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Therapeutic Implications of the Genetic Landscape of Head and Neck Cancer

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Large-scale sequencing studies of head and neck squamous cell carcinoma (HNSCC) have elucidated the genetic changes that characterize HNSCC. These findings have supported the development of therapeutic strategies that target key components of aberrant signaling pathways and immune dysregulation. Cumulative evidence suggests that these agents in combination with radiotherapy may have synergistic effects. This review highlights the predictive biomarkers that have been identified from HNSCC genomic studies and implications on the development of molecular-targeting agents that may effectively treat patients with HNSCC, especially when used in combination with radiation.
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Introduction

Head and neck squamous cell carcinoma (HNSCC) encompasses all cancers arising in the mucosa of the oral cavity, pharynx, and larynx. HNSCC is the sixth most common cancer worldwide and accounts for more than 600,000 new diagnoses annually as a consequence of tobacco and alcohol use, and human papillomavirus (HPV) infection.[1] Even with the current standard of care involving surgery, radiation, and chemotherapy, the 5-year mortality rate stands at approximately 50%.[2] In view of these rather modest survival outcomes, even following invasive and radical treatment modalities, the development of molecular-targeting agents for the treatment of HNSCC has garnered considerable momentum. Until recently, cetuximab, a monoclonal antibody against the epidermal growth factor receptor (EGFR), was the only molecular-targeting agent available for HNSCC. In 2016, the FDA approved the use of programmed death receptor-1 (PD-1) blocking antibodies, nivolumab and pembrolizumab, thus expanding the HNSCC treatment options. The identification of additional therapeutic agents may further revolutionize treatment and ultimately improve survival outcomes in HNSCC patients.

Leveraging information from elucidating the genomic landscape of HNSCC may also guide the development of more effective therapies to enhance radiation therapy for the treatment of HNSCC.[3] Although there have been significant advances in radiation technologies, such as the introduction of intensity modulated radiation therapy (IMRT), the effect of specific genetic alterations in HNSCC on response to radiotherapy has not been intensively investigated. Several studies first identified EGFR as a potential target for radiosensitization by demonstrating that cancer cells exposed to radiation expressed increased levels of EGFR.[4] In comparison to radiation alone, administration of the anti-EGFR monoclonal antibody cetuximab with concomitant high-dose radiotherapy improved locoregional control and reduced mortality.[5] Subsequent introduction of IMRT dramatically optimized radiation treatment modalities for HNSCC treatment by decreasing long-term side effects (eg, xerostomia and dysphagia) and extending cancer-specific survival in comparison to non-IMRT.[6-8] For example, in a prospective study of 73 patients with oropharyngeal cancer, combination chemotherapy (carboplatin and paclitaxel) with IMRT resulted in enhanced locoregional control of tumor growth while sparing important swallowing structures to reduce posttherapy dysphagia.[9] However, IMRT has its own limitations as high-dose volumes continue to correlate with chronic dysphagia leading to nutritional deficiencies, and higher risk for aspiration, anxiety, and

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depression.[10,11] In light of this, researchers discovered that reducing IMRT dose-volumes from 61-64 Gy to 52-55 Gy resulted in fewer swallowing disturbances.[7,12,13] A subsequent study indicated that patients with HPV-positive tumors who responded well to induction chemotherapy had favorable outcomes by combining cetuximab with reduced-dose IMRT ≤54 Gy (2-year progression-free survival = 96%, overall survival (OS) rate = 96%, swallowing difficulties = 40%, and impaired nutrition = 10%) vs cetuximab in combination with higher doses of IMRT, close to 70 Gy, in patients who did not achieve adequate response following induction (2-year progression-free survival = 80%, OS rate = 94%, swallowing difficulties = 89%, and impaired nutrition = 44%).[14] These results suggest that the addition of cetuximab to radiation in patients who responded well to induction chemotherapy may allow delivery of lower radiation doses with improved outcomes. However, concomittant administration of cetuximab and radiotherapy is not always curative, underscoring the need to identify other therapeutic agents that will improve HNSCC outcomes when combined with radiation.

This review will summarize our current understanding of the genetic alterations that characterize HNSCC, with a particular focus on aberrant signaling pathways and immunomodulatory mechanisms. We will highlight how this knowledge has direct implications for the development of therapeutic strategies to successfully treat these lethal cancers. We will focus on novel therapies in the context of radiation therapy with the ultimate goal of identifying genomic alterations that can serve as predictive biomarkers.

