

Review Article

Receptor-Tyrosine-Kinase-Targeted Therapies for Head and Neck Cancer

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Molecular therapeutics for treating epidermal growth factor receptor-(EGFR-) expressing cancers are a specific method for treating cancers compared to general cell loss with standard cytotoxic therapeutics. However, the finding that resistance to such therapy is common in clinical trials now dampens the initial enthusiasm over this targeted treatment. Yet an improved molecular understanding of other receptor tyrosine kinases known to be active in cancer has revealed a rich network of cross-talk between receptor pathways with a key finding of common downstream signaling pathways. Such cross talk may represent a key mechanism for resistance to EGFR-directed therapy. Here we review the interplay between EGFR and Met and the type 1 insulin-like growth factor receptor (IGF-1R) tyrosine kinases, as well as their contribution to anti-EGFR therapeutic resistance in the context of squamous cell cancer of the head and neck, a tumor known to be primarily driven by EGFR-related oncogenic signals.

1. Introduction

Squamous cell carcinoma of the head and neck (HNSCC) is a heterogeneous disease that includes tumors arising from the mucosal epithelial surface of the oral cavity, oropharynx, hypopharynx, and larynx. Although these tumors originate within different anatomic sites within the upper aerodigestive tract, they are histologically identical (95% of HNSCC are squamous cell carcinomas), share common etiologic risk factors and overlapping metastatic target site profiles (reviewed in [1–3]). Recent genetic analysis of human head and neck tumors has revealed common molecular alterations including p53 mutation, p14ARF, and p16 methylation, as well as Cyclin D and EGFR amplification [3–6]. Despite these similarities, the distinct anatomic subsites are associated with differing rates of regional metastasis—for example, vocal cord lesions tend to metastasize less frequently than oropharyngeal or hypopharyngeal lesions. This variation may be attributed to differing densities of lymph draining vessels within each of the relevant subsites. Patients who exhibit metastases into the regional nodal basin exhibit a 50% decrease in survival irrespective of treatment [7–15].

The incidence of HNSCC has continued to increase over the last 3 decades. Currently, it is the 5th leading cause of cancer by incidence and the 6th leading cause of cancer mortality in the world [16, 17]. Recurrent and/or metastatic HNSCC patients have a poor prognosis, with a median survival of less than 1-2 years [18, 19].

Several lines of evidence indicate that cancer is a disease resulting from dynamic changes in the genome that promote the progressive transformation of normal human cells into highly malignant derivatives [20, 21]. During this process, cancer cells acquire several unique capabilities including self-sufficiency in response to growth signals, insensitivity to antigrowth signals, evasion of programmed death (apoptosis), limitless replicative potential, sustained angiogenesis as well as invasion and metastasis, reprogramming of energy metabolism, and avoiding immune destruction [21, 22]. Detailed global genomic analyses of several human tumors has revealed that certain classes of signaling proteins appear to be targeted more frequently by oncogenic mutations [23]. Receptor tyrosine kinases (RTKs) are a good example. Of the 59 transmembrane RTKs identified to date, dysregulation of ~30 RTKs are associated with neoplastic transformation and

cancer progression [23–25]. Interestingly, ninety percent of primary head and neck squamous cell cancers, irrespective of subsite, have alterations in members of the epidermal growth factor (EGF) family of receptor tyrosine kinases (ErbBs), in particular ErbB1/EGFR [26]. Ten to fifteen percent of tumors will also have an alteration in another EGFR family member, the ErbB2/HER2/*Neu* receptor [27, 28]. These findings suggest a strong etiologic role for RTK dysregulation in this type of tumors. Given this association, patients with head and neck squamous cell cancers are well positioned to benefit from existing and future molecular targeted agents directed against oncogenic RTKs such as EGFR (reviewed in [29]).

