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Review

Cancer Research

Novel Insights into Head and Neck Cancer using Next-Generation "Omic" Technologies

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Abstract

Head and neck squamous cell carcinoma (HNSCC) is a highly heterogeneous disease that develops via one of the two primary carcinogenic routes: chemical carcinogenesis through exposure to tobacco and alcohol or virally induced tumorigenesis. Human papillomavirus (HPV)–positive (HPV^+) and HPV-negative (HPV^-) HNSCCs represent distinct clinical entities, with the latter associated with significantly inferior outcome. The biologic basis of these different outcomes is an area of intense investigation; their therapeutic regimens are currently also being reevaluated, which would be significantly facilitated by reliable biomarkers for stratification. With the advent of the omics era and accelerated development of targeted therapies, there are unprecedented opportunities to address the challenges in the management of HNSCC. As summarized herein, side-by-side molecular characterization of HPV^+ versus HPV^- HNSCC has revealed distinct molecular landscapes, novel prognostic signatures, and potentially targetable biologic pathways. In particular, we focus on the evidence acquired from genome-wide omics pertinent to our understanding of the clinical behavior of HNSCC and on insights into personalized treatment opportunities. Integrating, mining, and validating these data toward clinically meaningful outcomes for patients with HNSCC in conjunction with systematic verification of the functional relevance of these findings are critical steps toward the design of personalized therapies. *Cancer Res; 75(3); 480–6. ©2014 AACR.*

Oncogenic Role of Human Papillomaviruses

High-risk human papillomaviruses (HPV) are double-stranded DNA viruses that infect epithelial cells (1). Tumorigenesis by high-risk HPVs is driven by their two main viral oncogenes, *E6* and *E7*, which inactivate p53 and pRb, respectively, leading to cell-cycle deregulation and inhibition of p53-mediated apoptosis (1, 2). *E7* binds pRb, targeting it toward proteosomal degradation, in turn releasing the E2F transcription factor, resulting in *CDKN2A* (or p16) overexpression and cell-cycle progression (1).

High-risk HPVs, predominantly types 16, 18, 31, 33, and 35, are estimated to cause approximately 5% of cancers worldwide, including 99% of cervical, 25% of head and neck (or 60% oropharyngeal), 70% of vaginal, 88% of anal, 43% of vulvar, and 50% of penile cancers, in order of prevalence (1–6). A significant subset of approximately 500,000 annual cases of head and neck squamous cell carcinoma (HNSCC) include approximately 85,000 HPV-associated tumors, establishing this as the second most common HPV-associated tumor site (3, 4). HPV type 16 is identified in over 90% of HPV-associated HNSCCs (4). The majority of the remaining HNSCCs are attributed to exposure to chemical carcinogens such as tobacco and alcohol.

HPV-positive (HPV^+) and HPV-negative (HPV^-) HNSCCs are separate entities associated with distinct etiology, clinical behavior, treatment outcomes, imaging and pathology appearance, and molecular profiles (2, 7). HPV^+ HNSCC primarily involves the oropharynx (predominantly involving the tonsil and tongue base) while only a small fraction of other HNSCC subsites have been associated with high-risk HPVs (2). Unlike HPV^+ HNSCC, HPV^- HNSCC has been decreasing in incidence in the developed world owing to implementation of smoking cessation campaigns. Cervical cancers have also been declining due to screening for premalignant lesions and preventative vaccination (4, 8). In contrast, HPV-associated oropharyngeal cancer cases have been rising across North America, Europe, and Australia over the recent decades (8, 9). At the current pace, oropharyngeal cancer incidence is expected to surpass cervical cancer incidence by 2020 in the United States. Furthermore, HPV^+ oropharyngeal cancer is associated with younger age at diagnosis and significantly more favorable outcome (3-year overall survival rate of ~85% vs. ~60% for HPV^- patients; refs. 2, 10, 11). This can be attributed to superior locoregional disease control in HPV^+ patients. Tumor HPV positivity is also associated with better prognosis in recurrent/metastatic HNSCC (reviewed in detail in ref. 11), although few therapeutic options exist for these patients and long-term survival is uncommon. The biologic mechanisms for different outcomes in HPV^+ versus HPV^- HNSCC remain poorly understood.

