HHS Public Access

Author manuscript

Semin Radiat Oncol. Author manuscript; available in PMC 2018 December 14.

Published in final edited form as:

Semin Radiat Oncol. 2018 January ; 28(1): 2–11. doi:10.1016/j.semradonc.2017.08.005.

Therapeutic Implications of the Genetic Landscape of Head and Neck Cancer

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Abstract

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 utilized in combination with radiation.

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 five-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.

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No disclosure statements.

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 impact 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 (ex. xerostomia and dysphagia) and extending cancer-specific survival in comparison to non-IMRT[6,7,8]. 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 post-therapy 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 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 rate=96%, swallowing difficulties=40%, impaired nutrition=10%) versus 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%, overall survival rate=94%, swallowing difficulties=89%, 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 utilized 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 (e.g. anatomic 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[15,16,17]. In the first such studies, high-throughput next-generation sequencing of primary HNSCC tumors performed by two different groups identified the six 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 that were HPV-positive[18].

In 2015, The Cancer Genome Atlas (TCGA) reported a robust integrative multi-platform 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 differentation (*NOTCH1*), and adhesion and invasion signaling (*FAT1*)[15,16,17,19]. The top two mutated and/or altered genes from the TCGA cohort were *TP53* (72% mutated/87% altered) and *CDKN2A* (22% mutated/58% altered), with the top 10 mutations from this study presented in Table 1; 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 copy number alterations (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 1), *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/RNA structural aberrations and somatic mutations[17]. Recurrent focal amplifications were evident in receptor tyrosine kinases (*EGFR, ERBB2*, and *FGFR1*),

while 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 co-amplifications of chromosome 11q13 (*CCND1, FADD*, and *CTTN*) and 11q22 (*BIRC2* and *YAP1*) were detected[17]. Somatic mutations such as inactivating, non-synonymous mutations were notable in four primary genes: *CDKN2A, TP53*, and WNT signaling genes (*FAT1* and *AJUBA*)[17]. Genes involved in immune evasion (*HLA-A*) and chromatin remodeling (*KMT2D/MLL2*) also were found to be mutated to a statistically significant degree[17]. *CASP8*, a frequently mutated apoptosis gene, contained missense and other inactivating mutations in the death effector and caspase peptidase domains[17]. A subset of HPV-negative tumors, referred to as "M class" (driven by mutations but generally lacking CNAs), primarily originated in the oral cavity and were enriched for a novel three-gene constellation of wild-type *TP53*, and mutant *HRAS* and *CASP8*, which suggested a p53-independent tumorigenesis pathway[17,23,24]. Two HPV-negative tumors also possessed an alternative *MET* transcript with skipped exon 14, which had previously been shown to promote oncogenesis in non-small cell lung cancer[17,25]. Based on RNA-sequencing data, alternative splicing resulted in distinct mRNA transcripts for *TP63* and *kallikrein 12* (*KLK12*)[17]. Generally, HPV-negative HNSCCs were more broadly distributed among different anatomic sites, relative to HPV-positive tumors, and commonly occurred in the context of heavy alcohol and/or tobacco use[17].

HPV-positive tumors are clinically distinct from HPV-negative tumors[26]. HPV-positive tumors primarily originate in the oropharynx, specifically the tonsillar beds[26]. Patients with HPV-positive tumors tend to be younger and lack traditional risk factors such as alcohol and tobacco use, which may partly explain the improved survival outcomes associated with these tumors in comparison to their HPV-negative counterparts[26,27]. The presence of HPV viral DNA and expression of the HPV oncoproteins E6 and E7 that promote proteasomal degradation of p53 and Rb, respectively, characterize these tumors[26]. The TCGA data confirmed an earlier study that illustrated the absence of *TP53* mutations and *CDKN2A* alterations as well as a predominance of mutations and copy number alterations in genes encoding components of the PI3 kinase (PI3K) pathway in HPV-positive tumors[17,28]. In the TCGA cohort, loss of *TRAF3* (TNF receptor-associated factor 3) and amplification of the transcription factor *E2F1* additionally distinguished HPV-positive tumors[17]. *TRAF3* is a major regulator of the innate antiviral response against pathogens like HPV, acting by inducing type I interferon (IFN) production[29,30]. Although loss of *TRAF3* had been previously reported in hematological malignancies and nasopharyngeal carcinoma, the TCGA study first identified this mutation in HPV-positive HNSCC[17,19,31,32]. Unlike HPV-negative tumors, HPV-positive tumors noticeably lacked *RTK* amplification and *CCND1* amplification[17]. Two HPV-positive tumors in the TCGA cohort possessed *FGFR3-TACC3* fusion mutations that had been previously reported[17,33]. Beyond the actions of E6 and E7 viral oncoproteins, earlier studies identified cancers involving both integrated and nonintegrated forms of HPV, which displayed unique patterns of DNA methylation and consequential differences in human and viral gene expression[34]. In HPV-integrated HNSCC, viral DNA integration into the host genome has been commonly associated with alterations

