REVIEW

Novel genetic alterations and their impact on target therapy response in head and neck squamous cell carcinoma

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Abstract: Head and neck squamous cell carcinoma (HNSCC) is highly variable by tumor site, histologic type, molecular characteristics, and clinical outcome. During recent years, emerging targeted therapies have been focused on driver genes. HNSCC involves several genetic alterations, such as co-occurrence, multiple feedback loops, and cross-talk communications. These different kinds of genetic alterations interact with each other and mediate targeted therapy response. In the current review, it is emphasized that future treatment strategy in HNSCC will not solely be based on "synthetic lethality" approaches directed against overactivated genes. More importantly, biologic, genetic, and epigenetic alterations of HNSCC will be taken into consideration to guide the therapy. The emerging genetic alterations in HNSCC and its effect on targeted therapy response are discussed in detail. Hopefully, novel combination regimens for the treatment of HNSCC can be developed.

Keywords: head and neck malignancy, gene mutations, deletions, amplifications, genetic interactions, target therapy

Introduction

Head and neck squamous cell carcinoma (HNSCC) causes more than 300,000 deaths worldwide each year and its incidence is still growing.[1] HNSCC is a fatal heterogeneous disease, with highly variable tumor sites and biologic behaviors.[2] The Cancer Genome Atlas (TCGA) has profiled 279 of HNSCC, from the oral cavity (n=172/279, 62%), oropharynx (n=33/279, 12%), and laryngeal sites (n=72/279, 26%).[3,4] They generate comprehensive lists of genomic and epigenomic alterations present in diverse tumor samples. The MSK-IMPACT (Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets) assay is a targeted next-generation sequencing (NGS) assay approved through Clinical Laboratory Improvement Amendments.[5] There were 151 patients with advanced, treatment-resistant head and neck tumors, including HNSCC, adenoid cystic carcinoma, and other salivary and cutaneous cancers, whose tumors were sequenced.[6] Thus, it provided a comprehensive understanding of the molecular pathogenesis of HNSCC progression and treatment response.[7]

Comprehensive understanding of the biologic, genetic, and epigenetic alterations of HNSCC is necessary to guide the therapy.[8] Drug responses are determined largely by pharmacogenetics, cancer microenvironment, and genetic aberrations.[9] Identifying molecular aberrations that link to drug sensitivity helps to avoid unnecessary and unhelpful treatment.[10] Although associations between genomic alterations and drug response were previously observed in HNSCC, no therapeutically targetable genomic

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subtypes have been identified. Therefore, surgery, radiation, and chemotherapy still remain to be the standard therapy for the disease.

We reviewed the emerging genetic alterations that represent a novel therapeutic target in HNSCC. These include frequent mutations of several genes (*TP53*, *PIK3CA*, *CDKN2A*, *NOTCH1*, and *MET*) as well as copy number gain in EGFR, CCND1, and PIK3CA. Those genomic alterations have been analyzed in relevance of sensitivity to chemotherapy, targeted therapy, and ionizing radiation that could potentially guide the therapy (Table 1). It is essential to determine whether proteins or pathways are involved in the targeted therapy of HNSCCs, aside from gene mutation.[11] These genetic alterations may serve well as novel therapeutic candidates and predictive biomarkers for prognosis in HNSCC.

Based on the recently identified mutations in HNSCCs, the major pathologic pathways implicated in the tumorigenesis of HNSCC include dysregulation of four processes: 1) cellular survival and proliferation (eg, TP53, EGFR, MET, and PIK3CA); 2) cell-cycle control (eg, CDKN2A and CCND1); 3) cellular differentiation (eg, NOTCH1); and 4) adhesion and invasion signaling (eg, FAT1).[7] TP53, EGFR,

PIK3CA, CDKN2A, CCND1, and MET participate in several common signaling pathways (Figure 1). Alterations of these genes are most frequently seen in alcohol and tobacco-related HNSCC.[12] Genetic aberration interactions involve pathway reactivation (downstream re-engagement of original effectors), pathway bypass (recruitment of a parallel pathway converging on the same downstream output), and pathway indifference (development of a cellular state independent of the initial therapeutic target). Gene–drug interactions are analyzed in cBioPortal. Data were from TCGA Head and Neck and Recurrent and Metastatic Head & Neck Cancer (Figure 2).[3]
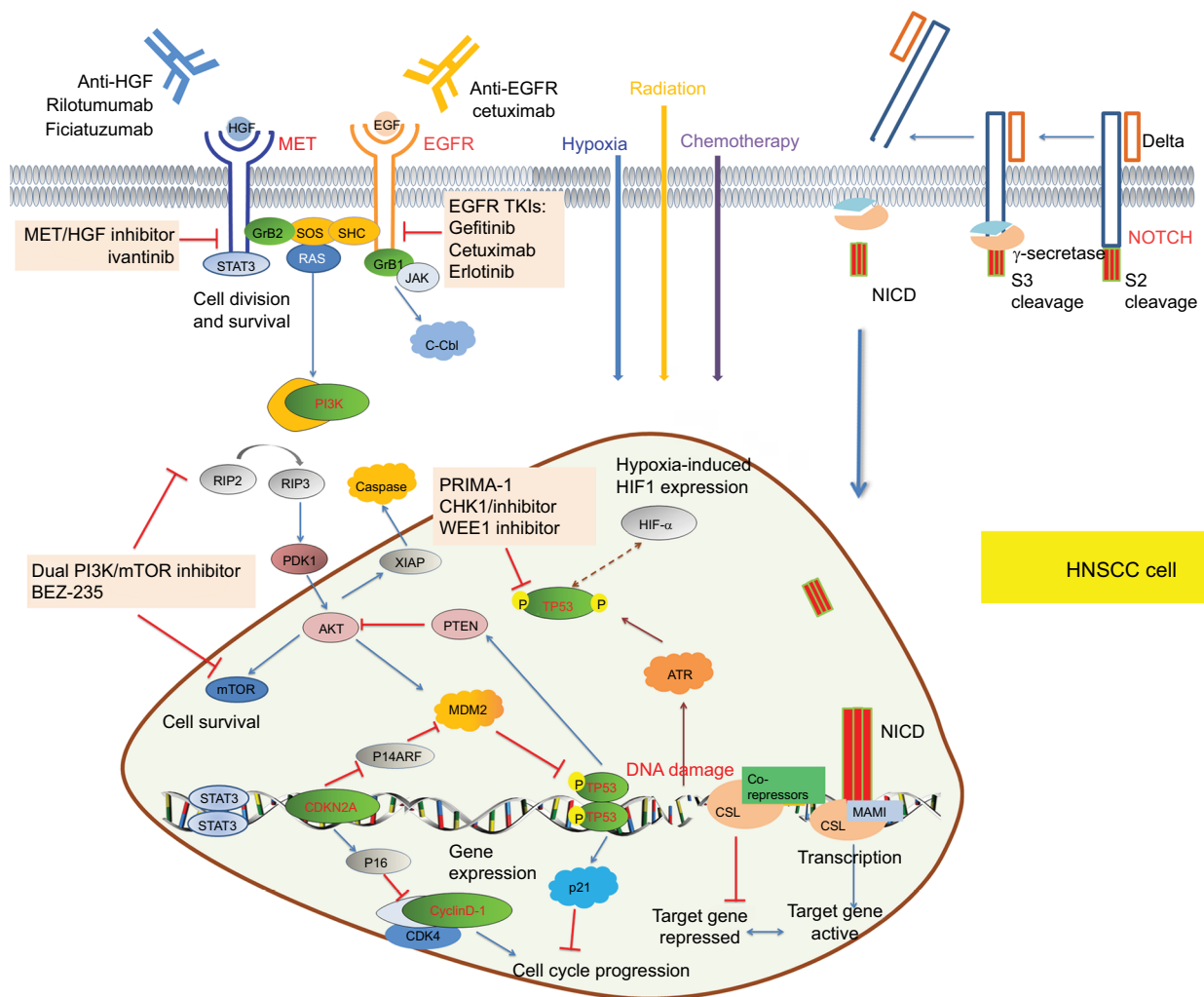
TP53
TP53 mutation
Mutations in the tumor suppressor *P53(TP53)* gene are present in about 70% of HNSCC.[13] Missense mutations in TP53, including those at codons R248, R273, G245, R175, R282, and H179, are the most frequent hotspot mutations in HNSCC.[7] Two thousand four hundred ninety-eight samples in seven studies (Head and Neck Squamous Cell Carcinoma [Broad, Science 2011]; Head and Neck Squamous Cell