HNSCC is typically treated with radiotherapy ± chemotherapy or surgery ± postoperative radiotherapy/chemotherapy, or surgery ± postoperative radiotherapy/chemotherapy, irrespective of tumor HPV status. However, as HPV^+ oropharyngeal cancers are highly responsive to radiotherapy/chemotherapy compared with

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HPV$^-$ oropharyngeal cancers, clinical trials are ongoing to determine the efficacy of treatment de-escalation in select HPV-associated HNSCCs (reviewed in ref. 12). Thus, these patients could be spared from acute and late toxicities associated with such aggressive combined treatments. Development of de-escalation strategies, however, can be complicated by approximately 10% of HPV$^+$ patients at high risk of developing distant metastases (13). Although the rate of distant metastasis is akin to HPV$^-$ oropharyngeal cancer, distant metastasis in HPV$^+$ oropharyngeal cancer may occur later with potentially more frequent dissemination to multiple organs and unusual sites (13–15). Cetuximab, a monoclonocal antibody therapeutic targeting EGFR, is the sole FDA-approved targeted therapy for HNSCC and has demonstrated a significant yet modest survival benefit with a single-agent response rate of approximately 15% (2, 16). Hence, biomarkers for distant metastasis risk identification, as well as prediction of response to targeted therapies are critically necessary to facilitate patient stratification toward specific treatment modalities.

Significant efforts have been undertaken to delineate the distinct molecular landscapes of HPV$^+$ and HPV$^-$ HNSCC, including genomic, epigenetic, transcriptomic, and proteomic profiles (Table 1), with three main expectations: (i) to achieve a better understanding of the molecular progression and biologic basis of the clinical behavior for these disease entities; (ii) to uncover potentially novel prognostic and predictive molecular signatures; and (iii) to identify therapeutic targets. In this review, we provide the highlights of such omics studies performed on primary HNSCCs, with a particular emphasis on the aforementioned objectives. Systematically deciphering the clinical relevance of these data is imperative for improving outcomes and reducing treatment-related morbidities in HNSCC.

Chromosomal Aberrations

HNSCCs have complex karyotypes with specific chromosomal gains and losses arising through premalignant, malignant, and metastatic progression (2, 17). HPV-associated tumors harbor significantly fewer chromosomal alterations overall; this is likely related to disease etiology whereby extensive genetic alterations are required to induce malignant transformation in the absence of high-risk viral infection as an oncogenic event (17, 18). Further supporting the distinct etiologic roles is the resemblance between copy number alterations of HPV-associated HNSCC and cervical cancers (19). The genome instability associated with the greater number of chromosomal aberrations in HPV$^-$ HNSCC might lead to a higher risk of developing treatment resistance (18, 20).

The genetic changes observed in premalignant lesions depend on the etiologic agents, which can start with 17q loss/*TP53* mutation followed by 9p loss/p16 inactivation in HPV$^-$ HNSCC, or *E6*/*E7*-mediated disruption of p53/pRb activities in HPV$^+$ HNSCC (17). Subsequently, amplification at 11q13, a locus harboring the *CCND1* oncogene and the candidate oncogenes *CTTN* and *FADD*, may lead to malignant transformation of precancerous cells (2, 17). The potent oncogenic role of these aberrations is supported by the immortalization of keratinocytes in response to disruption of the p53 and pRb pathways (21).

Significant differences exist in early carcinogenesis markers between HPV$^-$ and HPV$^+$ HNSCC; the former is frequently associated with gains at 11q12.1-13.4, and losses at 9p21.1-24, which often co-occur. Amplifications in 11q13 or *CCND1* overexpression as a therapeutic target for CDK4/6 inhibitors in HPV$^-$ HNSCC certainly warrant exploration (18, 22). Because of frequent losses in HPV$^-$ oropharyngeal cancer and overexpression in HPV$^+$ oropharyngeal cancer, p16 immunodetection is utilized clinically as a first-line test for determination of HPV positivity and has been shown to serve as an independent prognostic marker in oropharyngeal cancer (6, 23). P16-positive tumors are tested for viral presence by HPV DNA or RNA PCR or by *in situ* hybridization, thereby excluding specimens with p16 overexpression by mechanisms alternate to viral infection (2). Importantly, the utility of p16 as a prognostic or surrogate marker for HPV in nonoropharyngeal cancer HNSCC is controversial (24, 25). Another early event significantly more common in HPV$^-$ HNSCC is LOH at 3p (17, 19, 26). The minimally affected region, 3p11.2-26.3, encodes for the candidate tumor suppressors *FHIT* and *RASSF1A* (2, 17). However, this requires further evaluation as 3p loss may be relatively uncommon in oropharyngeal cancer as compared with other HNSCC subsites (27).

