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Molecular Characterization of Head and Neck Cancer: How Close to Personalized Targeted Therapy?

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Abstract

Molecular targeted therapy in squamous head and neck cancer (HNSCC) continues to make strides and holds much promise. Cetuximab remains the sole FDA-approved molecular targeted therapy available for HNSCC, though there are several new biological agents targeting the epidermal growth factor receptor (EGFR) and other pathways in the regulatory approval pipeline. While targeted therapies have the potential to be personalized, their current use in HNSCC is not personalized. This is illustrated for EGFR targeted drugs, where EGFR as a molecular target has yet to be individualized for HNSCC. Future research needs to identify factors that correlate with response (or lack of one) and the underlying genotype-phenotype relationship that dictates this response. Comprehensive exploration of genetic and epigenetic landscapes in HNSCC is opening new frontiers to further enlighten, mechanistically inform, and set a course for eventually translating these discoveries into therapies for patients. This opinion offers a snap shot of the evolution of molecular subytping in HNSCC, its current clinical applicability, as well as new emergent paradigms with implications for controlling this disease in the future.

Keywords

Cetuximab; EGFR; HPV; DNA methylation; HNSCC

1. Introduction

Targeted cancer therapies are drugs or other substances that block the growth and spread of cancer by interfering with specific molecules involved in tumor growth and progression. Current conventional modalities of therapy in use for the treatment of squamous cell carcinoma of the head and neck (surgery, radiation and chemotherapy) are non-selective, cause damage to normal tissue and may be associated with systemic toxicity. The introduction of cetuximab ushered in the era of targeted therapy in the field of head and neck cancer, but while this targeted therapy has the potential to become a personalized treatment option, its current use in head and neck cancer is not personalized. Identifying appropriate molecular markers that correlate with or predict response to a given therapy will help personalize targeted and untargeted therapies alike. This opinion highlights the progress in molecular subtyping of HNSCC and the limitations and challenges of molecular targets as selective (targeted) therapies with the potential of becoming more personalized.

2. Background

The overwhelming majority of mucosal head and neck cancers are squamous cell carcinomas (HNSCC) [1] that primarily develop in the oral cavity, pharynx and larynx. Accurate and reliable stratification of HNSCC for prediction of outcomes has been

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challenging, mainly because of the numerous anatomic sites and sub-sites from which tumors can arise. HNSCC affect more than 500,000 people worldwide each year, accounting for 5% of all malignancies, and a gradually increasing rate over the last three decades[2]. In the United States, approximately 40,250 cases of oral cavity and pharynx HNSCC are expected in 2012 with an estimated 7,850 deaths [3].

HNSCC has a high mortality rate and despite considerable efforts, the 5-year survival rate has not changed significantly. Lymph node metastases and distant metastases are the most important predictors of prognosis[2]. Early stage (I and II) patients have a 60% to 95% chance of cure with local treatment alone, but for the two-thirds of patients who continue to present with locally advanced disease[1], the risk of recurrence or development of distant metastatic disease is greater than 50%[2]. For these patients, the 5-year survival rates are < 50% with severely reduced post-treatment quality of life[4].

In the absence of a single risk factor attributable to developing HNSCC, the two well studied important risk factors, tobacco and alcohol[5], are responsible for 72% of HNSCC cases[6]. More recent epidemiological and laboratory evidence indicate the human papilloma virus (HPV) as a causative agent for some HNSCC [7] and an independent risk factor for oropharyngeal cancer (OPSCC)[8]. The biologic significance of HPV as another independent risk factor, is underscored by the improved prognosis for patients with HPV positive HNSCC relative to HPV negative HNSCC[7, 8], due in part to a better therapeutic response to chemoradiotherapy[9].

Tumor HPV status has been shown to be the single strongest predictor, followed by measures of tobacco exposure and tumor stage[10]. Tobacco exposure has been associated with clinical trial outcome[11] and nicotine has been reported to reduce the cytotoxic effects of cisplatin and radiation of HNSCC cell lines[12]. The latter indicate that the most important risk factors for development of head and neck cancer have utility as predictors of response to therapy and patient survival and likely determine the molecular profile of this disease.

2.1 Molecular heterogeneity of HNSCC

2.1.1 Influence of race—To achieve personalized medicine, a better understanding of patient and tumor characteristics is a key attribute for patient selection. In HNSCC, molecular subtyping has highlighted the importance of accounting for the influence of racial differences. A significant clinically relevant characteristic of HNSCC is its marked disparate unfavorable diagnosis and prognosis outcomes for African Americans (AA)[3, 13, 14]. There is no consensus on the causes of the differences in the higher incidence of and the mortality from HNSCC for AA when compared to Caucasian Americans (CA), but they can include differences in access to care, stage at diagnosis, insurance status, attitudes of health providers, as well as HPV infection status[15]. Recent studies found that the poorer survival outcomes for AA versus CA with OPSCC were attributable to racial differences in the prevalence of HPV positive tumors. HPV positivity was higher in CA as compared to in AA and HPV negative AA and CA patients had similar survival outcomes[15, 16].

