore, inhibition of E6 and E7 induces apoptosis and decreases cell viability, indicating that expression of the E6 and E7 oncoproteins is re-
quired for tumor maintenance in oropharyngeal cancers, as is seen in cervical cancers (27). In addition, E7 is found to be the major transforming oncogene during the early stages of car-
cinogenesis in HNSCC when compared with E6, which seems to function at later stage (28). When E6, E7, and both E6/E7 are expressed under keratin 14 (K14) promoter regulation in trans-
genic mice, mice develop tumors in their tongue and esophagus after treatment with 4-nitroquinolin-N-oxide (4-NQO) in their drinking water. The K14E6 mice developed tumors at a much lower rate than the K14E7 mice (22% versus 95%), although the nontransgenic mice had a tumor formation rate at 16%. The K14E6E7 bi-transgenic mice had a similar rate of tumor for-
mation as the nontransgenic mice had a tumor formation rate at 16%. On further examination, mitosis phase progression is found to be required for HPV infection and early gene expression. More specifically, the early prophase segment of mitosis is the critical phase for the infection, as determined by arresting the cell cycle at the G2/M phase using the cyclin-dependent kinase (CDK) 1 inhibitor, purvalanol A (12, 14).

At least 15 high-risk or oncogenic HPV types have been iden-
tified on the basis of associations with cervical cancer. HPV16 is the most common type found in 87 to 90% of HPV-positive oropharyngeal cancers (7, 15, 16). The role of high-risk HPV oncoproteins (E5, E6, and E7), in cellular transformation and maintenance of the malignant phenotype was first described in cervical cancers. The HPV E5 protein plays an important role during the early course of infection by binding to epidermal growth factor receptor (EGFR), platelet derived growth factor β receptor, and colony stimulating factor 1 receptor to promote cell proliferation (17). However, the E5 coding sequence is fre-
quently deleted from the episomal viral DNA during integra-
tion to host genome; therefore, E5 is thought not to be re-
quired for the late stage of carcinogenesis (18). The significant role for carcinogenesis and maintenance of malignant pheno-
type is played by E6 and E7.

The HPV E6 protein forms a complex with an E3 ubiquitin ligase, E6-associated protein (E6AP), and ubiquitinates the p53 tumor suppressor protein (Fig. 1D; refs. 19–21). The ubiquiti-
nation causes rapid degradation of p53, which results in dereg-
ulation of both the G1/S and G2/M cell cycle checkpoints upon DNA damage and other cellular stress leading to genomic insta-
bility (22). The HPV E7 protein binds to the cullin 2 ubiquitin ligase complex and ubiquitinates the retinoblastoma (pRb) tumor suppressor protein (Fig. 1A; refs. 23–25). Again, the ubiquitination induces degradation of pRb resulting in uncon-
cellular transformation in keratinocytes caused by the HPV oncoproteins, E6 and E7; clockwise from A, ubiquitination by E7 and the cullin 2 ubiquitin ligase complex leading to pRb degradation (22, 25, 56, 57); B, interaction between E7 and p27kip1 resulting in inhibition of cell cycle arrest contributing to carcinogenesis (58); C, interaction between E7 and p21Cip1 resulting in inhibition of cell cycle arrest contributing to carcinogenesis (31, 59); D, ubiquitination by E6 and ubiquitin ligase E6AP leading to p53 degradation (19–21); E, increased expression of p16INK4A by a consequent of feedback loops from the absence of pRb function (42); and F, degradation of NFX1, a transcriptional repressor of hTERT, by association with E6/E6AP resulting in hTERT activation and cellular immortalization (60).
tumorigenesis (28). For example, E7 interacts with the pRb-related "pocket proteins" p107 and p130, and the CDK inhibitors p21WAF1 and p27KIP1 (29–31). Inhibition of these key regulatory proteins for cell cycle arrest contributes to uncontrolled cellular proliferation and carcinogenesis (Fig. 1B and C). E6, in concert with E6AP, induces telomerase activity through activation of hTERT via degradation of NFX1, a transcription repressor of hTERT, thus contributing to cellular immortalization (Fig. 1F; ref. 32). In addition to exerting anti-apoptotic effects by degrading p53, E6 directly binds a pro-apoptotic protein Bak with E6AP, which further contributes to anti-apoptosis (33). These transforming effects work in concert such that E6 prevents E7-induced apoptosis by exerting anti-apoptotic effects degrading p53 and Bak, and that E7 rescues E6 from p16INK4A inhibition by direct activation of cyclins A and E and functional inactivation of p16INK4A bypassing its regulation (33, 34).