Late-stage chromosomal aberrations that differ with HPV status include alterations in 3q, 5q, 7p, 16q, 18q, and 20q. Significant enrichment of 3q24-27 amplifications in HPV$^+$ versus HPV$^-$ tonsillar carcinomas has been observed; potential oncogenes in this locus include *PIK3CA*, *TP63*, *SOX2*, *CCNL1*, *PARP1*, *hTERC*, and *DCUN1D1* (2, 22, 27). Interestingly, 3q amplification is an early event in cervical cancer development (27). Along the PI3K pathway, 10q deletions harboring the *PTEN* tumor suppressor were found to be more frequent in HPV$^-$ tumors (30% in HPV$^+$ vs. 20% HPV$^-$ HNSCC; ref. 22); although this difference was not statistically significant. Amplifications at chromosome 7p harboring the *EGFR* oncogene were found in 13% of HPV$^-$ HNSCC, yet absent in HPV-associated HNSCC (2, 22), which is consistent with the modest efficacy of cetuximab (28); although the utility of *EGFR* status in predicting response to anti-EGFR therapy remains inconclusive. In addition, *FGFR1* amplifications were found to be restricted to HPV$^-$ tumors (22).

In another study, loss of 16q12-24 was observed primarily in HPV-related HNSCC (26, 29). Amyloid β precursor protein-binding protein1 (*APP-BP1*) was proposed as a candidate downregulated gene, with the premise that it may contribute to increased responsiveness of HPV-positive tumors to RT by allowing induction of p53-mediated apoptosis, which is normally inhibited by *APP-BP1* (29). Chromosomal alterations in 18q21.1-23, a locus encoding for the established HNSCC tumor suppressor *SMAD4*, have been reported as lost in HPV$^-$ HNSCC and gained in HPV$^+$ HNSCC (17). Together with *SMAD2* and *SMAD3*, *SMAD4* is involved in mediating growth inhibitory effects of *TGFβ* in HNSCC (2). Therefore, loss of *SMAD4* may lead to abrogation of *TGFβ* signaling, thereby potentiating increased cell proliferation, cell survival, and apoptotic evasion (2). Focal amplifications in 20q, encoding for *E2F1*, were more commonly observed in HPV-associated HNSCC (19, 22). Additional chromosomal aberrations in HNSCC that may vary based on HPV status but require further validation involve aberrations in 7q, Xp, 8p, 11q22 (candidate gene: *ATM*), 13q, 14q32 (candidate gene: *TRAF3*), and 15q (19, 22, 26–28), whereas gains in 8q, 9q and 20p, and 11q14 losses may be common to both subtypes (17, 22). In summary, analysis of global chromosomal alterations support the presence of at least two distinct subtypes of HNSCC, HPV$^+$ and HPV$^-$, which follow unique routes of genetic progression. The genetic drivers associated with many of the aforementioned aberrations await identification and functional characterization.

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Table 1. Molecular differences between HPV$^+$ and HPV$^-$ HNSCCs: comparison of the various "omics" technologies