in DNA copy number, mRNA transcript abundance, and both inter- and intra-chromal rearrangements[34]. For most HPV-positive tumors in the TCGA cohort, viral DNA genome integration primarily occurred in a single genomic location and also in association with host genome amplification[17]. However, a single driver mechanism for HPV integration did not exist[17].

Since HPV-positive tumors are traditionally associated with more favorable outcomes in contrast to HPV-negative tumors, a number of studies have evaluated the effects of treatment de-escalation for HPV-positive HNSCC through reduced radiation and/or chemotherapy doses[2,35,36]. According to a preclinical study, HPV-positive HNSCC cell lines displayed greater sensitivity to radiation via reduced DNA double-strand break repair capacity in comparison to HPV-negative cell lines[37]. Additionally, patients with HPV-positive tumors had higher two-year overall survival rates after treatment with induction chemotherapy followed by chemoradiation in comparison to patients with HPV-negative tumors[36]. Based on these observations, several ongoing clinical trials have aimed to formulate protocols for treatment de-escalation, including reduction of radiation doses, without compromising disease control[35,38,39]. In a phase 2 clinical trial of forty-three patients with favorable-risk, HPV-positive oropharyngeal squamous cell cancer, decreased radiation dose of 60 Gy (vs. standard doses of 70 Gy) and concurrent administration with weekly low-dose cisplatin (vs. high-dose cisplatin) resulted in extremely favorable outcomes (86% pathologic complete response) as well as a decrease in treatment-related side-effects[40]. Futhermore, thirty-three patients with locally metastasized HPV-positive HNSCC and absence of tumor hypoxia (a well-established poor prognostic factor in HNSCC) received reduced radiation doses of 60 Gy; these individuals responded with a 100% two-year locoregional control rate and a 100% overall survival rate[41,42]. A well-defined manner of identifying candidates for treatment de-escalation will be essential for successful implementation of radiation doses tailored to patients with HPV-positive HNSCC.

Gene expression subtypes of HNSCC

In addition to classifying HNSCC based on genetic alterations, histology, and tumor sites, HNSCC can also be categorized into gene expression subtypes with potential biological and clinical relevance[43,44]. Despite some variability in nomenclature in different studies, four distinct subtypes have been proposed: atypical, mesenchymal, basal, and classical[43,44]. The atypical subtype involves a large majority of HPV-positive tumors that lack gain-of-function of the 7p chromosome (a region containing the *EGFR* gene), which was consistent with previous observations associating HPV-positivity to low EGFR expression[2,44,45]. In contrast to the atypical subtype, the other subtypes exhibit gain-of-function of 7p[44,45]. Basal and classical subtypes are characterized by loss of the 9p region, which contains the *CDKN2A* gene (encodes p16, a known biomarker of HPV-oncoprotein function); loss of 9p is not observed in the atypical subtype[2,45,46,47]. Data from the TCGA data also segregates into the atypical (24%), mesenchymal (27%), basal (31%), and classical (18%) subtypes, which have been analyzed for specific somatic alterations[17]. Consistent with previous observations, the atypical subtype possesses an enrichment of HPV-positive tumors with activating mutations of *PIK3CA* and lacking in chromosome 7 amplifications[17]. The mesenchymal subtype involves high levels of alterations to innate immunity genes such as increased expression of

CD56, a natural killer cell marker, and low frequency of HLA I mutations[17]. The basal subtype includes *NOTCH1* inactivation, *HRAS-CASP8* co-mutation, co-amplification of 11q13/q22, and fewer alterations of chromosome 3q containing the *SOX2* gene[17]. The classical subtype is notable for *TP53* mutation, loss of *CDKN2A*, chromosome 3q amplification, alteration of oxidative stress genes (*KEAP, NFE2L2* or *CUL3*), heavy smoking history, and predominance of the larynx sub-site[17]. DNA methylation, microRNA (miRNA), and reverse-phase protein array data from the TCGA cohort demonstrated *NSD1* hypomethylation and loss-of-function mutations in addition to wild-type *NOTCH1* in the atypical and classical subtypes[17]. *NSD1* encodes the histone 3 Lys 36 (H3K36 methyltransferase) and is associated with DNA hypomethylation. Since the TCGA report, a subsequent study defined a subset of HNSCC that possessed novel recurrent mutations encoding p.Lys36 Met (K36M) alterations in H3 histone genes, which in addition to NSD1 mutations, corresponded to a specific DNA methylation cluster responsible for blocking cell differentiation and promoting oncogenesis[48]. Furthermore, deletion of newly-identified miRNAs let-7c-5p and 100–5p in HNSCC tumors led to increased expression of the following target genes: cell cycle regulator *CDK6*, transcription factor *E2F1*, mitosis regulator *PLK1*, and transcription factor *HMG2A*[17]. The miRNA let-7c was most often altered in HPV-negative tumors[17].