Genetic Characterization of HNSCC

The limited survival benefits of surgery, chemotherapy, and radiation have led to the design of alternative approaches to treat HNSCC. One potential strategy has been the identification and molecular targeting of aberrant signaling pathways that promote HNSCC development. Although targeted drug therapies have successfully been used in the treatment of other cancers, these approaches have met with limited success to date in HNSCC. This is due, in part, to a limited understanding of the genetic and biologic mechanisms that contribute to HNSCC pathology as well as the heterogeneity of HNSCC tumors (eg, anatomical location, clinical characteristics, and risk factors). The elucidation of the key "driving" genomic changes that enable tumorigenesis will facilitate the development of novel therapies for patients with HNSCC.

Over the last 6 years, landmark advancements in whole-exome sequencing and gene copy number analyses in primary patient tumors have revealed an extensive network of molecular changes underlying HNSCC.[15-17] In the first such studies, high-throughput next-generation sequencing of primary HNSCC tumors performed by two different groups identified the 6 most frequently mutated genes that may potentially encode key signaling molecules for HNSCC tumorigenesis: *TP53*, *NOTCH1*, *CDKN2A*, *PIK3CA*, *HRAS*, and *PTEN* genes.[15,16] Notably, *NOTCH1* is a novel gene linked to squamous cell differentiation that had not previously been reported as a commonly mutated gene in other solid tumor types.[15,16,18] Risk factors including tobacco use and HPV exposure also impacted the mutational rate observed in the samples.[15,16] For instance, tumors from patients with a history of tobacco use without evidence of HPV infection harbored 3.2-fold and 4-fold, respectively, more mutations than tumors from nonsmokers who were HPV-positive.[18]

In 2015, The Cancer Genome Atlas (TCGA) reported a robust integrative multiplatform characterization of 279 primary HNSCC tumors from a cohort consisting of male (73%) heavy smokers, with tumors derived from the oral cavity (62%), larynx (26%), and oropharynx (12%); approximately 13% of the tumors were also positive for HPV.[17] In addition to validating the previously identified frequently mutated genes, the TCGA study also profiled copy number alterations (CNAs), gene and protein differential expression, and epigenetic changes.[15,16,17] TCGA and earlier studies grouped genes into four broad categories including genes important for cell survival and proliferation (*TP53*, *HRAS*, *EGFR*, and *PIK3CA*), cell-cycle control (*CDKN2A* and *CCND1*), cellular differentiation (*NOTCH1*), and adhesion and invasion signaling (*FAT1*).[15,16,17,19] The top 2 mutated or altered genes from the TCGA cohort were *TP53* (72% mutated or 87% altered) and *CDKN2A* (22% mutated or 58% altered), with the top 10 mutations from this study presented in the Table; HPV-positive tumors, however, largely lacked mutations and alterations in *TP53* and *CDKN2A*.[17] Generally, HNSCC genomes displayed high instability, as indicated by the presence of CNAs (amplifications or deletions) and chromosomal fusions.[17] Both HPV-positive and HPV-negative tumors possessed recurrent focal amplifications of chromosome 3q26/28, a region containing *TP63*, *SOX2*, and *PIK3CA*.[17] *PIK3CA* mutations were also commonly found in both tumors types, albeit at higher levels in HPV-positive HNSCC.[15,17,20] Consistent with changes that had been previously recognized in lung squamous cell carcinomas, HNSCC exhibited copy number alterations such as deletions of chromosome 3p and 8p as well as amplifications of the 3q, 5q, and 8q chromosomal regions.[17,21] Among the top mutations seen in HNSCC genomes (Table), *TP53*, *CDKN2A*, *CASP8*, and *NSD1* were differentially mutated across all anatomic sites; unlike the other gene mutations, *CASP8* mutations were additionally concentrated within the oral cavity.[17] In the TCGA cohort, HPV-negative tumors (87%; 243 of 279 tumors) demonstrated unique DNA or RNA structural aberrations and somatic mutations.[17] Recurrent focal amplifications were evident in receptor tyrosine kinases (RTKs) (*EGFR*, *ERBB2*, and *FGFR1*), whereas focal deletions were present in the nuclear set gene (*NSD1*) and tumor suppressor genes (*FAT1*, *NOTCH1*, *SMAD4*, and *CDKN2A*).[17] Another group found that Notch activation and subsequent *FGF1* transcriptional upregulation increased mortality in patients with oral cavity HNSCC.[22] The TCGA cohort also displayed genetic alterations in oxidative stress regulators (*NFE2L2*, *KEAP1*, and *CUL3*).[17] Further, novel coamplifications of chromosome 11q13 (*CCND1*, *FADD*, and *CTTN*) and 11q22 (*BIRC2* and *YAP1*) were detected.[17] Somatic mutations such as inactivating, nonsynonymous mutations were notable