Marker	Enriched in (+) or (−) subgroup	Chromosomal locus	Genetic	Epigenetic	Transcriptomic	Proteomic	Reference
Cell cycle							
TP53	−		mu				(22, 28, 31, 32, 34)
E2F1	+	20q	↑↑				(19, 22)
E2F targets (MCMs, CDC2/7, CCNA1, CCNE1)	+				↑	↑	(41)
CCND1	−	11q13	↑↑		↑		(17, 22, 28, 41)
CDKN2A	−	9p21	↓↓, mu	me	↓	↓	(7, 17, 28, 32, 41)
PCNA	+				↑	↑	(41, 51, 52)
APP-BP1	+	16q22	↓↓		↓		(26, 29)
miR family "miR15a, miR16, miR195, miR424 and miR497"	+				↑[a]		(42)
RASSF1A	−	3p	↓↓				(2, 17, 19, 26)
FHIT	−	3p	↓↓				(2, 17, 19, 26)
Receptor tyrosine kinases							
EGFR	−	7p12	↑↑, mu				(22, 28)
FGFR1	−	8p11	↑↑				(22, 28)
FGFR2/3	+		mu				(22, 28)
PI3K pathway							
PIK3CA	+	3q26	↑↑, mu		↑		(7, 22, 27, 36)
PIK3R1	+		mu				(22, 36)
MAPK pathway							
KRAS	+		mu				(28)
HRAS	−		mu				(22)
TGFβ pathway							
SMAD4	+ and −	18q21	↓↓ and ↑↑				(17)
Immune response							
TRAF3	+	14q32	↓↓				(22)
IFN-induced genes (IFIT1, IFITM1-3, IFI6-16, OAS2)	+				↓		(46)
IL6	+					↓	(50)
IL10	+				↓		(46)
IL13	+				↓		(46)
Immunoglobulins	+					↓	(51)
Lactotransferrins	+					↓	(51)
Lymphocyte activation induced (HLA-DRA, HLA-DRB1/3/5, CSK, ICAM1)	+					↑	(52)
JAK–STAT pathway							
JAK3, STAT5A	+			me			(40)
DNA repair or recombination							
ATM	+	11q22	↓↓				(28)
BRCA1/2	+		mu				(28)
Testis-specific genes (SYCP2, TCAM1, STAG3)	+				↑		(7, 41, 46)
SMG1	+			me			(37)
EMT							
Cadherin family members (CDH8/15, PCDH8-10, PCDHB3)	+			me			(39)
Tissue development and regeneration							
ALDH1A2	−			me			(38)
OSR2	−			me			(38)
GATA4	+			me			(38)
GRIA1	+			me			(38)
IRX4	+			me			(38)
Other							
FADD	−	11q13	↑↑				(22)
CTTN	−	11q13	↑↑		↑	↑	(22, 41, 52)
miR363	+				↑		(42, 43)
N/A[b]	−	5q	↓↓				(17, 19, 26)
N/A[b]	+	7q	↑↑				(27)
N/A[b]	+ and −	8p	↑↑ and ↓↓				(19)
N/A[b]	−	13q	↓↓				(19)
N/A[b]	−	15q	↓↓				(26)
N/A[b]	−	Xp	↑↑				(26)

NOTE: Chromosomal location for gained or deleted regions is indicated. ↑↑, gain or amplification; ↓↓, loss or deletion; ↑, upregulated; ↓, downregulated; mu, mutated; me, promoter methylated; (+), HPV+; (−), HPV−.

[a]miR15a and miR16 are upregulated in HPV$^+$ oropharyngeal cancer, whereas miR195 and miR497 are downregulated.

[b]No single molecular markers were mentioned in association with these aberrations.

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Mutational Landscape

Exome sequencing studies have contributed greatly to our understanding of the mutational landscape of HNSCC and revealed untapped opportunities for personalized therapy (reviewed in detail in ref. 18). Initial studies revealed that HNSCC has a relatively significant mutational load, ranking ninth highest among tumors from 27 anatomical sites (30). In concordance with chromosomal aberration patterns, HPV$^-$ or tobacco-associated HNSCCs were found to contain significantly higher number of mutations versus HPV$^+$ HNSCC (28, 31, 32). Two landmark whole-exome sequencing studies revealed a 2- to 5-fold increase in mutation rates between HPV$^-$ and HPV$^+$ HNSCC (31, 32). Moreover, mutational profiles of HPV-negative HNSCC resemble those of smoking-associated cancers, including lung and esophageal SCCs, and involve frequent transitions and transgressions at CpG sites (22, 28, 33). Meanwhile, HPV-positive HNSCCs reflect mutational profiles of cervical cancers; they both commonly contain Tp*Cp(A/C/T) substitutions caused by HPV-induced *APOBEC3B* cytosine deaminase activity (22, 28, 30, 33).