Epidemiologic considerations

A meta-analysis of HNSCC gene expression data validated by eleven independent microarray datasets has revealed six different subtypes by subdividing the aforementioned groups into classical, mesenchymal, HPV-like, hypoxia, defense response, and immunoreactive[49]. The six subtypes exhibit predicted differences in sensitivities to drugs in clinical use or under preclinical investigation for HNSCC, including paclitaxel, rapamycin, afatinib/pan-EGFR inhibitor, Nutlin3a, and Z-LLNle-CHO[49]. A recent study of twenty-eight HNSCC patient-derived xenografts (PDXs) distributed over three gene expression subtypes - mesenchymal/inflamed, basal, and classical - were subjected to treatment with various chemotherapy agents, including cetuximab[50]. The basal subtype strongly correlated with response to cetuximab whereas the mesenchymal subtype was cetuximab resistant[50]. No associations were evident with other subtypes and/or chemotherapy agents, suggesting that the basal subtype may be a predictive biomarker for cetuximab response[50].

It should be noted that the HNSCC TCGA study represented a typical US surgical cohort, largely limited to oral cavity tumors from white, male smokers[17]. Recent evidence incorporating additional genome-wide association studies have suggested that additional epidemiologic factors may impact HNSCC mutational landscapes. For example, a 2016 genome-wide association analysis of oral cavity and pharyngeal cancers in 6,034 cases and 6,585 controls from Europe, North America, and South America detected novel loci that were not previously highlighted in the TCGA study[51]. This study included a combination of oral, pharyngeal, and oropharyngeal cancers that unveiled significant associations with the genes *HLA-DQB1, LHPP, OR52N2-TRIM5*, *ADH1B*, *GPN1, LAMC3, CLPTM1L*, and *CDKN2B-AS1*; *CDKN2B-AS1* was the only gene also noted in the TCGA study[51]. Additionally, HPV-positive cancers had stronger gene loci associations than HPV-negative cancers[51]. In a separate study, tongue cancers from Asian patients exhibited lower *TP53*

mutational rates (10.6%), in stark contrast to the 86% mutation rate from TCGA data[17,31]. These findings support the notion that HNSCC mutations exhibit geographic and tumor heterogeneity that may lead to additional challenges in determining viable therapies for multiple tumor types and global populations.

Aberrant pathways implicated in HNSCC and subsequent therapeutic strategies

The HNSCC genomic landscape is highlighted by commonly mutated genes that may result in aberrant activation of signaling pathways that promote tumorigenesis. In the following section, we explore the consequences of oncogenic *PIK3CA* and *CCND1* genetic alterations, with a focus on their roles as potential biomarkers for targeted therapies.

PIK3CA

PIK3CA is a commonly altered (mutated and/or copy number amplified in 37% of the TCGA cohort) oncogene in HNSCC and is more frequently altered in HPV-positive tumors[17]. Approximately, 66% of HNSCC *PIK3CA* mutations cluster at three hotspots in the helical (E542K, E545K) and kinase domains (H1047R), which is consistent with the frequency of hotspot mutations found in other *PIK3CA*-altered tumors such as breast, lung, and colorectal cancers[17,20,52].

The phosphoinositol-3-kinase (PI3K) signaling pathway is frequently dysregulated in HNSCC, with PI3K-related gene mutations present in 30.5% of tumors[20]. Of the three different classes of PI3Ks, *PIK3CA* encodes the class I p110α subunit of PI3K that becomes activated by receptor tyrosine kinases (RTKs) such as epidermal growth factor receptor (EGFR)[52]. Activated class I PI3K (heterodimer of p85 and a p110 catalytic subunit) phosphorylates the lipid phosphatidylinositol 4,5-biphosphate (PIP_2) within the plasma membrane to generate phosphatidylinositol (3,4,5)-triphosphate (PIP_3)[52]. These events trigger a cascade of downstream signaling, including the activation of additional pathway components such as protein kinase B (Akt) and mechanistic target of rapamycin (mTOR) to promote cell growth, survival, motility, and metabolism (Figure 1)[52].