Another important consideration in assessing more accurately HNSCC racial disparities is the heterogeneity in the AA population due to population admixture[17, 18]. Ancestry informative markers (AIMs) to estimate the amount of population admixture can reduce potential confounding effects due to population admixture and control for heterogeneity in genetic studies in admixed populations like African Americans and Hispanic Americans[17] [18]. A recent study using AIMS to examine stage and survival outcomes in a primary HNSCC cohort, showed that only self-reported race as AA was associated with late stage[18]. Stratification within the AA group by West African genetic ancestry revealed no

correlation with stage or survival pointing to the causes of HNSCC disparities as likely due to social rather than biological factors[18]. Also, studies investigating the association of a broader spectrum of tumor and host factors, particularly in cohorts with an unusually higher proportion of AA indicated significant differences between AA and CA HNSCC for histopathology, treatment, smoking, marital status, type of insurance, as well as tumor gene copy number alterations[19]. These variables reiterate that for HNSCC as in the case of other complex diseases, tumor genetics or biology is only one of many potential contributors to differences among racial groups. Understanding and accounting for factors contributing to differences in HNSCC racial groups should provide much needed insights not only into disparities of incidence and mortality in AA, but also aid in the most efficacious application of molecular targets therapies.

2.1.2 Tumor heterogeneity—Tumor heterogeneity poses serious barriers to treatment of HNSCC. The extent of heterogeneity or lack of clonality of tumors is an important factor that underscores the biologic propensity of a cancer cell to persist, progress and metastasize. The latter has serious impact on treatment rationales and outcomes. In 1953, Slaughter and colleagues [20] introduced the concept of “field cancerization” as histologically altered epithelium surrounding tumor samples taken from the upper aerodigestive tract, with implications of an increased likelihood of concurrent or future disease in patients with head and neck lesions. They hypothesized that constant bathing of the epithelium with carcinogens results in multiple foci of transformed cells resulting in multicentric tumor development over the carcinogen-exposed mucosal surface. Molecular genetics support for field cancerization in synchronous primary cancer of the oral cavity was evident from the finding of different p53 (*TP53*) mutations in the right tonsillar pillar-soft palate tumor, and a left retromolar trigone tumor[21]. In studies of X chromosome inactivation in second primary cancers arising in women, Bedi and colleagues[22] demonstrated inactivation of the same allele in both tumors. Other evidence employing fluorescence in situ approaches suggests a monoclonal origin of second primary tumors[23]. Knowledge of whether a tumor has a single or multiple cell origin can provide important information about its etiology and pathogenesis with implications for most effective treatment outcomes.

2.2 Enhancing the HNSCC staging system

Given the ample evidence that tumor behavior is dependent on a complex interrelationship between the tumor and patient factors, the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) {AJCCUICC} TNM staging system used for staging HNC worldwide[24][2], has been periodically revised not only to incorporate information available from advances in diagnosis (e.g., endoscopy and radiologic imaging) but also from improved understanding of the biologic behavior of the numerous tumors that occur in this anatomic area[2].

The highly disfigurative nature of HNSCC surgical treatment and typically repeated exposure to high-dose radiation due to over- or under-treatment highlight the flaws in this clinicopathological staging method with respect to the balance between risk of treatment toxicities from surgery and radiation versus the need for adequate tumor control. This underscores the urgent need for identification of primary HNSCC tumors with enhanced metastatic potential by molecular means to aid clinicians in tailoring appropriate treatment strategies, especially in cases that have no apparent nodal involvement. Improvements in our ability to diagnose, evaluate, and stage patients would improve management and offer individualization of treatment.

3. Molecular subtyping of squamous head and neck cancer

The greatest advances in understanding the origin and progression of cancer during the past decade have occurred in the field of molecular genetics. Genomic instability, a hallmark of malignant transformation, promotes a wide range of mutations, including chromosome deletions, gene amplifications, translocations and polyploidy[25]. More recently, gene transcriptional inactivation via hypermethylation at CpG islands within promoter regions without changing the DNA sequence has been shown to lead to transcriptional repression akin to other abnormalities such as a point mutation or deletion [26] and is an important mechanism in cancer including HNSCC[30]. HPV infection has added another dimension to the molecular pathogenesis of HNSCC tumors.

Knowledge of genetic, epigenetic, and viral mechanisms that drive cancer growth and development can provide better diagnostic and prognostic information as well as more appropriate selection of therapy. Dissecting out processes specific to the pathogenesis of malignancy has distilled several genomic markers and profiles of HNSCC etiology, transformation, and progression.

3.1 Genetic alteration signatures in HNSCC

Cytogenetic analysis of HNSCC is well advanced in contrast to other solid tumors. The karyotype is typically very complex, but common features in SCC at one anatomic site are often very similar to SCC at other anatomic sites such as esophagus [27], skin[28], and vulva [29] irrespective of the initiating changes (tobacco and alcohol, human papilloma virus). These common changes strongly suggest that initiation, development, and progression of squamous epithelial neoplasia have common genetic pathways irrespective of anatomic site.