Table 1 Genomic expression and alterations associated with drug resistance in HNSCC studies

Markers	Expression/ alteration	Therapy resistance		Patients/xenografts/cell lines	Study
TP53	MT	Cisplatin-based treatment		p53-null or -mutant TP53 HNSCC cells, HNSCC patients	Gadhikar et al[22] Neskey et al[32] Niehr et al[21]
EGFR	Nuclear EP/OP	Radiation and hypoxia		Patients with advanced-stage LSCC	Nijkamp et al[95]
CCND1	EP	EGFR inhibitor gefitinib		Cisplatin-resistant cell line, derived from moderately differentiated tongue SCC	Zhang et al[60]
	AP	Platinum		Testicular germ cell	Noel et al[61]
	AP	EGFR inhibitor gefitinib		HNSCC cell lines	Kalish et al[64]
NOTCH1	MT	PI3K inhibitor BEZ-235		Breast adenocarcinoma-like cell line MCF7, ductal carcinoma-like cell lines BT474, HCC70, and BT549	Muellner et al[10]
MET	EP/AP	EGFR inhibitors	Cetuximab	R/M HNSCC patients	Madoz-Gúrpide et al[116]
			Erlotinib	HNSCC cell lines	Seiwert et al[118] Stabile et al[119]
			Dasatinib	Oral SCC cell lines	Sen et al[125]
	AP	Cisplatin		SCC35/HN5 cell lines	Seiwert et al[107]
	EP	VEGFR inhibitors cediranib and vandetanib		Murine xenograft models of NSCLC	Cascone et al[126]
PIK3CA	EP	Cetuximab-containing chemoradiation		Patients with operable stage III/IV HNSCC	ECOG 2303 Phase II trial Psyrri et al[82]
	MT	MET inhibitor tepotinib		Detroit 562 (PIK3CA H1047R), SCC-61 (PIK3CA E542K) cells	Nisa et al[77]
	AP/MT	PI3K inhibitors		HNSCC PDX model	Herzog et al[38]

Abbreviations: AP, amplification; ECOG, Eastern Cooperative Oncology Group; EP, expression; HNSCC, head and neck squamous cell carcinoma; LSCC, laryngeal squamous cell carcinoma; MT, mutation; NSCLC, non-small-cell lung cancer; OP, OverEP; PI3K, phosphoinositide 3-kinase; R/M, recurrent/metastatic; SCC, squamous carcinoma; VEGFR, vascular endothelial growth factor receptor.

Figure 1 Schematic diagram of signaling pathways that emerging biomarkers TP53, EGFR, PIK3CA, CDKN2A, CCND1, and MET participated in.

Notes: EGFR is a member of the HER family of cell-surface receptor tyrosine kinases. Ligand binding triggers EGFR and activates downstream effectors, thus promoting cell proliferation. Hypoxia induces the expression of EGFR and enhances the phosphorylation of EGFR thereby regulating intrinsic DNA-repair mechanisms, meanwhile EGFR can stabilize HIF-1a. MET is a receptor tyrosine kinase associated with enhanced migration, invasion, and angiogenesis when overexpressed in cancer. PIK3CA encodes p110α, a catalytic subunit of PI3K, which is a heterodimeric kinase with enzymatic activity on lipid and protein substrates. The TP53 has a vital role in the regulation of genes responsible for cell-cycle arrest, senescence, and apoptosis. MDM2 promotes the rapid degradation of the TP53 protein. TP53 missense mutations cause single-amino-acid substitutions that lead to loss of DNA-binding capability. Cyclin D1 is a cell-cycle protein that regulates the key G1-to-S phase transition through formation of complexes with CDKs, such as CDK4 and CDK6. Upon ligand binding, NOTCH receptors undergo a conformational change enabling cleavage and nuclear translocation of the intracellular domain to release the transcriptional repression of downstream target genes.

Abbreviations: CDKs, cyclin-dependent kinases; HIF-1a, hypoxia-inducible factor 1a; PI3K, phosphoinositide 3 kinase.