However, despite having multiple binding partners with oncogenic effects as well as enabling the cells to acquire genetic alterations that cause genomic instability, expression of E6 and E7 alone is not sufficient to cause malignant progression or oncogenic transformation (reviewed in ref. 1). Currently, the additional genetic events that are required for the development of cancer are unknown. Also unclear is whether these genetic events will be common among specific HPV-associated cancers (for instance, oropharyngeal cancers) or common to all HPV-associated cancers (e.g., cervical, vulvar, vaginal, anal, penile, and oropharyngeal cancers). Compared with HPV-negative head and neck cancers, HPV-positive cancers have fewer genome-wide DNA copy number alterations, less genome-wide hypomethylation, less frequent TP53 mutations, and lower expression of EGFR (7, 35–38). For instance, HPV-negative tumors have losses at 3p11.2–26.3, 5q11.2–35.2, and 9p21.1–24, and gains or amplifications at 11q12.1–13.4, which are absent in HPV-positive tumors. Interestingly, 18q12.1–1.23 is gained in HPV-positive tumors and lost in HPV-negative tumors (35). In gene expression analyses, the most prominent differences between HPV-positive and -negative tumors are found in cell cycle regulatory pathways (39, 40). HPV-positive tumors have up-regulation of cyclins E and B as well as multiple S-phase proteins that are responsive to activation by the E2F transcription factors, likely as a consequence to E7-induced pRB loss (41). As a consequence of feedback loops from pRB loss, HPV-positive tumors have up-regulation of CDKN2A (p16INK4a; Fig. 1E; ref. 42). In addition to the cell cycle-regulated genes, up-regulation of testis-specific genes (e.g., SYCP2, TCAM1, and STAG3) is observed in HPV-positive tumors. Although SYCP2 and TCAM1 expression are synergistically up-regulated by E6 and E7, STAG3 expression increase is not an immediate effect of the viral infection; rather, it is a delayed response that is passage-dependent (40).

Further understanding of these additional genetic alterations leading to malignant progression is critical to future secondary prevention strategies as well as rationally targeted therapeutic interventions for patients with HPV-positive cancers. The prevalence of genital HPV infection among women aged 14 to 59 years in the United States is ~26.8% (43), with HPV16 prevalence being ~1.5%. Among men, initial estimates for genital HPV infection in the U.S. population are currently unknown, but it is assumed from cervical data that the majority of individuals with an oral HPV16 infection will not develop cancer. Identification of the additional factors that promote cancer among those with an oral HPV infection may result in novel screening methods or novel therapeutic targets in selected high-risk subpopulations.

**Clinical-Translational Advances**

To investigate the effects of HPV in human tumors, optimization of HPV detection methods with both high sensitivity and high specificity is crucial. Several well-established methods exist. Although detection of HPV E6 and E7 expression is the gold standard for classifying a tumor as HPV-positive, detecting viral RNA in existing clinical samples (e.g., formalin-fixed paraffin-embedded tumors or cytologic specimens from fine-needle aspiration) is impractical for cancer diagnostics at this time. Therefore, several PCR-based, as well as in situ hybridization (ISH) assays, are currently used to detect HPV DNA in tumors. The p16INK4A immunohistochemical (IHC) staining of tumors has also been used as a surrogate marker for HPV presence (7, 8, 46, 47). Overall, the PCR-based assays have higher sensitivity with lower specificity owing to the presence of transcriptionally inactive viral DNA or cross-contamination of samples that may cause falsely positive results (48). Commercial ISH assays capable of detecting multiple high-risk HPV types have lower sensitivity but can be used to visualize the HPV genome specifically within tumor cell nuclei (49). IHC staining for p16INK4A is an excellent surrogate marker for HPV infection, reflecting the functional effects of E7-induced inactivation of pRB (Fig. 1D; refs. 47, 49, 50). Some investigators have proposed p16INK4A IHC staining as an initial screen, followed by HPV detection with more specific assays in tumors that are p16INK4A IHC positive (51).