Due to the frequency of PI3K-AKT-mTOR pathway alterations in HNSCC, multiple preclinical studies and clinical trials have attempted to delineate the therapeutic efficacy of targeting various components of the PI3K pathway albeit with mixed results (Figure 1)[53]. A screen of 130 anti-cancer agents in 639 cancer cell lines listed *PIK3CA* mutations as significant biomarkers of sensitivity to drugs targeting the PI3K pathway in 23 HNSCC lines[54]. However, *in vitro* studies of HNSCC cell lines with *PIK3CA* amplifications, unlike *PIK3CA* mutations, were not sensitive to PI3K pathway inhibitors and did not predictably activate downstream components of the PI3K pathway such as Akt and mTOR[55]. HNSCC cells resistant to PI3K inhibition have also been classically associated with mutations within the PI3K pathway, thus implementing the incorporation of multi-targeted pathway inhibition[54]. Based on *in vivo* studies with HNSCC PDXs, *PIK3CA* mutations were associated with greater sensitivity to treatment with the dual PI3K/mTOR inhibitor (BEZ-235) in comparison to *PIK3CA* wild-type tumorgrafts[20]. A Phase 1 trial of patients

with *PIK3CA* cancers, including HNSCC, reported greater response to PI3K/AKT/mTOR inhibitors than those without *PIK3CA* mutations; this was especially true in patients with the hotspot *PIK3CA* mutation, H1047R, relative to other *PIK3CA* mutations[56,57]. However, to date, the cumulative results from i*n vitro, in vivo*, and clinical trials analyzing the utility of PI3K/AKT/mTOR pathway inhibition have been inconsistent[53].

Trials are now combining PI3K inhibition with chemotherapy and/or radiation in the context of encouraging findings with treatments of other cancer types[58]. Buparlisib (BKM120) is a pan-PI3K inhibitor (targets more than one PI3K p110 isoform) that has been extensively studied. Based on preclinical data, buparlisib sensitized squamous lung cancer cells to radiotherapy, especially previously radiation-resistant cells with *NEF2L2* or *KEAP1* mutations[59]. While monotherapy generally had comparable effects, treatment of three different HNSCC cell lines with cetuximab (anti-EGFR) followed by buparlisib synergistically inhibited tumor proliferation[60]. Another group uncovered a similar finding with HNSCC xenografts after incorporating radiotherapy in conjunction with buparlisib and cetuximab[61]. Currently, there are five ongoing clinical trials for buparlisib (BKM120), including one involving combined IMRT and cisplatin. Of note, additional pan-PI3K inhibitors include copanlisib (BAY 80–6946), which has not been effective in clinical trials, as well as PX-866 and SF1126[53]. One of the more promising PI3K inhibitors is alpelisib (BYL719), a p110α-specific inhibitor that has exhibited dose-dependent inhibition of *PIK3CA*-dependent murine xenografts[62]. Preliminary data from a Phase IB/II study of alpelisib in conjunction with cetuximab for the treatment of recurrent/metastatic HNSCC suggested potential antitumor activity of this regimen[63]. There are four active clinical trials with alpelisib underway, with two of the four trials incorporating combined IMRT. NCT02282371 is a clinical trial involving the treatment of Stage III/IVb HNSCC with triple therapy BYL-719, cetuximab, and IMRT. NCT02537223 involves triple therapy with BYL-719, cisplatin, and IMRT in patients with locoregionally advanced HNSCC.

Inhibitors targeting downstream components of the PI3K pathway, such as Akt (MK-2206) and mTOR (sirolimus/rapamycin, everolimus, temsirolimus), have reported limited efficacy in conjunction with toxicities, especially when combined with radiotherapy[53,64]. Although preclinical data supports targeting the PI3K pathway, the clinical benefit of agents that inhibit nodes in this pathway has been limited in *PIK3CA*-mutated patients to date[65]. Additional studies are needed to identify treatment strategies to increase the effectiveness of PI3K inhibition, especially in combination with radiation.