A universal class of cytogenetic change is deletions, also observed as loss of heterozygosity (LOH). Losses of segments of 3p, 5q, 8p, 9p, 10p, and 18q and gains of segments within 3q, 5p, 7p, 8q, distal 1q, and 11q13–23 are among the most common in head and neck cancers [30–36].

Fluorescence in situ hybridization (FISH) analysis of SCC[23, 37] and comparative genomic hybridization (CGH) studies in HNSCC [38, 39] have provided further genome-wide loci resolution that has paralleled classical cytogenetics[23, 28, 34, 37, 40–47].

Chromosome aberrations have clearly served as landmarks to identify cancer genes in many tumor types, however, individual gene loci altered in tumors cannot be deduced solely from the type of chromosome rearrangement[30]. Perturbations detected at the level of individual genes include structural mutation (with presumed altered function), gene amplification, and gene deletion or loss. Although many of these changes are relatively common, none is unique to HNSCC and none is found in all HNSCC. Investigations based on the polymerase chain reaction, gene sequencing for mutation detection, and recent genome wide search efforts to explore more thoroughly HNSCC genetic landscapes are beginning to provide considerable new information.

Mutations in the tumor suppressor gene *TP53*, encoding tumor protein p53, occur in 45 to 70% of HNSCC and strategies targeting the *TP53* gene and protein may halt or reverse the process of tumorigenesis[48]. Another important gene in HNSCC pathogenesis is *CDKN2A*, which is located at 9p21 and encodes cyclin-dependent kinase inhibitor 2A (also known as $p16^{INK4a}$). CDKN2A inhibits phosphorylation of the retinoblastoma protein (RB1) and blocks cell cycle progression at the G1 to S check point[49]. Loss of CDKN2A expression by deletion, mutation, or hypermethylation is common in HNSCC[34, 50] and is associated

with worse prognosis in some HNSCC[51]. CDKN2A overexpression, on the other hand, has been correlated with improved outcome in OPSCC[52]. This occurs as a result of functional inactivation of RB1 by the HPV E7 protein, resulting in the upregulation of CDKN2A[9]. Thus, HPV positive tumors are characterized by high expression of CDKN2A, indicating that CDKN2A positivity may be a biomarker for tumors harboring clinically and oncogenetically relevant HPV infections[9, 53].

The epidermal growth factor receptor (*EGFR*) gene is located at 7p12 and makes a 170-kD transmembrane glycoprotein[54]. It is a member of the receptor protein tyrosine kinase family with several extracellular growth factor ligands, including epidermal growth factor (EGF) and transforming growth factor (TGF)-α. Overexpression of EGFR is observed in 42%–80% of HNSCC studied [55, 56], and *EGFR* gene amplification occurs in up to 30% of HNSCC tumors[57, 58]. The majority of evidence suggests that increased EGFR expression and gene copy number are linked to poorer patient outcomes in HNSCC[59–62]. Quantifying EGFR and TGF-α protein levels in primary HNSCC may be useful in identifying subgroups of patients at high risk of tumor recurrence and in guiding therapy[55, 63, 64].

3.2 High-throughput strategies for gene biomarker discovery

Historically, the molecular pathogenesis of cancer has been teased out one gene at a time. Recent high-throughput genome-wide candidate strategies such as the Multiplex Ligation-dependent Probe Amplification (MLPA) assay[65] showed that loss or gain of genes concurred with chromosomal aberrations, and provide a novel index to estimate the extent of genomic abnormality with disease progression[30]. Genetic alterations that discriminate malignant and non-malignant tissue in HNSCC include a 16-gene signature spanning loci along 7 chromosomes: 3p21: *CTNNB1*, 3q27: *BCL6*; 4q26: *IL2*; 6p21.3: *BAK1* and *LTA*; 8p12: *FGFR*; 8q11: *PRKDC*; 8q24.12: *MYC*; 8q24.3: *PTP4A3*; 9p21: *CDKN2A*, *CDKN2B*; 11p13: *LMO2*; 11q13: *CCND1*, *FGF3*; and 21q11.1: *STCH*; 21q22.3: *TFF1*. Alterations of loss or gain at these gene loci support cytogenetic [23, 28, 34, 37, 40–47] and molecularly altered regions by LOH and array CGH studies in HNSCC [30–36, 38, 39] underscoring finely choreographed genomic instability events to achieve biological distinctiveness, providing clues to the drivers in invasive cancers as well as insight into gene rearrangements that might arise in non-malignant lesions.

The complexity and intricacies of molecular subtyping of HNSCC were recently highlighted utilizing whole-exome (protein coding genes) mutational profiling[66, 67]. These ground-breaking studies provide evidence that HNSCCs, although morphologically similar, are comprised of distinct diseases at the molecular level and that unraveling this heterogeneity is key to obtaining biological insights. In addition to *TP53* mutations, both groups[66, 67] reported mutations in genes involved in the differentiation pathway involving NOTCH 1. Tobacco exposure increased the number mutations compared to tumors with no tobacco exposure, and HPV expressing tumors had fewer mutations than HPV negative tumors, reiterating the importance of these risk factors in prognosis and treatment outcomes.