Exome sequencing confirmed the complexity of mutational profiles and validated the prominent role of disrupted p53 and Rb tumor suppressor pathways as driver events in HNSCC. At an occurrence of approximately 70% in HPV$^-$ HNSCC, *TP53* mutations are by far the most common; however, they are rare in HPV-associated HNSCC as p53 is targeted by an alternate route through *E6* (22, 28, 31, 32, 34). *TP53* mutations are associated with poor patient outcome and inferior therapeutic response (2, 18). Importantly, wild-type p53 reactivation in response to radiation leading to cell death has been shown to play a role in the increased sensitivity of HPV$^+$ cells to RT (35). Gene therapy approaches that restore wild-type p53 expression demonstrated safety and efficacy and may serve as viable treatment modalities for HPV$^-$ HNSCC (18). In addition, *CDKN2A* was found to be mutated in approximately 21% of HNSCC, but the inactivation rate was approximately 80% due to other mechanisms that include chromosomal deletions and promoter hypermethylation (28, 32). The consequence of p16 inactivation is cell-cycle progression via disruption of the Rb pathway.

As compared with HPV$^-$ tumors, HPV$^+$ HNSCCs have higher mutation frequencies in a number of clinically relevant pathways, including components of the PI3K pathway (*PIK3CA*, *PTEN*, *PIK3R1*), receptor tyrosine kinases (*FGFR2* and *FGFR3*), MAPK pathway (*KRAS*), and DNA repair genes (*BRCA1*, *BRCA2*; refs. 22, 28, 36). *PIK3CA* mutations were found in 37% of HPV$^+$ versus 18% of HPV$^-$ HNSCC (22, 36). It is the most commonly mutated oncogene in HNSCC and occasionally the only altered oncogene in some HPV-associated HNSCCs, rendering it as a highly-actionable target in HNSCC (36). Administration of *mTOR/PIK3CA* inhibitor in HPV$^+$ and HPV$^-$ HNSCC patient-derived xenografts expressing mutant *PIK3CA* led to tumor growth inhibition and abolished PI3K signaling while no effect was observed in patient-derived xenografts with wild-type *PIK3CA*, indicating the potential for *PIK3CA* mutation status to serve as a biomarker for patient selection toward *mTOR/PI3K* inhibitors. Furthermore, alterations in DNA damage repair genes may be associated with increased susceptibility of HPV-associated HNSCCs to RT (28). On the other hand, HPV$^-$ HNSCCs appear to be enriched for *EGFR* and *HRAS* mutations (22, 28). In summary, HPV$^+$ and HPV$^-$ HNSCC potentially harbor both common and unique actionable targets; preclinical studies validating their therapeutic relevance are highly anticipated.

Epigenetic Profiles

Epigenetic modulation of gene expression plays a critical role in tumorigenesis and is particularly therapeutically attractive due to its potential amenability to pharmacologic reversal. However, the epigenetic changes induced by HPV in the tumor genome have been largely uncharacterized. Gubanova and colleagues demonstrated a causative role between HPV oncoprotein expression and promoter DNA methylation of the DNA damage response gene, *SMG1* (37). *SMG1* promoter hypermethylation was associated with increased radiation sensitivity in HNSCC cell lines and improved outcome in patients with HNSCC, underscoring that epigenetic regulation may be a significant contributor to the clinical behavior associated with HPV status (37). Global DNA methylation profiling revealed that HPV$^+$ HNSCC is a unique molecular entity that exhibits hypermethylation in relation to HPV$^-$ tumors (22, 38, 39). Ectopic expression of *E6* but not *E7* in HNSCC cell lines led to global hypermethylation, pointing to *E6* as the primary effector of methylation in the host genome, although the mechanism remains unknown (39).

Key gene families found to be differentially modulated in HPV$^+$ versus HPV$^-$ HNSCC through epigenetic silencing include cell-cycle genes (e.g., *CDKN2A*, *CCNA1*), JAK–STAT pathway genes (e.g., *JAK3*, *STAT5A*), cadherin family members (e.g., *CDH8*, *CDH15*, *PCDH8-10*), and tissue development and regeneration genes (38–40). Hypermethylation of cadherin family members may be indicative of HPV-mediated tumorigenic progression via epithelial-to-mesenchymal transition (EMT; ref. 39). In this study, two potentially prognostic subgroups of HPV$^+$ HNSCC were identified, one of which was characterized by a potential CpG island methylator phenotype (CIMP), indicating heterogeneity in this subgroup. CIMP is defined by DNA hypermethylation in CpG-rich promoters typically associated with "epigenetic instability" and improved outcome, although in this exceptional case, CIMP in HPV$^+$ HNSCC was associated with worse outcome (39). In another study, promoter methylation signature of five tissue development genes (*ALDH1A2*^{low}, *OSR2*^{low}, *GATA4*^{high}, *GRIA1*^{high}, and *IRX4*^{high}) has shown striking independent prognostic utility across three independent patient cohorts, suggesting that this could be utilized to classify treatment responders in oropharyngeal cancer (38).