CCND1

Cell cycle control genes, which include *CCND1, CDKN2A*, and *CDK4/6*, are commonly mutated in HNSCC. In particular, nearly 31% of all HPV-negative tumors have mutations in the oncogene *CCND1* (encoding cyclin D1)[17]. Additionally, HPV-negative tumors are associated with novel co-amplifications of chromosome 11q13m (containing *CCND1, FADD* and *CTTN*) with chromosome 11q22 (containing *BIRC2* and *YAP1*)[17]. *CCND1* encodes cyclin D1, which enables cyclin-dependent kinases (CDKs) 4 and 6 to phosphorylate and inactivate RB (retinoblastoma)[66]. This eventually leads to the release and activation of E2F transcription, facilitating the transition of the cell from G1 to S phase[66].

P16 (*CDKN2A; cyclin-dependent kinase inhibitor 2A*) is a potent inhibitor of CDK4/6 that blocks the interaction of cyclin D1 with CDK4/6, preventing RB phosphorylation and inhibiting cell cycle progression[66]. Alterations in this pathway promote carcinogenesis and include amplification, which lead to overexpression of cyclin D1, amplification and activating mutations of *CDK4*, and loss-of-function mutations in p16 (*CDKN2A*)[67]. Notably, *CDKN2A* inactivation is commonly associated with smoking-related HNSCCs and present in 57% of HPV-negative tumors[17].

A recent study illustrated that *CCND1* amplification and a subsequent increase in cyclin D1 expression predicted occult nodal metastasis in early oral cancer, supporting *CCDN1* and CDK4/6 as potential biomarkers[68]. A preclinical study of the CDK4/6 inhibitor abemaciclib (LY2835219) demonstrated potent activity in HNSCC cells and xenografts when utilized in combination with an mTOR inhibitor, relative to either drug alone[69]. In 2015, the FDA approved palbociclib, a CDK4/6 inhibitor, for the treatment of breast cancer after studies indicated positive responses to this agent in patients with high rates of *CCND1* amplification[70]. Initial results from a Phase I trial of patients with cetuximab- or platinum-resistant HNSCC indicated that the combination of palbociclib and cetuximab safely reduced target lesions in 56% of patients and exhibited a disease control rate of 89%[71]. Given the small sample size of this trial (n=9), additional studies are needed to support the utility of this agent for the treatment of HNSCC. One study (NCT03024489) is testing palbociclib in combination with cetuximab and IMRT for the treatment of locally advanced squamous cell carcinoma.

Genomic-based radiation sensitivity of HNSCC

Genomic studies may additionally identify subpopulations of HNSCC patients with optimal responses to radiotherapy. Currently, the administration of radiotherapy for HNSCC treatment is based on a “one-size fits all” model with patients receiving standard doses of radiation irrespective of tumor heterogeneity. A handful of non-genomic based parameters have been used to predict HNSCC radiosensitivity, with fairly limited success. For example, analysis of components such as survival fraction at 2 Gy (SF2) following growth of HNSCC colonies *in vitro* predicted local control but not overall survival, while cell kinetic parameters measured through flow cytometry only weakly predicted response to radiotherapy[72,73]. However, a systems biology approach utilizing a gene expression-based radiosensitivity index (RSI) to estimate SF2 *in vitro* reliably predicted response to preoperative radiotherapy and clinical outcomes in patients with various cancers, including HNSCC[74,75]. More recently, the genomic-adjusted radiation dose (GARD), a value derived from RSI and the linear quadratic model (basis for dose and fractionation in clinical radiation oncology), effectively predicted the responses of multiple tumor types, including HNSCC, to radiotherapy[76]. Higher median GARD values predicted greater response to radiotherapy and also specifically correlated with oropharyngeal over non-oropharyngeal HNSCC; the latter finding was consistent with more favorable outcomes for patients with oropharyngeal cancer who underwent radiotherapy based on a prior study[76,77]. Evidence from this study implicates the utility of GARD in guiding genomically-based radiation therapy for HNSCC.

An analysis of 82 primarily (chemo)irradiated HNSCC patients confirmed previously acknowledged biomarkers of radiosensitivity and radioresistance (p16 and c-Met, respectively) and identified new prognostic biomarkers including survivin, programmed death-1 (PD-1), and programmed death-ligand 1 (PD-L1)[78]. Expression of p16, PD-1, and PD-L1 corresponded with radiosensitivity while survivin and c-Met predicted radioresistance[78]. Given the recent approval of PD-1 blocking antibodies for HNSCC treatment, this finding suggests a possible enhanced radiotherapeutic effect if these agents are supplemented with radiation. The optimization of radiation doses dictated by genomic differences between HNSCC will be necessary to effectively optimize precision radiation therapy in a clinical setting.