3.3 Epigenetic signatures in HNSCC

3.3.1 Epigenomics and Cancer—The study of human disease has focused primarily on genetic mechanisms. Dispelling the belief that the only way to treat such conditions is by fixing or replacing damaged genes, scientists are instead focusing on the field of epigenetics. Perhaps the best known epigenetic process, in part because it has been easiest to study with existing technology, is DNA methylation. This is the addition or removal of a methyl group (CH_3). Hypermethylation is a well described DNA modification that has been implicated in normal mammalian development, [68, 69] imprinting [70] and X chromosome inactivation

[71]. However, recent studies have identified hypermethylation as a probable cause in the development of various cancers [72–74]. Aberrant methylation by DNA-methyltransferases in the CpG-rich sequences ('CpG islands') of a gene's promoter region can lead to transcriptional repression akin to other abnormalities such as a point mutation or deletion [26]. Gene transcriptional inactivation via hypermethylation at the CpG islands within the promoter regions is an important mechanism [75]. This anomalous hypermethylation has been noted in a variety of tumor-suppressor genes, whose inactivation can lead many cells down the tumorigenesis continuum [75–78]. In many cancers, aberrant DNA methylation of CpG islands is associated with the inappropriate transcriptional silencing of critical genes [79–81]. These DNA methylation events represent an important tumor-specific marker occurring early in tumor progression and one that can be easily detected by PCR based methods in a manner that is minimally invasive to the patient.

3.3.2 Significance of DNA Methylation—When compared to the genome, which is identical in every cell and tissue in the human body, the epigenome is highly variable over the life course, from tissue to tissue and from environment to environment [82]. Also, unlike genes that are inactivated by nucleotide sequence variation, genes silenced by epigenetic mechanisms are still intact and, thus, retain the potential to be reactivated by environmental or medical intervention[82]. There are several current human therapeutic intervention trials to reverse deleterious epigenetic changes. Some examples include epigenetic therapeutic trials to treat T-cell lymphoma based on reactivation of tumor suppressor genes[83] and similar trials to prevent colorectal cancer by inhibiting the enzyme responsible for DNA methylation[84]. Such therapies have shown promise in halting tumor growth by reactivation of the tumor suppressor gene or by blocking progression of precancerous epigenetic lesions.

3.3.3 DNA Methylation in HNSCC—Gene silencing via hypermethylation is still a relatively new idea with regard to the development of HNSCC. Promotor hypermethylation of genes in HNSCC have been reported for *CDKN2A* (encodes p14 and p16), *DAPK*, *RASSF1A* [85–91], *RARB2* [92–94], *MGMT* [95], and E-cadherin (*CDH1*) [96]. In primary HNSCC promoter hypermethylation of *RARB* and *APC* in early-and late-stage tumors and of *CHFR* only in late-stage tumors suggested *CHFR* as a putative diagnostic biomarker for late-stage disease[92]. In a retrospective multi-ethnic primary laryngeal squamous carcinoma (LSCC) cohort, aberrant methylation of *ESR1* was an independent predictor of late stage LSCC[97]. DNA methylation patterns also have utility in determining whether a second tumor represents a recurrence of the original malignancy or a second primary cancer[98].

In benign papillomas, the high frequency of DNA hypermethylation events supports the utilization of gene silencing mechanisms as one of the driving forces behind their growth, reiterating DNA hypermethylation events as hallmarks of sinonasal and laryngeal papilloma pathogenesis, some of which are initiating clonal alterations in the recurrence continuum in some sinonasal[99] and recurrent respiratory papilloma (RRP) cases [100]. Aberrant methylation of *BRCA2, APC*, *CDKN2A* (p16) and *CDKN2B*, detected in the initial and all subsequent transformation biopsies in some RRP, appears to be an early event in the pathogenesis of laryngeal papillomatosis tracing a monoclonal progression continuum to SCC[78]. Epigenetic alterations identified in precancerous lesions with biomarker potential would have high clinical significance in risk assessment and early detection, and may also serve as molecular targets for chemopreventive interventions.

3.4 HPV

For HNSCC, epidemiological and laboratory evidence now warrant the conclusion that the human papilloma virus (HPV) is a causative agent for some HNSCC [7, 101] and an independent risk factor for oropharyngeal SCC[8, 102, 103]. A systematic review of 5046 patients with HNSCC reported an overall prevalence of HPV infection of 25.9% and concurs with a more recent meta-analysis of 5681 HNSCC[104]. The prevalence of HPV infection was significantly higher among patients with oropharyngeal SCC (35.6%) than among those with oral (23.5%) or laryngeal (24.0%) SCC [105]. Approximately 95% of these HNSCC subgroups contain high-risk HPV type 16 (HPV-16) genomic DNA sequences [106]. Its contribution to neoplastic progression is predominantly through the action of the viral oncoproteins E6 and E7 [107]. Expression of these proteins is sufficient for the immortalization of primary human epithelial cells and induction of histologic atypia characteristic of pre-invasive HPV-associated squamous intraepithelial lesions [108].