Carcinoma [Johns Hopkins, Science 2011]; Head and Neck Squamous Cell Carcinoma [TCGA, Nature 2015]; Head and Neck Squamous Cell Carcinoma [TCGA, PanCancer Atlas]; Head and Neck Squamous Cell Carcinoma [TCGA, Provisional]; Oral Squamous Cell Carcinoma [MD Anderson, Cancer Discov 2013])[4,14,15] showed 62.7% of somatic mutation and 45.2% of missense mutations (http://www.cbioportal.org/). TP53 mutation was markedly higher in metastatic HNSCC.[6] TP53 mutation involves varieties of proteins that contribute to tumorigenesis and tumor progression.[4,16] It occurs early in carcinoma progression and more frequently in those with greater histologic severity.[17,18]
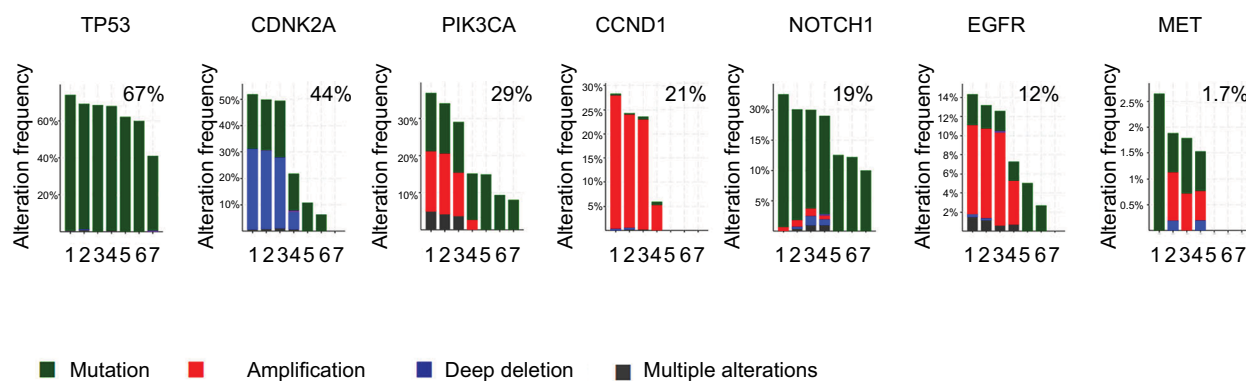
Data TCGA Head and Neck and Recurrent and Metastatic Head & Neck Cancer (MSKCC, JAMA Oncol 2016) analyzed by cBioPortal (detailed description of data mining could be found in the figure legends) demonstrated that only TP53 mutation is a predictor for overall survival (OS) rate and disease-free survival rate (Figure 4). Moreover, tumors of the larynx and hypopharynx have the highest TP53 mutation rate (83.5%). Tumors of the tongue and oral cavity have a TP53 mutation rate of 75.6%, and those of the oropharynx (including the tonsils), and base of the tongue have the lowest TP53 mutation rate (28.6%).[13] Previous studies have shown that TP53 mutation correlated with resistance to chemotherapy
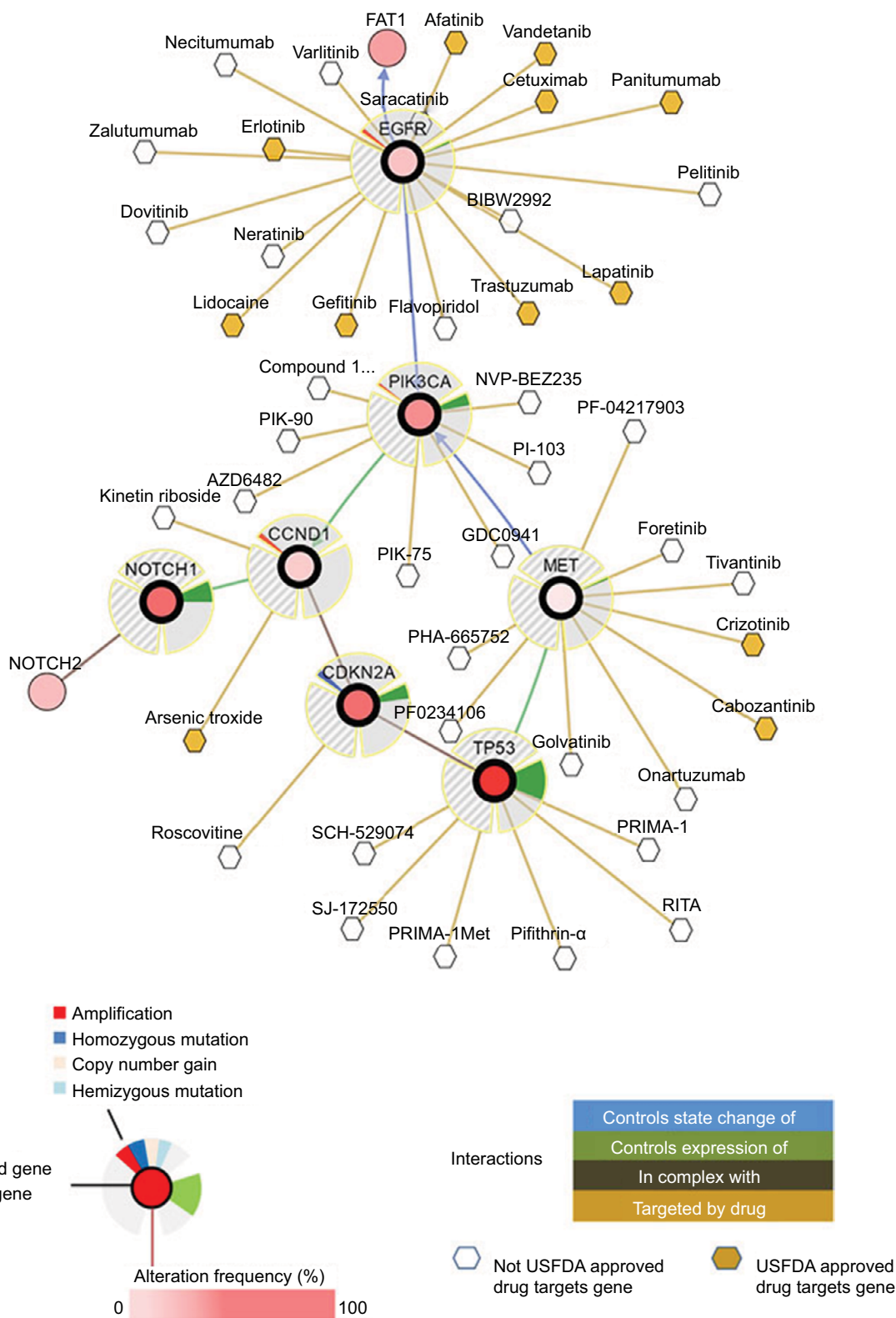
Figure 2 General interactions of genomic mutations (TP53, CDKN2A, CCND1, NOTCH1, PIK3CA, EGFR, and MET) and current target drugs.
Notes: Primary data are from tumor samples with sequencing and CNA data (132 patients/samples) and could be accessed in cBioPortal.[6] Detailed description of data mining is (filter neighbors by max 28.8% alteration) found in http://www.cbioportal.org/results/network?Action=Submit&RPPA_SCORE_THRESHOLD=2&Z_SCORE_ THRESHOLD=2&cancer_study_list=hnc_mskcc_2016&case_set_id=hnc_mskcc_2016_cnaseq&data_priority=0&gene_list=TP53%2520CDKN2A%2520CCND1%2520 NOTCH1%2520PIK3CA%2520EGFR%2520MET&geneset_list=%20&genetic_profile_ids_PROFILE_COPY_NUMBER_ALTERATION=hnc_mskcc_2016_gistic&genetic_ profile_ids_PROFILE_MUTATION_EXTENDED=hnc_mskcc_2016_mutations&tab_index=tab_visualize.[134,135]
Abbreviations: US FDA, US Food and Drug Administration.