In summary, significant differences in DNA methylation patterns and their potential prognostic utility underscore the importance of examining chromosomal modifications as the second major arm of epigenetic regulation. Furthermore, the mechanism by which the virus exerts epigenetic changes in the host genome, and relation to differences in clinical behavior requires further elucidation. Finally, it remains unclear as to whether pharmacologic reversal of epigenetic silencing is a viable therapeutic option in HPV$^+$ or HPV$^-$ HNSCC.

Transcriptional Profiles

Global mRNA and miRNA characterization of primary HPV$^+$ and HPV$^-$ HNSCCs has led to identification of differentially regulated pathways, discovery of potentially prognostic patient subgroups or molecular signatures, and determination of HPV-associated genes/miRNAs. Comparison of mRNA and miRNA

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expression profiles of HPV$^+$ HNSCC, HPV$^-$ HNSCC, and cervix cancer revealed that HPV$^+$ HNSCC is more closely associated with cervix cancer than HPV$^-$ HNSCC (41, 42). Cell-cycle and DNA replication genes were commonly upregulated among HPV$^+$ tumors, whereas epidermal development/cell differentiation and hormone activity genes were downregulated (7, 41). Differential abundance of cell-cycle genes reflected the consequences of *E7*-mediated inhibition of pRB such as induction of *E2F* transcription factor target genes, including multiple minichromosome maintenance genes, *CDC2*/*7*, cyclin E, *PCNA*, *CDKN2A*, and *CCND1*. Upregulation of *PCNA*, a proliferation marker, points to a higher proliferative state of HPV$^+$ tumors, thereby rendering them more susceptible to RT and anti-DNA replication chemotherapy (41). In accordance with gene expression studies, the miRNA family "miR15a, miR16, miR195, miR424, and miR497" with a reported role in cell-cycle progression and interaction with members of the p53 and pRB pathway, exhibited differential abundance in HPV$^+$ versus HPV$^-$ tumors (42). In addition, HPV-dependent upregulation of miR363 was consistently observed in patient specimens and cell lines (42, 43). Other potentially HPV-associated miRNAs were miR9, miR9*, miR34a, miR223, miR31, miR18a, and miR155, among others (44, 45). Considering the limited overlap between the results of miRNA profiling studies, added validation is definitely required. Furthermore, HPV$^+$ HNSCC and cervical cancers exhibited differential expression of testis-specific genes (*SYCP2*, *TCAM1*, *STAG3*) involved in promoting DNA recombination, potentially mirroring virally-induced genome instability (41). Also, downregulation of IFN-induced genes (*IFIT1*, *IFITM1-3*, *IFI6-16*, *OAS2*) and ILs (*IL10* and *IL13*) in HPV$^+$ HNSCC was evident, possibly reflecting impaired immune response that allows persistent HPV infection, shown to be a necessary step in cervical carcinogenesis (46).

Transcriptional profiles of primary HNSCC specimens segregate into at least three potentially clinically relevant subgroups (22, 47). Chung and colleagues were the first to classify HNSCC into four prognostic groups termed "classical," "basal," "mesenchymal," and "atypical" subtypes (47). The presence of these subgroups was corroborated in two independent cohorts, although not their prognostic utility; HPV-associated HNSCCs classified within the "atypical" subgroup characterized by absence of *EGFR* amplification (22, 48). In another study, analysis of publicly available microarray gene expression data for 371 HNSCCs resulted in three prognostic gene expression subtypes: "mesenchymal," "classical," and "basal," which were then validated in two independent cohorts (49). HPV$^+$ HNCs were equally proportioned between classical and mesenchymal subtypes, with the latter group experiencing significantly worse prognosis, characterized by enrichment in EMT markers (*MMP9*, *S100A4*, *VIM*), immune-related genes, and high levels HPV *E5* mRNA. Although the association between the two modes of classification of HNSCCs is unclear, both approaches highlight potentially clinically relevant molecular heterogeneity in HNSCC that extends beyond HPV status.