3.4.1 Characteristics of HPV positive and HPV negative HNSCC—Molecular subtyping has shown that HPV positive HNSCC differ from HPV negative HNSCC in several ways. HPV positive HNSCC have genetic alterations that are indicative of HPV oncoprotein function [106] and are characterized by wild-type TP53 [101, 109], wild-type CDKN2A (p16)[110], and infrequent amplification of cyclin D [111–113], whereas the converse is true for HPV negative HNSCC. High-risk types of HPV encode E6 and E7, two viral oncoproteins that promote tumor progression by inactivating two well-characterized tumor suppressor proteins, TP53 and RB1, respectively [107, 114]. Underphosphorylated RB1 plays an important role in the negative regulation of cell proliferation, causing cell cycle arrest in mid to late G_1 [115]. Wild-type TP53 acts as a cell cycle checkpoint after DNA damage and induces G_1 arrest or apoptosis, required to maintain genomic stability [116]. However, HPV-associated cancers generally do not exhibit *TP53* mutations [117–119]. In cervical carcinomas, where HPV is found in over 90% of cancer specimens [120], *TP53* is very rarely mutated [121]. A subset of HNSCC with *TP53* mutations rarely carries HPV, while tumors with E6-protein expression lack *TP53* mutations [117–119]. A consistent cluster of HPV16 DNA, wild-type p53, and lack of exposure to smoking. was reported for oral and oropharyngeal SCC [122]. By inactivating TP53 and RB1, E6 and E7 functionally disrupt the same cell cycle regulatory and DNA repair pathways that are frequently inactivated via genetic or epigenetic alterations during molecular progression of HNSCC [123]. HPV positive HNSCC also differ from HPV negative HNSCC in their patterns of allelic [117] and chromosomal [124, 125] loss and in their global gene expression profiles [111, 126].

The vast majority of HNSCC arise in smokers or chewers [101, 127]. These individuals are chronically exposed to high levels of chemical mutagens, and hence *TP53* mutations may act in conjunction with non-TP53-mediated mechanisms of HPV carcinogenesis [122]. The association of *TP53* mutations with tobacco and alcohol in HNSCC [128] and an absence of an association between tobacco or alcohol and HPV+ HNSCC suggests that these risk factors and HPV may act at the same step of stepwise carcinogenesis[129]. These observations provide support for the existence of at least two separate pathways for multistage carcinogenesis of HNSCC: one driven primarily by the mutagenic effects of tobacco and alcohol and the other driven by HPV-mediated transformation.

Recent data reveal that the biologic behavior of an HPV positive tumor may be altered by tobacco use. Genetic alterations induced by tobacco-associated carcinogens may render HPV positive tumors less responsive to therapy, with the likelihood of such genetic alterations appearing to increase as the number of pack-years of tobacco smoking increases[8]. Also, HPV appears to play a role in cell mediated immunity against the viral

tumor-specific antigens E6 and E7, contributing to improved patient prognosis. HPV transformed mouse tonsillar epithelial cell tumors were cleared after exposure to cisplatin or radiation only in immune competent mice[130]. Changes in HPV specific CD8+ and CD4+ T cells before and after therapy in human subjects are currently under investigation[131].

The biologic significance of HPV as another independent risk factor is underscored by the improved prognosis for patients with HPV positive oropharyngeal SCC (OPSCC) relative to HPV negative OPSCC[8, 101, 132, 133], due in part to a better therapeutic response to chemoradiotherapy[9]. HPV positive OPSCC has been noted as a distinct variant of HNSCC characterized by high prevalence of HPV infection, better patient outcome, nonkeratinizing histology, and overexpression of CDKN2A[134]. Currently, HPV status is the most valid and robust molecular diagnostic and prognostic biomarker to date for HNSCC[135]. Despite the fact that HPV positive HNSCC are more likely to be detected as late-stage cancers, survival has been shown to be better for patients with HPV positive HNSCC when compared with HPV negative HNSCC. This indicates that HPV can be used as a biomarker not only to help diagnose HNSCC, but alsoto stratify patients by risk and help direct treatment plans based on the disease behavior and prognosis[9].

4. Translational Research in HNSCC: Clinical applications for molecular targeted therapies

Molecularly targeted therapy is the application of molecular biomarkers as clinical diagnostic, prognostic, and treatment tools. Given the arsenal of molecular markers in HSNCC, their utility as targeted therapies to affect personalized treatment is an area of active investigation. EGFR is the only proven molecular target for HNSCC therapy. There are two different approaches to inhibit EGFR. One is the inhibition of its tyrosine kinase domain with a small molecule[136] to block the ATP binding domain of EGFR and second is inhibition of the extracellular ligand binding using monocloncal antibodies (mAbs) to specifically inhibit ligand binding. In general, EGFR targeted drugs inhibit cellular proliferation, survival, invasion and angiogenesis as well as act synergistically with chemoradiation therapies [137, 138]. An attribute of mAbs against EGFR is the induction of immune mediated antitumor processes such as antibody-dependent cellular cytotoxicity[139, 140]. The anti-EGFR mAb cetuximab is currently the only FDA-approved molecularly targeted HNSCC therapy.