Figure 3 Alteration profiles of the emerging biomarkers from querying 1,627 patients/1,629 samples in 7 studies analyzed in cBioPortal.
Notes: Data from 11,000 cases and all TCGA tumor types from TCGA. Detailed description for data mining could be found in http://www.cbioportal.org/results/cancerTypesSummary?Action=Submit&RPPA_SCORE_THRESHOLD=2&Z_SCORE_THRESHOLD=2&cancer_study_list=hnc_mskcc_2016%2Chnsc_broad%2Chnsc_jhu%2Chnsc_tcga_pub%2Chnsc_tcga_pan_can_atlas_2018%2Chnsc_tcga%2Chnsc_mdanderson_2013&case_set_id=all&data_priority=0&gene_list=TP53%2520CDKN2A%2520CCND1%2520NOTCH1%2520PIK3CA%2520EGFR%2520MET&geneset_list=%20&tab_index=tab_visualize.[134,135]
Abbreviation: TCGA, The Cancer Genome Atlas.

drugs such as cisplatin, doxorubicin, and paclitaxel.[19–22] It has been recently further demonstrated that cisplatin resistance was associated with aneuploidy of chromosome 17, increased TP53 copy numbers, and overexpression of mutant variant R248L.[21]

TP53 mutation has also been indicated for assessment of postoperative radiotherapy.[23] If there are no histologically detectable tumors and no TP53 mutations in the surgical margin, patients can be spared postoperative radiotherapy. It is supported by the studies that show the absence of TP53-mutated DNA in surgical margins was significantly associated with local recurrence-free survival.[24,25]

Classification of TP53 mutation

The value of TP53 mutation in diagnosis is different between subtypes. Some are associated with more aggressive HNSCC phenotypes, whereas others are linked with a more indolent pattern of tumor progression.[26]

Perrone et al categorized TP53 mutation significance based on the transactivation activity as functional, partially functional, or nonfunctional.[27] Loss of function of TP53 (transactivation activities) predicts a significantly low rate of pathologic complete remission and suboptimal response to cisplatin-based neoadjuvant chemotherapy in patients

with oral squamous cell carcinoma (OSCC).[28] Accumulating evidence suggests that gain of function (GOF) of TP53 mutants as well mediates drug resistance. The underlying mechanisms include apoptotic proteins inhibition and gene regulations.[29,30]

Another large study classified TP53 mutation as disruptive and nondisruptive, based on alteration of DNA binding.[31] The disruptive is defined as any mutation in L2 or L3 loop of the DNA-binding domain or stop codon, resulting in a polarity change within the protein. Disruptive TP53 mutation strongly predicted locoregional recurrence driven by tumor cell radioresistance. The radioresistance is measured by SA-β-gal staining, p21 expression, and release of ROS.[31]

Evolutionary action (EATP53), a novel computational approach, has been applied to stratify tumor patients with TP53 mutation as high- or low risk. This system was validated both in vivo and in vitro[32] (available at http://mammoth.bcm.tmc.edu/EATP53). High-risk mutations promote invasion, metastasis, as well as cisplatin resistance in head and neck cancer cell lines, as they acquired oncogenic GOF properties, while low-risk mutations retained wild-type (WT) TP53 activity.[32,33] Different effect of TP53 mutation on cisplatin response has been observed in vitro and in vivo. Mice with HNSCC harboring WT or low-risk mutations responded

well to cisplatin treatment. Quite the opposite, TP53 null type or high-risk TP53 mutations failed to show any growth inhibition with cisplatin therapy.[34] Similar results were seen in patients with those characters. The high-risk TP53 mutations were associated with decreased OS.[32]

Targeting TP53 mutation and other coexisting alterations to induce synthetic lethality

Researchers have explored introduction of exogenous WT TP53 into HNSCC cells, or reactivation of some level of WT function in mutant p53-bearing cells, otherwise promotion of mutant TP53 degradation. Of all the compounds that restore WT activity, the only drug directly targeting mutant TP53 that has reached the clinical stage is PRIMA-1(met)/APR-246.[35–37] It also induced apoptosis and enhanced the cytotoxicity of standard chemotherapy in HNSCC cells.

In addition to targeting mutant p53 directly, investigators have used strategies targeting mutant TP53-regulated downstream targets and signaling pathways. Mutant TP53 proteins are believed to achieve GOF activity by interacting with other molecular alterations that coexist in HNSCC. Understanding the impact of TP53 mutation on cellular growth and survival signaling pathways can help design more effective therapeutic strategies that target TP53 mutation-bearing HNSCC.

CDKN2A deletions

TP53 and CDKN2A are involved in cell cycle.[33] A genomic analysis of human HNSCCs detected that CDKN2A suppresses the oncogenic function of TP53 mutation that promotes malignant progression, and the prognostic value of mutant p53 needs to be considered in the context of CDKN2A.[26] The survival of patients with HNSCCs bearing co-occurring high-risk TP53 mutation and CDKN2A homozygous deletions was extremely lower than that of patients with tumors in which high-risk TP53 mutation did not contain CDKN2A homozygous deletions, or that of patients with tumors in which homozygous CDKN2A deletions coexisted with either low-risk TP53 mutation or potential loss-of-function mutations in TP53 (high- and low risk according to the EATP53 classification system).[32]

PIK3CA activation

Preliminary findings from TCGA Head and Neck Cancer have detected amplifications or putative activating mutations in PIK3CA in 30% of 279 HNSCC tumors, which overlap with WT and mutant TP53 subsets.[38] The aforementioned studies indicate that increase in phosphoinositide 3-kinase (PI3K)/Akt activation has been linked to frequent altera-

tions in TP53 via multiple mechanisms.[10,38–40] Comparing half-maximal inhibitory concentration in a wider panel of HNSCC cell lines suggests that TP53 status contributes to the sensitivity of PI3K/mTOR inhibitor.

In human xenograft, PI3K/mTOR inhibition has been proven to enhance WT TP53 expression, enhance the TP53 DNA-damage response, and further delay tumor regrowth with radiation;[38] thus, the combination of PI3K/TP53 events seems to be necessary for PI3K inhibitor sensitivity. The dual PI3K/PLK inhibitor rigosertib has such potent antiproliferative effects on patient tumor xenografts, which carried combination of a PIK3CA-activating event driven by amplification or mutation and a TP53-inactivating event driven by either human papillomavirus (HPV)-negative or nonsense TP53 mutation.[41]

CDKN2A
CDKN2A mutations

Global genomic analyses identified the tumor suppressor CDKN2A as the second most commonly altered gene in HNSCC.[13] CDKN2A (also known as P16, INK4, p16INK4A, and MTS1) is allelic to chromosome 9p21 and encodes a CDK4/CDK6 kinase inhibitor that constrains cells from progressing through the G1 restriction point. It is thought to be involved in early stages of HNSCC development. It is affected in up to 80% of HNSCC – often deleted, hypermethylated, or, much rarely, mutated.[42] CDKN2A mutation is considered as "noncoding mutations", "inactivation", or "loss of function".[43] It is associated with worse oversurvival in patients with recurrent and metastatic HNSCC (MSKCC, JAMA Oncol 2016) (Figure 4).[13] Most of the mutations in CDKN2A were found in exon 2 of the gene.[42] However, these are likely insufficient to drive tumorigenesis by CDKN2A mutations themselves.[44] For example, CDKN2A mutations in benign epithelial lesions have low potential to transform into malignancy.[44] Therapeutic targeting of CDKN2A presents the challenge of restoring tumor suppressor activity or inhibiting downstream targets that have been rendered overactive.