Immunotherapies

Overview

Another promising avenue for the treatment of HNSCC is immune checkpoint inhibition. In light of the 2016 FDA approval of anti-PD1 antibodies pembrolizumab and nivolumab, tremendous enthusiasm currently surrounds immune modulatory agents. Immune dysregulation has been commonly cited in tumorigenesis by its propagation of angiogenesis, invasion, and metastasis[79]. Impairment of the host immune system in HNSCC occurs via several mechanisms, including downregulation of human leucocyte antigen (HLA) class I molecules[80,81,82], development of T-cell tolerance to overexpressed antigens[82,83], inhibitory cytokine production[84], and increased programmed death ligand-1 (PD-L1)/programmed death-1 (PD-1) expression[85]. These studies have implemented the development of immune checkpoint inhibitors such as ipilimumab (anti-cytotoxic T-lymphocyte associated protein/CTLA-4), pembrolizumab (anti-PD-1), and nivolumab (anti-PD-L1)[79]. Cumulative evidence indicates that immune checkpoint inhibitors and radiotherapy have synergistic effects that enhance antitumor immunity by inducing immunogenic cell death and promoting recruitment of T cells within the tumor microenvironment[86].

CTLA-4 blockade

CTLA-4, a receptor commonly found on the surface of T-cells, downregulates helper T-cell activity and upregulates the immunosuppressive effects of regulatory T (Treg) cells[87]. In principle, CTLA-4 inhibition induces tumor regression by promoting T-cell activation against cancer cells and preventing Treg cells from inhibiting immune activity[87]. Although the survival benefits of the anti-CTLA-4 monoclonal antibody ipilimumab have been primarily demonstrated for metastatic melanoma, its role in the treatment of HNSCC has yet to be elucidated[88]. There is some evidence to suggest that blocking CTLA-4 may reverse treatment-resistant HNSCC. In the context of the limited survival benefits of cetuximab for HNSCC, one study noted that cetuximab-treated HNSCC patients exhibited increased Treg expression of CTLA-4 with higher levels of Treg correlating with poor prognosis[89]. Furthermore, ipilimumab notably reversed these effects *ex vivo* by eliminating intratumoral Treg cells through the assistance of natural killer (NK) cells, indicating a possible role for ipilimumab in enhancing treatment response to cetuximab[89]. Based on this study, the unremarkable response of HNSCC to cetuximab monotherapy may have been secondary to the recruitment of Treg cells to the tumor microenvironment, which subsequently suppressed

antitumor effects[89]. In preclinical models of lung cancer and melanoma, the addition of radiotherapy to CTLA-4 blockade demonstrated synergism likely secondary to radiation-induced T-cell release of antigens that boost the immune system and increase responsiveness to ipilimumab[86]. Abscopal effects (regression of metastatic cancer at a site distant from irradiation) were noted in patients with melanoma who were initially unresponsive to ipilimumab[86]. Several ongoing clinical trials are aimed at establishing use of ipilimumab in combination with IMRT and other molecular targeted agents, namely nivolumab, in HNSCC (ex. NCT01860430, NCT02741570, NCT01935921)

PD-1 or PDL-1 pathway blockade

There is considerable interest in the role of the PD-1/PDL-1 pathway in HNSCC. PDL-1 serves as one of two ligands for the PD-1 receptor, whose activation limits T-cell activity in peripheral tissue during inflammation, thereby restricting autoimmunity[87]. The recruitment of Treg cells expressing high levels of PD-1 to the tumor microenvironment may in turn further suppress effector immune responses[87]. HNSCC tumor cells exhibit high levels of PD-L1, with 66% of HNSCC expressing either membrane and/or intracytoplasmic PD-L1[87]. HPV-positive tumors are more commonly associated with higher levels of PD-L1 expression in comparison to HPV-negative tumors[90,91]. It is interesting to note that PD-L1 is upregulated after activation of the PI3K pathway in human glioma cell lines, suggesting a potential role for administering PI3K inhibitors in conjunction with PD-L1/PD-1 blockade[92].

In a Phase 3 trial of nivolumab in 361 patients with platinum-therapy resistant metastatic HNSCC, overall survival (OS) in the nivolumab group was 7.5 months (95% CI= 5.5–9.1) versus OS in standard treatment group of 5.1 months (95% CI= 4.0–6.0)[93]. Furthermore, nivolumab-treated patients had a 30% lower risk of death, more than double the estimated rate of OS at 1 year, and a higher rate of progression-free survival at 6 months (19.7% with nivolumab vs 9.9% w/ standard therapy)[93]. From the Phase 1b KEYNOTE-012 trial, 60 patients with PD-L1-positive HNSCC treated with pembrolizumab exhibited overall response (OR) by central imaging review of 18% (eight of 45 patients; 95% CI 8–32) in all patients, 25% (four of 16 patients; 95% CI 7–52) in HPV-positive patients, and 14% (four of 29 patients; 95% CI 4–32) in HPV-negative patients[94]. In cervical cancer, which is almost exclusively associated with HPV infection, novel amplifications in immune target PD-L1 were noted further, emphasizing the association between PD-L1 expression and HPV[95].