Of the numerous potential EGFR targeted drugs, those that are currently in Phase III clinical trials in the US for HNSCC include the mAb panitumab and the tyrosine kinase inhibitors (TKIs) erlotinib, gefitinib and lapatinib. Two additional mAbs, zalutumumab and nimotuzumab, are currently in Phase III trials outside the US. Clinical development of TKIs have not progressed as well as mAbs[141]. This is illustrated for gefitinib, the first TKI to reach a Phase III investigation in HNSCC. However, due to recent study failures, gefitinib has been withdrawn from new drug consideration in the US.

Erlotinib, however, remains in active development for HNSCC because it has demonstrated encouraging results in several phase II studies[142, 143]. Phase III trials of erlotnib have been fraught with failures. Of the three phase III trials of erlotinib, two have been recently terminated (NCT00448240, NCT00412217)[141]. The third Phase III study NCT00402779) is an ongoing examination of erlotinib as a chemopreventative agent in high risk patients with previously treated oral intraepithelial neoplasia (IEN) without progression to cancer[141].

Unlike erlotinib and gefitinib, which are EGFR specific, lapatinib has dual specificity for EGFR and HER2, and is currently being evaluated in several phase II trials[144] in HNSCC with continued investigation as a phase III trial (NCT00424255).

In HNSCC, VEGF and VEGF receptor expression are associated with poor prognosis[145]. VEGF is up-regulated in HNSCC by hypoxic stimulation by hypoxia-inducible factor -1α (HIF1A)-dependent and independent processes, both of which involve phosphatidylinositol 3-kinase (PI3K) and AKT[146, 147]. Antiangiogenic therapies against the VEGF ligand include bevacizumab, a humanized mAb that binds and sequesters all five isoforms of VEGF[148], reducing the total amount of circulating VEGF. Sorafenib and sunitinib are multikinase inhibitors with specificity for a broad array of tyrosine kinases including VEGFR [149], and are currently under investigation for use in HNSCC[150–152].

The AKT pathway is a potential target for therapeutic intervention in HNSCC[153]. AKT, also known as protein kinase B, is a serine–threonine kinase activated by PI3K and PI4K under the influence of EGFR and HER3/ERBB3 activation [154]. Deletions in the phosphatase and tensin homolog (PTEN) and 'hot-spot' mutations of the *PI3K* gene lead to increased AKT signaling in HNSCC [155] contributing to the development and progression of HNSCC as well as resistance to radiation therapy and/or chemotherapy [147]. A downstream effector of AKT, the mammalian target of rapamycin (mTOR), a kinase, regulates cell growth, cell proliferation, cell motility, protein synthesis, and transcription[156]. Rapamycin, a potent inhibitor of mTOR, demonstrated synergistic effects with carboplatin and paclitaxel[157], resulting in inhibition of tumor growth, reduced angiogenesis, and the induction of apoptosis[155]. Presently, for renal cell carcinoma, the rapamycin analogs temsirolimus and everolimus are FDA approved as a first-line and second-line treatments, respectively[141]. Everolimus and temsirolimus are being evaluated in combination with other therapies in several phase II studies to treat HNSCC as well[141].

The contribution of HPV is of clinical significance because HNSCC patients whose tumors test positive for HPV have at least half the risk of death and respond better to treatment than those who test negative[8, 9]. This includes selection of patients for organ preservation therapy, which may be more successful in patients with HPV+ve HNSCC [158]. Also, a recent study found that poorer survival outcomes for African American (AA) versus Caucasian American (CA) with oropharyngeal tumors was attributable to racial differences in the prevalence of HPV positive tumors; HPV negative AA and CA patients had similar outcomes[159].

The rapid rise in the incidence of HPV-associated carcinoma of the oropharynx and its recognition as an etiological agent has prompted a re-evaluation of past trial outcomes and a call for HPV-specific studies to rigorously evaluate new prognostic factors and new treatment approaches with less morbidity. A Phase III clinical trial, RTOG 1016 (the randomized trial conducted by the Radiation Therapy Oncology Group (RTOG) is currently underway, in which HPV positive patients are randomized to receive biological therapy vs standard chemotherapy, concurrently with radiation[131]. The overall goal of this trial is to identify a less toxic approach in HPV-associated cancer of the oropharynx with the high survival currently associated with aggressive chemoradiation approaches. RTOG 1016 will test the hypothesis that targeted bioradiation will substantially reduce the burden of acute toxicity, result in faster recovery and return to function, carry lower rates of late effects, with similar rates of long-term survivorship, compared to conventional chemoradiation. RTOG 1016 is expected to provide significant knowledge with regard to epidemiological and molecular differences between HPV-positive and HPV-negative patients and identify predictors of response to radiotherapy, cisplatin and cetuximab chemotherapy. This landmark clinical trial will test the hypothesis that tobacco exposure is the strongest

predictor of overall and progression-free survival in patients with HPV-positive cancer; the risk of death is expected to increase per unit increase in tobacco exposure (measured in pack-years or years of smoking) and be independent of treatment assignment. It also presents a unique opportunity to identify distinct molecular biomarkers predictive of progression after treatment with cisplatin or cetuximab therapy.