CDKN2A mutation reflects p16INK4a function

p16INK4a immunohistochemical expression has been proposed as a surrogate marker for viral oncogene activity in HNSCC.[45] The overexpression of p16INK4a is an outstanding surrogate for HPV positivity extensively documented in OSCC.[46,47] Numerous studies have reported a considerably improved survival (death risk reduced by 40%–80%) or lower locoregional recurrences (reduced by 60%–70%) and better response to chemo- and radiotherapy in HPV-positive
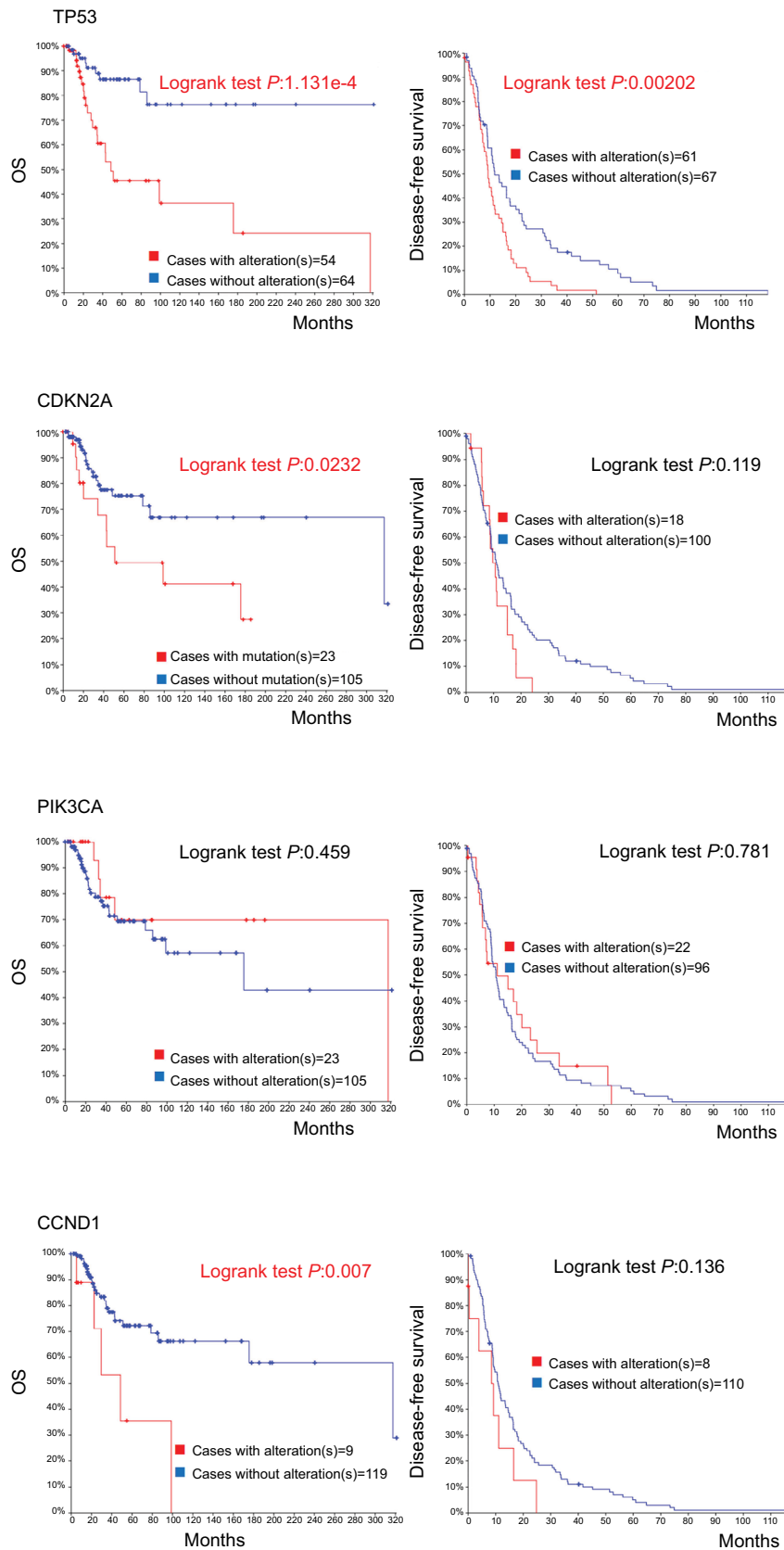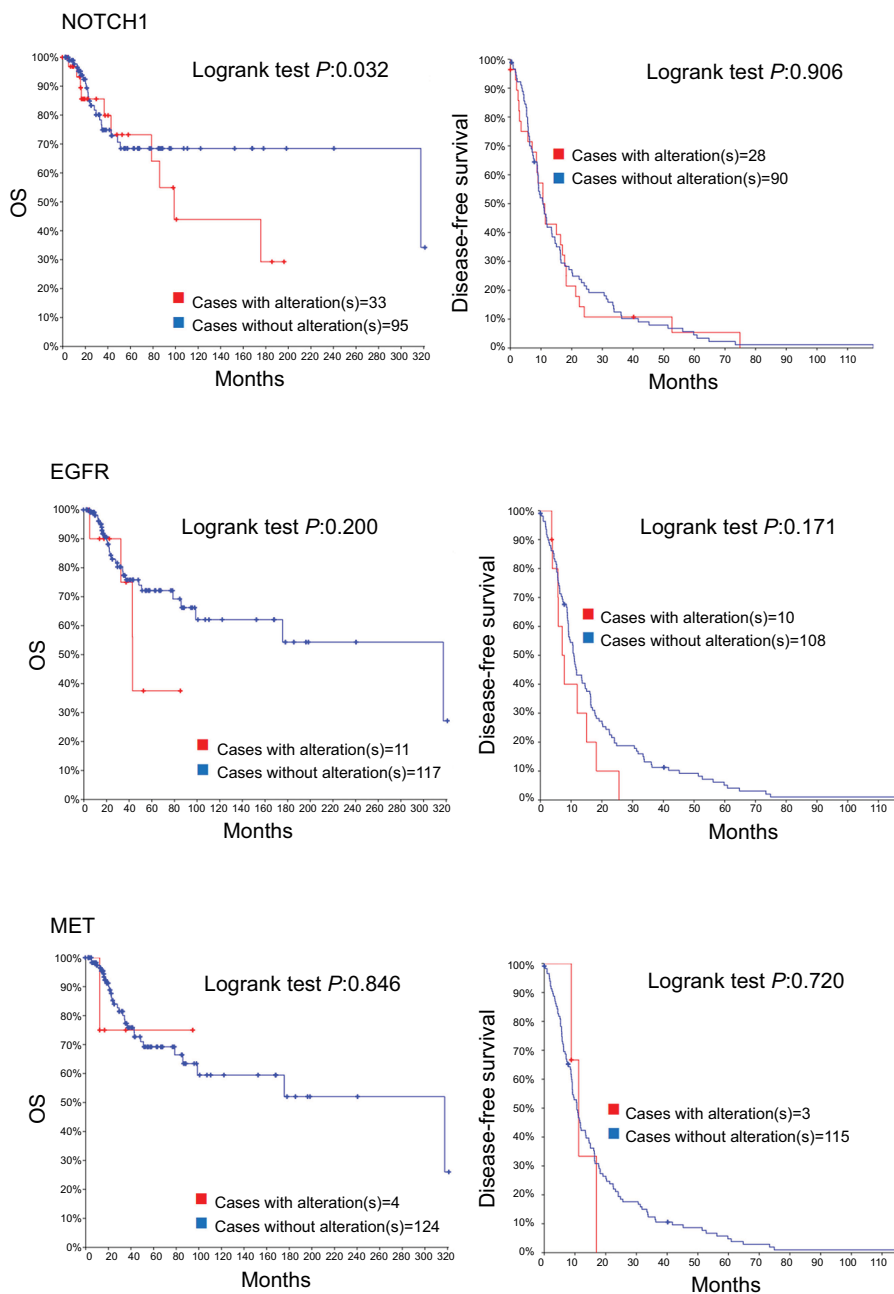
Figure 4 (*Continued*)