The utility of PD-L1 and PD-1 inhibition in combination with radiotherapy is the focus of several preclinical studies aimed at analyzing potential synergism between these two treatment modalities. The anti-tumor efficacy of low dose radiotherapy, which results in the upregulation of PD-L1 on tumor cells, can be improved by combining with antibodies against PD-L1 and PD1 in murine models[96]. In mice with intracranial gliomas, the administration of anti-PD-1 therapy alongside stereotactic radiation improved survival by 25–28 days when compared with either modality alone[97]. A syngeneic colon cancer model had similar improvement in outcomes when treated with dual anti-PDL1 and radiation therapies[98]. There are two ongoing clinical trials involving combination nivolumab with either stereotactic body radiotherapy (NCT02684253) or IMRT/cisplatin (NCT02764593).

Although additional supporting preclinical and clinical data are required in the context of HNSCC, the aforementioned studies underscore the potential utility of combining radiotherapy with immunotherapeutic agents

Conclusion

Despite increased understanding of the genetic underpinnings of HNSCC and the 2016 FDA approval of immune checkpoint inhibitors, approximately 50% of HNSCC patients still succumb to their disease. The incorporation of targeted agents such as cetuximab has not consistently resulted in significant increases in survival and predictive biomarkers are lacking. Genomic studies such as TCGA, among others, have contributed to a more comprehensive integrative genomic characterization of HNSCC. Additional sequencing studies have also identified HNSCC-mutated genes based on diverse worldwide cohorts. The development of novel drug therapies targeting frequently mutated candidate genes and their resultant aberrant signaling pathways may improve outcomes in oncogene-driven HNSCC. Promising candidate targets include PI3K/AKT, CDKs, and immune checkpoints. Additional studies aimed at understanding the effects of targeted therapeutic agents administered with radiation therapy will also dictate how these two modalities improve survival and reduce toxicity for HNSCC treatment. Successful completion of preclinical studies that can be readily translated to the clinic will have immediate impact on patient outcomes for HNSCC. The identification of alternative therapeutic targets will be critical to improve survival outcome in patients affected by this otherwise lethal diseae.

Acknowledgments

This work was supported by R01 DE24728 (to DEJ), R01 DE023685 and the American Cancer Society (to JRG) and Howard Hughes Medical Institute Medical Fellows Program (to JC)

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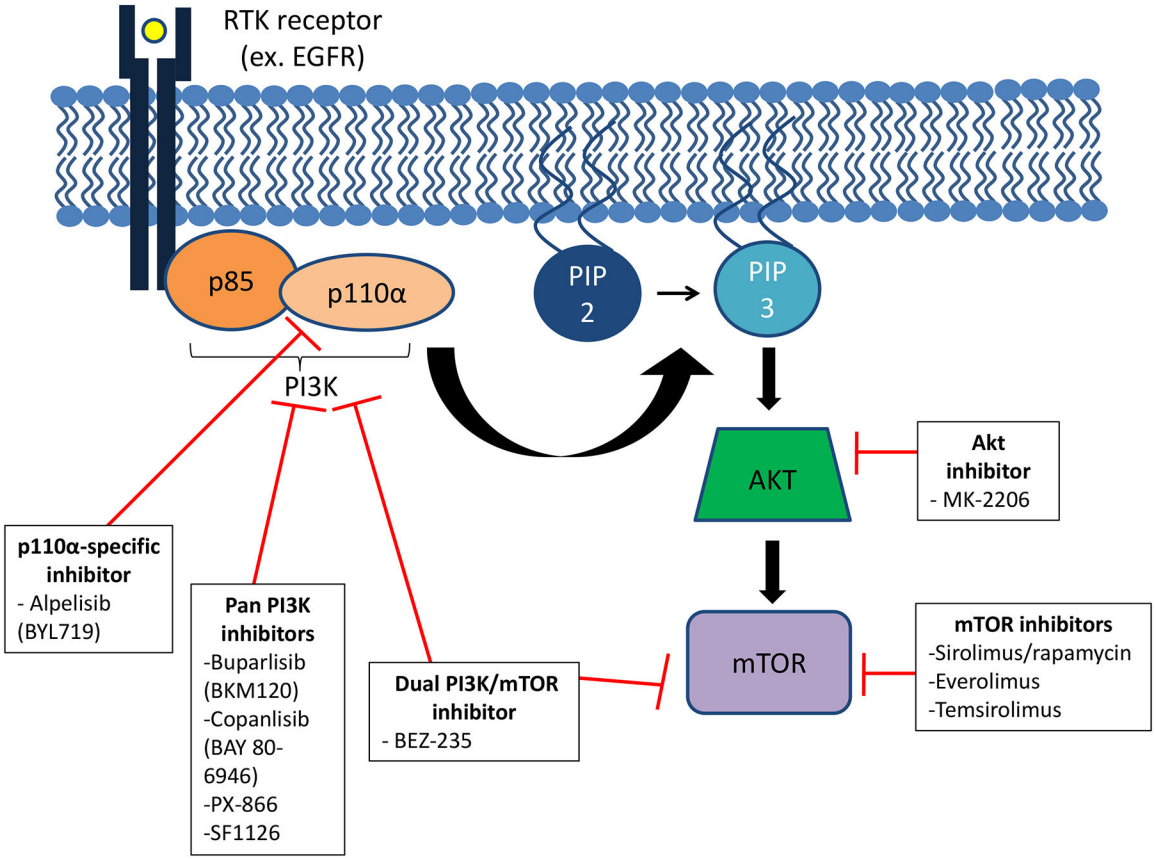