RTKs are a family of transmembrane proteins that mediate many important physiological processes in both normal and cancerous cells. Ligand binding to the extracellular domain of RTKs induces receptor dimerization and activation of RTK activity. Subsequent autophosphorylation of the receptor at specific tyrosine residues within the cytoplasmic domain generates binding sites for proteins that relay downstream biological signals to regulate protein function, protein-protein interactions, and gene expression. Under physiological conditions, RTK signaling is temporally and spatially regulated. However, RTKs that become dysregulated can contribute to cellular transformation. RTK dysregulation can occur through several mechanisms including gene amplification or RTK overexpression, chromosomal translocation to produce constitutively active RTKs, gain of function mutations or deletions that promote ligand-independent RTK activity, escape from negative regulatory mechanisms or local environmental changes, all of which lead to potent oncogenic signaling and hence neoplastic growth. These complex signaling networks use multiple factors to drive the outcome of RTK signaling. Although often depicted as linear pathways, they actually represent an integrated network with various modes of cross-talk, overlapping and distinct functions. Known signaling pathways involved in head and neck tumorigenesis include the phosphatidylinositol-3-kinase (PI3K)-AKT-mammalian target of rapamycin (mTOR), signal transducer and activator of transcription (STATS) and Raf kinase-mitogen-activated protein kinase (MEK)-p42/p44 mitogen activated protein kinase (MAPK) signaling pathways [1, 30]. This review highlights three RTK signaling pathways involved in head and neck squamous cell carcinoma; EGFR, the type 1 insulin-like growth factor receptor (IGF-1R) and the hepatocyte growth factor (HGF) receptor (Met). This short review will explore the relative contribution of each signaling axis to disease progression, potential modes of cross-talk, and targeted clinical approaches under investigation for disease management.

2. EGFR Amplification in Head and Neck Cancers

The EGFR family of RTKs is comprised of four different receptors known as ErbB1 (also referred to as EGFR), ErbB2 (HER2/*Neu* in rodents), ErbB3 (Her3), and ErbB4 (HER4) (reviewed in [31–33]). Each receptor, with the exception

of ErbB3, contain an intracellular tyrosine kinase domain that is activated by binding to extracellular EGF-like ligands, which result in receptor dimerization and hence activation of downstream signaling cascades including MAPK, PI3K/AKT and Stat signaling. Eleven EGF-like ligands have been identified to date that can be categorized into four groups—those that bind EGFR only (EGF, Transforming Growth Factor alpha (TGF α), and amphiregulin), those that bind to EGFR and HER4 (heparin binding-EGF, betacellulin and epiregulin), those binding directly to either HER3 and HER4 (neuregulin 1 and neuregulin 2) and HER4 binding only (neuregulin 3 and neuregulin 4) (reviewed in [34]). Epigen, the most recently discovered member of the EGF-like ligand family appears to be a low affinity and broad specificity ligand that effectively activates EGFR [35]. Epigen is unable to activate HER3 and HER4 in the absence of ErbB2 expression. ErbB2 is considered a ligand-less coreceptor as it does not have any known ligands that bind directly with high affinity, despite its established role as a potent oncogene in several cancer types including breast, colorectal, non-small cell lung carcinoma (NSCLC) and HNSCC [36, 37].

Aberrant EGFR activity has been strongly linked to the etiology of 58–90% of HNSCC [26, 38]. These rates can vary due to the inclusion of cancers from different subsites within the head and neck, methods used to assess gene amplification and tumor scoring methods. In contrast to lung adenocarcinomas in which activating EGFR mutations result in ligand-independent signaling [39–43], such activating EGFR mutations are infrequent in HNSCC [44, 45]. EGFR gene amplification resulting in upwards of 12 copies per cell has been reported in HNSCC patients compared to copy numbers detected in normal mucosa from noncancer patients [46]. This and other pathways of ligand-independent receptor activation that do not require EGFR overexpression have been characterized as the likely drivers of EGFR activity in HNSCC.