Figure 4 Recurrent and metastatic head and neck cancer samples with sequencing and CNA data (132 patients/samples) analyzed in cBioPortal.
Notes: OS/recurrence-free survival, for cases with/without alteration(s) in query gene. Survival probabilities were calculated with the Kaplan–Meier method, according to the original article.[6] Detailed description of data mining could be found in http://www.cbioportal.org/results/survival?Action=Submit&RPPA_SCORE_THRESHOLD=2&Z_SCORE_THRESHOLD=2&cancer_study_list=hnc_mskcc_2016&case_set_id=hnc_mskcc_2016_cnaseq&data_priority=0&gene_list=TP53&geneset_list=%20&genetic_profile_ids_PROFILE_COPY_NUMBER_ALTERATION=hnc_mskcc_2016_gistic&genetic_profile_ids_PROFILE_MUTATION_EXTENDED=hnc_mskcc_2016_mutations&tab_index=tab_visualize.[134,135]
Abbreviation: OS, overall survival.

patients with p16INK4a expression compared to HPV-negative patients, when treated with standard multimodality treatments.[48–53]

HPV-positive HNSCC rarely has CDKN2A mutation and thus has the p16INK4a function;[26,54] those patients are significantly less likely to develop a locoregional recurrence.[43] On the other hand, up to 90% of HPV-negative HNSCCs have loss of p16INK4a function as a result of CDKN2A mutation, promoter methylation, or gene/chromosome 9p21 deletion.[45] But the recent study found that CDKN2A mutation still reflects the p16INK4a expression in HPV-negative HNSCC. Loss of CDKN2A function as a result of CDKN2A mutation induced

p16INK4a mRNA transcription strongly.[45] Hence, p16INK4a overexpression is associated with CDKN2A mutation in a subset of cases. In those cases, the value of p16INK4a for inducing HPV infection was not reliable.

CCND1
CCND1 amplification
CCND1 encodes cyclin D1 on chromosome 11q13, and regulates the key G1-to-S phase transition through formation of complexes with cyclin-dependent kinases (CDKs), such as CDK4 and CDK6.[55] Mutations, amplification, and overexpression of this gene, which alters cell-cycle progression, are observed frequently in a variety of tumors and may contribute to tumorigenesis. CCND1 is amplified in as many as 30%–40% of head and neck cancers with cyclin D1 overexpression.[56,57] Comparative studies have either shown no prognostic advantage of protein expression over gene amplification or have favored cyclin D1 gene amplification as a better marker of poor prognosis. CCND1 amplification had a statistically significant association with recurrence, distant metastasis, platinum and cisplatin resistance, and EGFR-inhibitor resistance.[58–61] Allele A may be a risk factor for upper aerodigestive tract cancer, especially in nonalcoholics. Patients of recurrent and metastatic head and neck cancer with CCND1 alterations have poor OS rate (Figure 4). CCND1 may be a valuable marker for predicting regional response to radiotherapy in metastatic HNSCC and might assist when deciding on appropriate primary therapy.[62] Kinetin riboside, an inhibitor of CCND2 transactivation, rapidly suppressed cyclin D1,[63] which is a promising CCND1 inhibitor.

CCND1 amplification confers to EGFR inhibitor resistance
Maintenance of cyclin D1 levels is critical to the resistance HNSCC cells display to gefitinib.[54] Sensitivity of the six HNSCC cell lines (FaDu, Detroit 562, SCC 9, SCC 15, SCC 25, and CAL 27) to gefitinib was related to cyclin D1 overexpression. These cell lines that overexpressed cyclin D1 continued to proliferate when treated with up to lethal level of gefitinib, but cell numbers remained static or decreased after gefitinib treatment in the non-overexpressed cyclin D1 cell lines.[65] Furthermore, the most resistant cell line displayed the highest level of CCND1 amplification and cyclin D1 overexpression. Cyclin D1 upregulation also combines with cortactin overexpression to promote resistance to gefitinib.[66] Cortactin overexpression attenuated ligand-induced downregulation of EGFR, leading to sustained signaling.[66]

A study in vitro explored the relationship between deregulated cyclin D1 expression and sensitivity to gefitinib to determine whether this frequently occurring oncogenic change affected the cellular response to gefitinib. Three of six cell lines displayed cyclin D1 amplification and/or overexpression, and these cell lines were resistant to gefitinib.[65] No preclinical studies have yet addressed whether cyclin D1 expression is maintained in CCND1-amplified cancers after EGFR inhibition, and this is an issue warranting investigation.

NOTCH1
NOTCH1 mutations
The NOTCH pathway consists of four receptors (NOTCH1–4) and two families of ligands: the Jagged (JAG1 and JAG2) and the Delta-like (DLL1, DLL3, and DLL4) proteins. NOTCH1 is a transmembrane receptor and controls cell differentiation and embryonic development. Upon ligand binding, functions of NOTCH1 in oral SCC are considered tumor-suppressive. In tongue cancer xenografts, the presence of NOTCH1 caused a dramatic tumor reduction compared to tumors in which this gene was absent.[67] Higher expression and activity of NOTCH1 have previously been associated with radio- and chemoresistance.[68]

Point mutations affecting this gene occurred in 11% of the HNSCC tumors.[13] It is considered to represent "loss of function".[13,69,70] Missense mutations in NOTCH1 are considered tumor-suppressive in OSCC. Notably, OSCC patients with NOTCH pathway mutations (AR, ARNT, EP300, CREBBP, JAK2, JAK, NOTCH1, NOTCH2, NOTCH3, and PARP1) were 3.3 times more likely to die with recurrent disease compared to those who did not have these alterations.[71] The NOTCH1 mutation status recently served as biomarker for the identification of HNSCC with higher sensitivity to radio- and chemotherapy, as significant association of NOTCH1 mutations with improved localregional control and increased overall survival was observed after chemoradiotherapy.[70] Using of gamma-secretase inhibitors supposed to be an attractive strategy for improvement of chemoradiotherapy in malignancies harboring wild-type NOTCH1. For HNSCC it is should be carefully evaluated given evidence of an increased frequency of squamous cell carcinoma in vivo with genetic reductional γ-secretase activity.[133]

Analysis of recurrent and metastatic head and neck cancer suggests a similar trend toward better 5-year (60 months) OS in patients with alterations in NOTCH1, but after 5 years the OS in patients with alterations was worse; however, this was not statistically significant (Figure 4).