Figure 1. Components of the phosphoinositide 3-kinase (PI3K) signaling pathway and its associated therapeutic targets.

Binding of growth factors and cytokines to a receptor tyrosine kinase (RTK) located on the cell membrane activates class I PI3K, which is a heterodimer of p85 and a p110 isoform (p110α is illustrated here). The catalytic subunit of PI3K (p110α) phosphorylates phosphatidylinositol 4,5-biphosphate (PIP_2), generating phosphatidylinositol (3,4,5)-triphosphate (PIP_3). PIP3 activates protein kinase B (Akt), which subsequently triggers mTOR (mechanistic target of rapamycin) activity. Therapeutic targets that inhibit various components of this signaling pathway include the following: p110α inhibitor (alpelisib/BYL719), pan PI3K inhibitors (buparlisib/BKM120, copanlisib/BAY-80–6946, PX-866, and SF1126), dual PI3K/mTOR inhibitor (BEZ-253), Akt inhibitor (MK-2206), and mTOR inhibitors (sirolimus/rapamycin, everolimus, and temsirolimus).

Table 1.

Common genetic mutations in head and neck cancer noted from TCGA.

Gene	Function	Type of genomic change	Frequency of mutations from TCGA (%)	Frequency of alterations based on HPV type (%)
TP53	Cellular survival and proliferation	Loss-of-function: structural alterations *, inactivating mutations **	72	HPV(−): 84 HPV(+): 3
CDKN2A	Cell-cycle control	Loss-of-function: structural alterations *, inactivating mutations **	22	HPV(−): 58 HPV(+): 0
FAT1	Adhesion and invasion signaling; cadherin superfamily; suppresses cancer growth by binding to B-catenin and inhibiting its nuclear localization	Loss-of-function: structural alterations *, inactivating mutations **	23	HPV(−): 32 HPV(+): 3
PIK3CA	Cellular survival and proliferation	Activation: amplification and/or mutation	21	HPV(−): 34 HPV(+): 56
NOTCH1	Cell differentiation and embryonic development	Loss-of-function: structural alterations *, inactivating mutations **	19	HPV(−): 26 HPV(+): 17
KMT2D (MLL2)	Chromatin remodeling		18	N/A
NSD1	Chromatin remodeling	Loss-of-function: structural alterations *, inactivating mutations **, hypomethylation	10	N/A
CASP8	Apoptosis	Loss-of-function: 3 gene pattern w/ HRAS activation and TP53 wild-type, inactivating (clustered missense); Co-amplification w/ or w/o HRAS	9	HPV(−): 11 HPV(+): 3
AJUBA	Adhesion and invasion signaling	Loss-of-function: structural alterations *, inactivating mutations **	6	HPV(−): 7 HPV(+): 0
NFE2L2	Oxidative stress	Activation	6	HPV(−): 14 HPV(+): 30

* Structural alterations include focal deletions, intra-and interchromosomal fusions;

** Inactivating mutations include nonsense, frameshift, and splice; HPV= human papillomavirus; HPV-positive and negative status are noted by the (+) and (−), respectively.