When clinical outcomes are evaluated on the basis of tumor HPV status, patients with HPV-positive oropharyngeal cancers have favorable outcomes compared with patients with HPV-negative cancer. In a phase III trial, Radiation Therapy Oncology Group 0129, 64% of 323 oropharyngeal tumors analyzed for HPV were HPV positive using ISH capable of detecting multiple high-risk HPV types (52, 53). However, a subset of the HPV-positive tumors were HPV positive using ISH capable of detecting the most common 13 high-risk HPV types. These patients with HPV-positive tumors were younger, had less extensive tobacco exposure, better performance status, and smaller primary tumors compared with HPV-negative patients (49). The clinical outcomes after treatment with cisplatin and radiation therapy were significantly better in patients with HPV-positive compared with HPV-negative tumors (2-year overall survival 87.9% versus 65.8%, *P* < 0.001; progression-free survival 71.8% versus 50.4%, *P* < 0.001; and local-regional failure 13.6% versus 24.8%, *P* 0.004). Favorable outcomes with HPV-positive patients have been observed independent of the treatment modalities used including chemotherapy, radiation, and/or surgery (52–54). However, a subset of the HPV-positive patients experience worse outcomes when compared with the average HPV-positive patient, resembling more of the clinical course in HPV-negative patients. This subset of patients has more extensive smoking histories, TP53 mutations, and higher EGFR and Bcl-xL expressions suggesting that the HPV status alone is not an adequate prognostic marker to perfectly segregate patients (38, 49, 55).
Because of the distinct biology and clinical behavior within subgroups of oropharyngeal cancer patients, clinician-scientists now propose to do clinical trials separately or at least stratify based on HPV status. For example, when a phase III clinical trial is done to compare drug X versus drug Y, the drug X arm may falsely show survival benefit if there are disproportionately more HPV-positive patients in the drug X arm compared with the drug Y arm. The apparent survival benefit of drug X may be entirely driven by a disproportionate number of patients with good prognosis, which may lead to the erroneous conclusion that drug X is better than drug Y. In addition, the distinct molecular mechanisms underlying HPV-positive and -negative tumors may lead to interaction effects whereby responses to drug X and/or drug Y are influenced by HPV status, which may also give the appearance that drug X and drug Y have different treatment responses. Therefore, the investigators have to ensure that the same number of HPV-positive patients is included in comparison groups by using the recent data for stratification. Currently, many of the completed clinical trial data that enrolled unselected patients are undergoing retrospective analyses in order to see the effect of HPV status versus treatment variables with respect to their survival outcomes. Furthermore, investigators are reevaluating current approaches for intensification of treatment by stratifying risk groups in order to avoid unnecessary toxicities among patients with favorable prognoses. How best to treat these patients is the subject of ongoing clinical trial design discussions and will best be determined through the cooperative groups. Additional correlative studies are required to further delineate the subset of patients with HPV-positive tumors that have a worse prognosis than would have been expected from their HPV status. The role of the currently available HPV vaccines should be also investigated for cancer prevention.

Disclosure of Potential Conflicts of Interest

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References

37. Westra WH, Taube JM, Poeta ML, Begum S, Sidransky D, Koch WM. Inverse relationship between human papillomavirus-16 infection and
Molecular Pathways

Human Papillomavirus in Head and Neck Cancer: Its Role in Pathogenesis and Clinical Implications

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