Activated NOTCH1 contributes to resistance of PI3K inhibitors

Active domain of NOTCH1 conferred resistance to the dual PI3K/mTOR inhibitor BEZ-235 in breast adenocarcinoma-like cell line MCF7 and the ductal carcinoma-like cell lines BT474, HCC70, and BT549.[10] They showed resistance to BEZ-235 treatment upon expression of ICN1 (intracellular active domain of NOTCH1).[72] Furthermore, in NOTCH1 mutant breast cells that lack the extracellular domain, BEZ-235 sensitivity could also be restored by inhibiting γ-secretase, indicating that naturally cleaved NOTCH1 also confers resistance to PI3K inhibition. A significant correlation was detected between low expression of negative regulator of NOTCH (NUMB) and resistance to PI3K/mTOR inhibition in cell lines derived from various tumor types, including melanoma and hepatocellular carcinoma. These results suggest that uncoupling proliferation from the PI3K pathway via NOTCH1 activation may be a general phenomenon across cancer cell lines.

PIK3CA

PIK3CA mutations

The PI3K pathway (PIK3CA, PTEN, AKT, and STK11) was the most frequently somatically mutated oncogenic pathway in HNSCC tumors.[73–75]

PIK3CA alterations encoding the key catalytic subunit of PI3K pathway were subsequently found to be prevalent. Data from the querying 2,498 samples showed PIK3CA alterations around 29%, including copy number gain (high polysomy or amplification) or mutations[13] (Figure 3).[4,14,15,67] However, PIK3CA alteration has no significant prognoses value for recurrent and metastatic head and neck cancer analyzed in cBioPortal (Figure 4).

PIK3CA mutations seem to favor advanced HNSCC, which harbor multiple PI3K-pathway mutations.[38] Lui et al detected that 100% HNSCC (10/10 cases) with concurrent PI3K mutations were advanced (Stage IV).[75] PIK3CA copy number gain (high polysomy or amplification) but not PIK3CA mutation was detected to be associated with significantly lower disease-free survival, resulting from an NGS.[77] The most frequent mutations occur in exons 9 and 20, with hotspots at H1047R (eight mutations total), E542K (three mutations), and E545K/G (four mutations).[78] H1047R was more potent than E545K at inducing resistance in PI3K pathway activation.[10] PIK3CA canonical and novel mutations increase survival. These canonical hotspot mutations showed significantly enhanced HNSCC growth compared to overexpression of WT PIK3CA.[74] This observation implies that the PI3K pathway-mutated HNSCC tumors have "oncogenic" advantage even with genomic instability, which can be partly explained by PIK3CA "driver" mutations' growth-promoting activity.

Although there is not enough evidence associated with oversurvival and recurrence rates, PIK3CA alterations are providing a potential approach to treat a substantial subset of patients in advanced stage.

Targeted PIK3CA activation

There are currently a variety of different inhibitors that are being studied for targeting the PI3K pathway.[74,79] PIK3CA activation either caused by mutation or copy number gain is oncogenic and is thus a commonly activating point mutation with targeted agents.[73,74] Targeting the PIK3CA activation has emerged as one of the most promising therapeutic targets in HNSCC.[75] They are already in clinical development for other cancer types.[10]

HNSCC cell lines containing hotspots endogenous PIK3CA (H1047R) mutations demonstrated increased sensitivity to PI3K pathway inhibition by the mTOR/PI3K inhibitor BEZ-235 compared to the representative HNSCC cells with WT PIK3CA.[80] Furthermore, they were more sensitive to the combination of BEZ-235 plus cetuximab compared to cetuximab alone.[80] PIK3CA mutations are more common in HPV-positive HNSCC and are associated with activation of mTOR.[4,74,81] Patient-derived HPV-positive OPSCC tumorgrafts in vivo with PIK3CA (E542K) mutation were also sensitive to a dual mTOR/PI3K inhibitor (BEZ-235), in contrast to PIK3CA WT tumorgrafts.[76,80] These results suggest that mTOR/PI3K inhibitors may have activity against PIK3CA mutant HNSCC, particularly in HPV-positive HNSCC.

Activated PI3K/Akt confers resistance to MET inhibitors

PIK3CA and MET may play an important role in oncogenesis of certain specific subtypes of head and neck cancer. In several cancers, gene copy numbers of MET and PIK3CA have been found to be prognostic and predictive for therapy response.[82] The MET receptor tyrosine kinase represents a promising target in cancer. PIK3CA activating mutations are common in several tumor types and can potentially confer resistance to anti-receptor tyrosine kinase therapy.[78] Hotspots E545K and H1047R confer resistance to MET inhibition in MET-driven models. Hence, resistance to MET inhibition could be synergistically overcome by co-targeting PI3K. Combination of PI3K/MET inhibitors led to more-than-additive effects.

Combined MET/PI3K inhibition leads to enhanced antitumor activity in MET-expressing HNSCC harboring endogenous PIK3CA (H1047R).[78] PIK3CA mutations can lead to resistance to MET inhibition, supporting future clinical evaluation of combinations of PI3K and MET inhibitors in common scenarios of malignant neoplasms featuring aberrant MET expression and PIK3CA mutations. In conclusion, PI3K signaling pathway was a potential target for treatment optimization across all risk groups.

Activated PI3K/Akt causes resistance of EGFR inhibitors

Activated PI3K/Akt and RAS/MAPK/ERK pathways are associated with resistance to cetuximab-containing chemoradiation (weekly cetuximab, paclitaxel, and carboplatin followed by chemoradiation with the same regimen) in operable stage III/IV HNSCC, implicated by Eastern Cooperative Oncology Group (ECOG) 2303 Phase II trial.[82] Inferior OS and/or PFS were observed in patients harboring activated PI3K/Akt and RAS/MAPK/ERK and maintained significance in multivariable analysis.[83] Preclinical studies suggest that PIK3CA and RAS mutations may predict intrinsic resistance to cetuximab, which may be prevented by combination of cetuximab and PI3K and/or mTOR inhibitors in HNSCC patients.[84]

EGFR

EGFR plays an important role in the emergence and progression of epithelial malignancies and is the most frequently activated receptor tyrosine kinase in HNSCC.[85] The first molecular targeting approach to demonstrate a survival advantage for HNSCC patients has emerged in the context of EGFR biology. TCGA data have detected EGFR alteration in 14.34% cases including 65.0% with EGFR amplification. High-level EGFR amplifications in ~10% of HNSCC, and polysomy are present in even more.[13] Primary somatic mutations in EGFR are uncommon in HNSCC.[86] Most of the previous studies found that EGFR overexpression was associated with radioresistance, and worse locoregional and disease-free survival.[87–90] However, EGFR alteration also has no significant prognoses value in metastatic head and neck cancer analyzed in cBioPortal (Figure 4). And because there has been no consistently identifiable alteration in the EGFR that correlates to sensitivity to EGFR inhibitors, EGFR analysis has not been incorporated into the pathologic evaluation of HNSCC.[85]