Currently, the role of two preventative HPV vaccines, Gardasil and Cervarix, approved by the FDA for use in preventing cervical cancer, has not yet been evaluated in HNSCC. Two Phase I studies are examining the use of HPV-16 peptide epitopes in recurrent HPV-16 positive HNSCC (NCT00257738, NCT00704041)[141]. Vaccines targeted against HPV, in addition to a preventative role, may also have therapeutic applications via the induction of cell meditated immunity against HPV positive E6 and E7 expressing tumor cells[141]. Other strategies might employ small molecule inhibitors against either HPV E6 or E7 that could also potentially sensitize HPV positive tumor cells to other therapies and/or be used to treat premalignant lesions[141]. The drug discovery investigational pipeline includes screening techniques to search for compounds that can inhibit the protein–protein interactions of E6 and E7 [160, 161], blocking peptides specific for E6[162] and organic disulfide compounds that disrupt the zinc binding domains of E6[163], as well as peptide aptamers, which upon binding to HPV E6 protein can induce apoptosis in HPV positive tumor cells[164].

4.1 Tailoring nonselective conventional modalities (surgery, radiation, chemotherapy) using molecular targeted therapy

Cetuximab appears to have less toxicity than high dose cisplatin. Preclinical studies have shown that cetuximab enhances the cytotoxic effect of radiation in squamous cell carcinoma[165, 166] and offers proof-of-principle for selective tumor targeting in the treatment of locally advanced HNSCC. This has led to confirmational clinical trials showing an advantage in locoregional tumor control of radiation therapy given concurrently with cetuximab vs. radiation therapy alone[167, 168]. This combined regimen of cetuximab and radiation has an advantage over radiation therapy with concurrent standard chemotherapy (platinum-based chemoradiotherapy) in that it is well tolerated and considerably less toxic[131]. In platinum-refractory recurrent/metastatic HNSCC, the addition of cetuximab to 5- FU/platinum significantly improved overall survival, providing further clinical evidence that it is working via a pathway (or pathways) distinct from DNA damaging agents such as platins or radiotherapy [169, 170]

An analysis of associations between patient and tumor factors and overall survival (Bonner et., 2010)[168], illustrates how molecular subtyping is impacting patient selection. Patients in the oropharyngeal group demonstrated benefit from the addition of cetuximab to radiation, supporting the hypothesis that cetuximab may be preferentially beneficial to this group of patients presenting with clinical factors associated with an HPV associated HNSCC (EGFR expression but not HPV status was assessed)[168]. Also interesting is the observation of a paradoxical inverse association between HPV presence and EGFR expression, suggesting that EGFR expression may be associated with poor local-regional control only in the HPV-negative patient population[171].

Initial results of RTOG 0522 showed no survival benefits by the addition of cetuximab to chemoradiation treatment for patients with locally advanced HNSCC[172]. RTOG 0522 did not include prospective HPV testing (HPV analysis of 0522 expected in 2012), and is not expected to lead to a clear answer regarding best management in the HPV subset.

5. Limitations and challenges to tailoring molecular targeted therapies in HNSCC

EGFR gene amplification and protein overexpression are associated with an unfavorable prognosis but no predictive significance thus far[60, 62]. Thus, despite provocative evidence supporting the role of EGFR overexpression adversely influencing relapse and survival in HNSCC patients treated with surgery, chemotherapy, or radiation, clinical trials of EGFR targeted therapies have not consistently demonstrated a correlation between EGFR overexpression and the efficacy of EGFR targeted therapies[173].

As an alternative to identifying positive predictive markers of response to anti-EGFR agents, it may be more fruitful to look for negative predictive factors to identify EGFR-independent tumors that are not sensitive to EGFR inhibition. Emerging data suggest that *KRAS* mutations confer EGFR resistance across tumor types[174, 175] and that a hyperactive mutant *KRAS* is likely to be a powerful negative predictive factor of EGFR response. The RAS proteins, members of a large superfamily of guanosine-5′-triphosphate (GTP)–binding proteins, play a complex role in the normal transduction of growth factor receptor–induced signals[176]. RAS is downstream from EGFR and aberrant RAS signaling in cells with mutant *KRAS* can lead to dysregulation of RAS-dependent pathways and downstream signaling, even if the upstream receptor is silenced by anti-EGFR monoclonal antibodies. This is supported by studies that show a lack of benefit with cetuximab in patients with colorectal cancer (CRC) harboring *KRAS* mutations[177, 178]. In HNSCC, however, *KRAS* mutations are rare and show no association with response or resistance to *EGFR*-TKIs in HNCC[179].