EGFR gene amplification remains a strong indicator for poor patient survival, radioresistance, and locoregional failure [47–49]. EGFR overexpression is detected in healthy mucosa in cancer patients (field cancerization) that will increase in proportion to observed histological abnormalities such as hyperplasia, carcinoma *in situ* and invasive carcinoma, indicating that it is an early event in HNSCC. Accordingly, significant effort has focused on EGFR signaling as a therapeutic target for treating HNSCC patients. The FDA has approved several EGFR-targeted reagents for treating HNSCC. Cetuximab, matuzumab and nimotuzumab represent humanized antiEGFR antibodies, whereas gefitinib and erlotinib are small tyrosine kinase inhibitors (TKIs) (Figure 1). Cetuximab (Erbbitux) competitively inhibits endogenous ligand-binding to EGFR and thereby inhibits subsequent receptor activation [50–53]. Cetuximab is a valuable treatment option in head and neck patients as it synergizes with current treatment modalities. Cetuximab enhances the effects of many standard cytotoxic agents, including cisplatin (the conventional platinum-fluorouracil chemotherapeutic), and in combination with chemotherapy it can elicit antitumor responses in tumors that previously failed to respond to that chemotherapy [54]. Cetuximab has

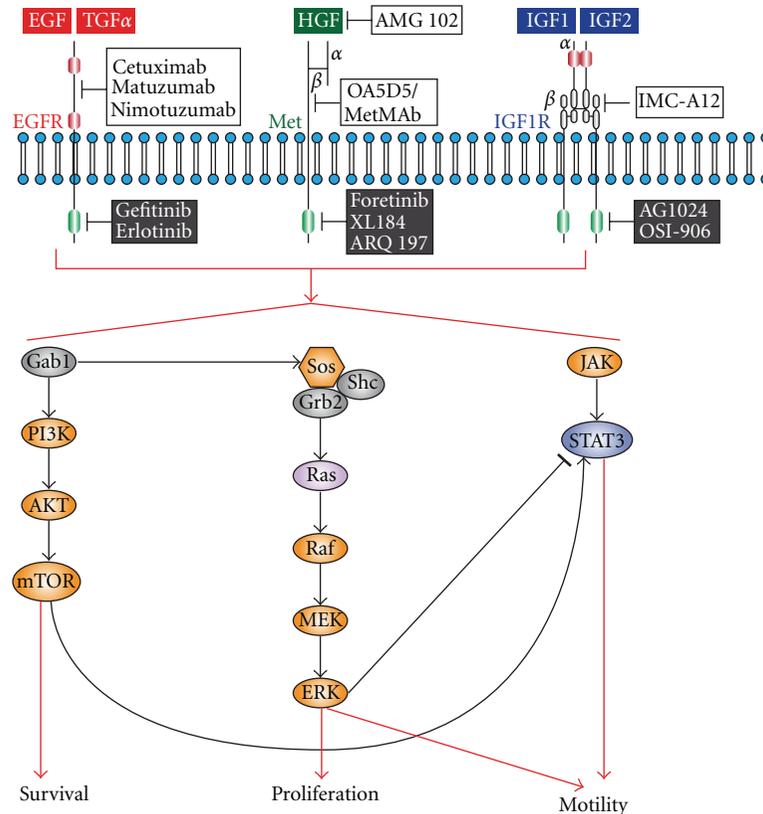


FIGURE 1: Targeted RTK and their signal transduction routes in head and neck cancer. The EGFR, Met, and IGF-1R receptors and their prototypic ligands are shown. Cysteine-rich domains (red box) and fibronectin type III-like domain (grey box) are indicated in the extracellular domains of the EGFR and IGF-1R, respectively. Cytoplasmic tyrosine kinase domains for each receptor are indicated (green boxes). The symbols α and β denote distinct RTK subunits. Targeted humanized monoclonal antibodies for extracellular components (white box) and TKIs (black box) for each receptor signaling axis is shown.

also been reported to enhance radiation-induced apoptosis. Notably, cetuximab did not dramatically exacerbate the common toxic effects associated with radiotherapy of the head and neck, including mucositis, xerostomia, dysphagia, pain, weight loss, and performance status deterioration [55]. Cetuximab has been approved for use in combination with radiation for treating patients with locally advanced HNSCC [56] and as monotherapy for patients with recurrent HNSCC [57]. Matuzumab (formerly EMD 72000) binds to EGFR with high specificity and affinity to block receptor signaling, and also modulates antibody-dependent cellular cytotoxicity (ADCC) when combined with cetuximab [58–60]. Phase I clinical trials report excellent antitumor activity of matuzumab against several human tumor types including head and neck cancers [61]. A randomized Phase IIb, four-arm, open-label study recently assessed the safety and efficacy of nimotuzumab in combination with radiation therapy (RT) or chemoradiation therapy (CRT) in patients with advanced (Stage III or IVa) HNSCC [62]. The addition of nimotuzumab to both the radiation and chemoradiation regimens was reported to improve the overall response rate, survival rate at 30 months, median progression-free survival and median overall survival. A combined group analysis of the nimotuzumab arms versus the non-nimotuzumab