Proteomic Profiles

Global proteomic characterization of HNSCC in association with HPV status has only scratched the surface of the proteome-wide differences. Nonetheless, consistent with gene expression studies, HPV-dependent upregulation of proteins involved in cell cycle, DNA replication processes, and downregulation of epidermal development/cell differentiation pathways was corroborated, and downregulation of keratinization and extracellular matrix proteins was newly observed (50–52). Pertinent to clinical outcome, the proteomic studies highlighted potential radiation response mechanisms and the central role of the immune system. First, HPV$^-$ HNSCC expressed significantly higher levels of *CTTN* and respective activation of downstream transcriptional targets (52). *CTTN* can mediate radiation resistance in HNSCC via the β1-integrin/FAK/cortactin/JNK1 axis, suggesting a mechanism for increased radiation resistance in HPV$^-$ HNSCC (53). Interestingly, pharmacologic inhibitors for β1-integrin are in clinical development; it remains to be determined as to whether they have differential efficacy based on HPV status. Second, downregulation of the *IL6* proinflammatory cytokine in primary tumor specimens and sera from HPV$^+$ HNSCC patients was observed (50). This may facilitate immune escape in HPV-associated cancers; whereas relatively high *IL6* abundance in HPV$^-$ HNSCC may enable treatment resistance. In support of the notion of reduced immune surveillance, underrepresentation of inflammatory pathway proteins, immunoglobulins, and lactotransferrins has been reported in HPV$^+$ versus HPV$^-$ oropharyngeal cancer (51). Third, proteins induced in response to lymphocyte activation appear to be enriched in HPV$^+$ versus HPV$^-$ oropharyngeal cancer (52). These observations point toward differences in the tumor microenvironment that may influence clinical behavior. Indeed, increased tumor-infiltrating lymphocyte levels have been ascribed to HPV$^+$ status as well as improved outcome in oropharyngeal cancer (54). Taken together, proteomic investigations appear to reflect the histopathology of HPV$^+$ and HPV$^-$ HNSCC and provide valuable hypotheses for further mechanistic exploration in association with clinical behavior.

Summary and Future Perspective

Multidimensional molecular investigations of HNSCC have led to improved understanding of molecular landscapes, identification of targetable pathways and promising biomarkers, and potential for classification of HNSCC into clinically relevant subgroups. It has been consistently shown that lung squamous and HPV$^-$ HNSCCs share molecular features, whereas HPV$^+$ HNSCCs share similarities with cervical cancers, suggesting opportunities for knowledge translation (55). Furthermore, the complementarity among the various "omes" of HNSCC appears to offer an unparalleled opportunity for integration to achieve improved patient classification. In fact, a recent study has demonstrated the exquisite prognostic utility of 3p loss and *TP53* mutations to classify HNSCCs (56, 57). Underrepresentation of oropharyngeal cancers, particularly HPV$^+$ tumors, is one potential caveat of these molecular investigations. Oropharyngeal cancers appear to be associated with unique biology from other HNSCC subsites in which the etiologic role of HPV is not well established. Therefore, it is necessary to expand the analyses focusing on carefully selected oropharyngeal cancer cohorts as well as to standardize techniques for HPV status determination across studies. Additional future challenges include the systematic validation of the functional relevance of the aforementioned molecular differences on tumor progression, particularly within the scope of treatment modalities for HNSCC. The lack of appropriate *in vitro* and *in vivo* models with genetic backgrounds that reflect primary tumors poses as a potential obstacle. To address these issues, much work is underway to develop

patient-derived xenograft models (58). Proteomics studies have been underrepresented in characterization of oropharyngeal cancer; however, as proteins represent cumulative effects of all upstream genomic aberrations and can provide indication of protein function, signaling, and cellular localization, their further application is warranted in the elucidation of clinical phenotypes (52). Finally, it is important to bear in mind that the molecular spectrum of HNSCC reflects strong influences by what appear to be significantly different tumor microenvironments in HPV^+ versus HPV^- tumors, which likely also play a critical role in therapeutic response, and undoubtedly require further interrogation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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