EGFR amplification is highly associated with resistance to chemotherapy and radiation in HNSCC.[22,91] Hypoxia induces radioresistance, directly as DNA damage is maximized in the presence of oxygen and indirectly by promoting genetic instability[92] (Figure 1). Hypoxia induces the expression of EGFR and enhances the phosphorylation of EGFR thereby regulating intrinsic DNA-repair mechanisms, meanwhile EGFR can stabilize hypoxia-inducible factor 1a.[93,94] These radioresistance mechanisms play a role in HNSCC because EGFR is overexpressed in most of these tumors. That is why EGFR inhibition has subsequently been explored as a potential therapeutic adjunct to radiotherapy in HNSCC.[95,96]

Targeting EGFR is lack of response evaluation

Anti-EGFR agents are largely divided into two classes of drugs: monoclonal antibodies and tyrosine kinase inhibitors (TKIs). EGFR inhibitors have been used to treat both lung and head and neck cancers with squamous cell histology.[97] These tumors often show high EGFR expression and/or increased gene copy number. The efficacy of cetuximab is demonstrated in the recurrent/metastatic (R/M) HNSCC settings of clinical trials: ECOG, Erbitux in First-line Treatment of Recurrent or Metastatic Head and Neck Cancer (EXTREME) trial. Recent developments included US Food and Drug Administration approval of pembrolizumab and cetuximab. Cetuximab has conferred a survival advantage when combined with platinum-based combination chemotherapy in the first-line R/M setting.[98] However, only a fraction of HNSCC are sensitive to the cetuximab, 5-year survival of HNSCC is not improved by the cetuximab, which is currently <40%.[82,99]

The use of EGFR as a biomarker for response to EGFR inhibitor is limited by the semiquantitative nature of and subjectivity of immunohistochemical scoring.[100–102] It might be due to inaccurate assays as well as the nature of increased gene copy number being a less predictive biomarker compared to activating mutations.[55] Several studies explored the biology, genomics, and patterns of response to EGFR inhibitors to inform identification of potential biomarkers that have shown promise in preclinical studies and clinical trials.[85,97,101,103–105] But no biomarkers are known to predict response to the treatment.

Vermorken et al demonstrated the superiority of incorporating cetuximab, in combination with cisplatin/carboplatin and fluorouracil in patients with recurrent/metastatic HNSCC.[97] Genomic studies suggest that EGFR alteration is more common in HPV-negative tumors compared to HPV-positive tumors.[74] Clinical data further contradict the HPV

positivity as a biomarker for EGFR-inhibitor resistance.[97] Development of EGFR inhibitors could be more relevant in treating HPV-negative tumors.[81,106,107]

MET

MET alterations as oncogenic driver of HNSCC

Hepatocyte growth factor (HGF) receptor (MET) is functionally novel in HNSCC with prominent overexpression, and increased copy number and mutations.[95,108] It regulates cancer cell plasticity through reversible reprogramming of epithelial–mesenchymal transition (EMT).[109,110] It is a process considered a critical step in the progression of HNSCC. Accumulating evidence suggests that EMT and its ligand HGF favor the distant dissemination of single carcinoma cells from the site of the primary tumor.[109,111]

MET/HGF pathway activation driven by MET overexpression correlated with worse outcome in R/M HNSCC patients for progression-free survival and OS.[112] Although MET alterations are relatively low from querying 2,498 samples in 7 studies in TCGA head and neck carcinoma (1.3%) (Figure 3), MET is proposed as an escape mechanism mediating drug resistance.[109,110,113] MET copy number gain or somatic mutations drive metastatic spread of HNSCC[108,114] and are significantly associated with shorter disease-specific survival.[99] Therefore, MET may be a substantial ontogenetic driver of certain specific subtypes of HNSCC, which should be further explored.

Hence, multiple clinical scenarios targeting the MET in HNSCC have been undertaken because of significant preclinical work demonstrating a relationship between MET/ HGF signaling and cancer cell survival (eg, tivantinib, cabozantinib, and crizotinib).[114] HGF/c-MET pathway mediates vascular endothelial growth factor receptor (VEGFR) inhibitor resistance and vascular remodeling in non-small-cell lung cancer (NSCLC). Forced HGF expression in NSCLC reduced tumor sensitivity to VEGFR TKIs and produced tumors with increased blood vessels. Dual VEGFR/c-MET signaling inhibition could prevent the vascular morphology alterations.[115] These preclinical results indicated that the level of MET expression is a determinant of outcome.[112]

MET mutations associated with resistance to EGFR inhibitor

The cross-talk of MET and its ligand with EGFR, such as coactivation of alternative kinases, may contribute to EGFR inhibitor resistance.[82,112] For example, MET mutations are present in up to 20% of patients with resistance to EGFR inhibitors, like cetuximab.[116] In head and neck cancer cell lines MET was shown to mediate resistance to dasatinib and erlotinib.[117,118] HGF/MET pathway activation is associated with poorer outcome in R/M HNSCC patients. Therefore, patients treated with EGFR inhibitors have a worse prognosis.[119,120] Experimental studies in vitro and in vivo of R/M HNSCC documented a synthetic lethal effect of dual blockade using c-MET (SU11274, PF2341066, and PF04217903) and EGFR TKIs (cetuximab, erlotinib, and gefitinib).[118,121,122]

Conclusion

Precision medicine in oncology seeks to match each patient with the most effective and appropriate targeted therapies.[6] NGS data-guided therapy is promising, in revealing novel molecular alterations, which might be used as drug targets.[6]

Since the last decade, concepts of cross-talk, co-occurrence, and interactions have been intensively introduced into biochemical research. Various etiologic factors are implicated in the genesis of distinct molecular subsets of HNSCC. Apparently, signaling pathways and transcription factors are not regulated by mutations of a specific gene. Multiple gene mutations, along with the corresponding protein dysfunction or molecular pathway dysregulations, are causative in the process of carcinogenesis or drug resistance.

HPV-positive and -negative HNSCCs are quite distinct clinically and biologically. However, they both can have deregulations within the PI3K pathway.[123,124,81] Our review paid less attention on HPV-positive HNSCC, because studies of the HPV-positive HNSCC are scarce. The TCGA cohort comprised 85% HPV-negative HNSCC.[3] HPV-negative HNSCC featured novel focal deletions in tumor suppressor genes (eg, NOTCH1 and CDKN2A).[13] Also, mutations of TP53, CDKN2A, PIK3CA, and NOTCH genes are enriched. Combination therapy is required. The major challenge is to identify and make good use of the novel genetic interactions, and develop more accurate model systems and innovative clinical trials.

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Disclosure

The authors report no conflicts of interests in this work.

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