Biomakers of response to chemoradiation in HNSCC have been disappointing. This is illustrated for excision repair cross complementing-group 1 (ERCC1), which plays a critical role in by the nucleotide excision repair (NER) pathway. ERCC1 dimerizes with xeroderma pigmentosum complementation group F to form a prerequisite complex for the successful excision of damaged DNA[180]. Cisplatin induced DNA intra-strand crosslinks are repaired by the NER pathway in cells[181]. Pre-clinical data suggest that increased *ERCC1* mRNA expression levels or ERCC1 protein expression levels correlate with cisplatin resistance in human cancer in ovarian, cervical, colon, testis, and lung cancer cell lines[181], where high levels of ERCC1 are associated with an increased rate of NER and reduced sensitivity to cisplatin, and low levels of ERCC1 are associated with higher platinum sensitivity. In HNSCC, there have been conflicting results regarding ERCC1 as a predictive marker for response and survival with platinum-based chemotherapy[182, 183]. In a recent larger study of HNSCC patients receiving concurrent cisplatin and radiation, ERCC1 expression was not a significant predictor of survival or response[184].

Conclusion

There is a general agreement that the selection of patients most likely to benefit from molecularly targeted therapies is not well established for HNSCC. Improved outcomes are attributable to advances in therapy as a result of a greater understanding of the molecular mechanisms underlying HNSCC pathogenesis. Although several biomarkers have been associated with prognosis for HNSCC patients (e.g. *TP53* mutations, EGFR expression, *EGFR* gene amplification), there are no biomarkers predictive of response to a specified therapy. The revelation that NOTCH1 [66, 67] functions as a tumor suppressor gene in HNSCC, unlike its role as on oncogene in several other cancers, has important implications for expanding the arena of newly identified targeted therapies for HNSCC patients. This is because the new generation of molecularly-targeted therapies, directed toward activated oncogenes, cannot directly target mutated tumor suppressor genes because they are already

inactivated. The realization of tumor suppressor gene predominance in HNSCC further complicates and limits accessibility and applicability of molecularly targeted therapies directed toward activated oncogenes, setting the stage for further work in the areas of target identification and therapeutics pertaining to personalized medicine.

Bolder and newer horizons for targeted therapies include the therapeutic control of cancers through epigenetic regulation, considered to be more amenable to fixing than irreversible genetic changes. In HNSCC, identification of epigenetic events of promoter hypermethylation is emerging as one of the most promising molecular strategies for cancer detection, and represents an important tumor-specific marker occurring early in tumor progression. The DNA methyltransferase inhibitors, azacitidine and decitabine, are two of a growing number of drugs designed to target epigenetic processes commonly deregulated during the development and progression of cancer. These demethylating agents are well tolerated and effective in low doses, and will continue to have clinical relevance as novel therapeutic interventions for cancer patients, including those with HNSCC.

As the body of molecular evidence grows for biological distinctiveness of tumor subtypes, such as the prognostic advantage of HPV positive over HPV negative HNSCC[9], this subset of HPV positive tumors is likely to be tested for response to agents directed against additional molecular targets such as the E6 and E7 proteins. Global characterization of the HNSCC methylome is beginning to uncover differential landscapes in HPV positive versus HPV negative tumors. Decreased genome-wide methylation has been found to be more pronounced in HPV negative HNSCC[185] and suggests additional treatment options for HPV positive tumors with demethylating drugs. Additionally, demethylating drugs in combination with therapeutic HPV DNA vaccines have been found to control more effectively a variety of HPV-associated malignancies[186]. This is due to the fact that DNA methylation is capable of decreasing expression of the encoded antigen of the DNA vaccines[186]. In fact, preliminary studies already suggest that there is promise of improving preventative HPV DNA vaccine therapy by the addition of the demethylating drug decitabine[186].

The promise of biologic therapy is to increase therapeutic gain: decreasing toxic effects on normal tissues while increasing tumor-specific effects[187]. Biologic therapy for HNSCC as the fourth major modality of cancer treatment (surgery, RT, and chemotherapy being the other three), exemplified by cetuximab, continues to gain momentum. However, as the sole targeted therapy for HNSCC currently, cetuximab is not individualized for patient treatment. Although tumor HPV status is predictive of improved survival, it is not an indication for selection of any particular therapeutic agent or modality. Personalized therapy for HNSCC patients continues to be hampered by the lack of proven biomarkers for predicting clinical outcomes and response to treatment as the development of prognostic tests has not progressed in conjunction with new therapies. Ongoing challenges for further research in molecular subtyping and personalizing therapy include availability of good quality tumor tissue for evaluation for molecular markers, and good clinical trial designs to incorporate biomarker correlates and to evaluate treatments in more homogeneous cohorts of patients as well as multi-ethnic, racially diverse cohorts for better elucidation of intra-patient and inter-patient tumor heterogeneity for patient selection.

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