arms demonstrated a significant difference in overall survival favoring nimotuzumab. This study is compelling as patient response rates compare favorably with studies combining cetuximab with radiotherapy, but with fewer side effects [62]. Gefitinib (Iressa) is a small molecule TKI-targeted to the intracellular active site for phosphorylation that has been tested in clinical trials involving HNSCC patients, as a single agent or in combination with radiation treatment. Unfortunately, gefitinib has shown limited clinical efficacy with response rates of 10–15% [63, 64]. Erlotinib is a selective inhibitor of the EGFR that also shows antitumor activity in HNSCC comparable to standard combination chemotherapy [65].

3. Targeting IGF-1R Signaling in Head and Neck Cancers

Another promising RTK under preclinical and clinical evaluation for head and neck cancers includes the IGF-1R (reviewed in [66, 67]). Two ligands, insulin-like growth factor 1 (IGF1) and IGF2 bind to IGF-1R. Ligand binding to the IGF-1R stimulates its intrinsic tyrosine kinase activity, activating downstream signaling networks including Ras-Raf, MAPK and ERK, and PI3K (Figure 1) to drive cellular

functions such as cell growth, survival and differentiation. It is widely accepted that the IGF-axis activates antiapoptotic signaling, which in turn upregulates the PI3K-Akt and MAPK pathways in cancer cells [68]. Additionally, IGF-1R also regulates vascular endothelial growth factor (VEGF) production, suggesting a role in tumor angiogenesis [69]. Several studies indicate that IGF-1R is overexpressed and functional in 94% of HNSCC patient samples [70, 71]. Consistent with this, IGF-1R signaling significantly enhances the proliferation, motility and tumorigenicity of human head and neck cancer cell lines [71]. IGF-1R down regulation in a HNSCC cell line using antisense oligonucleotides resulted in a dose-dependent decrease in cellular proliferation, induction of apoptosis, caspase activation and reduced expression of proangiogenic cytokines such as VEGF. Interest in targeting the IGF-1R in HNSCC was bolstered by the observation that treatment of head and neck cancer cells with either IGF or EGF resulted in IGF-1R and EGFR heterodimerization [71, 72]. However, only IGF resulted in the phosphorylation of both receptors. Using a mouse xenograft model for HNSCC, treatment with antibodies against IGF-1R, EGFR or both receptors resulted in significant differences in median tumor volume. It remains to be determined whether cellular cross-talk between IGF-1R and EGFR has an important role in determining the biological aggressiveness of HNSCC or resistance to EGFR-targeted therapies.

Several monoclonal antibodies and TKIs for IGF-1R have been tested in preclinical studies and early phase clinical studies. However, the efficacy of IGF-1R-targeted therapy for treating patients with HNSCC, particularly cross-talk with EGFR, warrants further investigation. To date, the effect of blocking oncogenic IGF-1R and EGFR signaling have been studied more extensively in breast cancer cell lines [73–75]. Treatment with gefitinib and AG1024, a TKI for IGF-1R reduced cell proliferation when used as single agents and showed an additive effect when used in combination [76, 77]. Targeting IGF-1R and EGFR signaling is currently under evaluation in hormone-sensitive metastatic breast cancer using the IGF-1R inhibitor OSI-906 and the EGFR TKI erlotinib, although results are not yet available (<http://www.clinicaltrials.gov/>, Identifier NCT01205685). Similarly, an exploratory study to assess the modulation of biomarkers in HNSCC patients treated preoperatively with cetuximab and/or IMC-A12, a humanized anti-IGF-1R monoclonal antibody is currently underway (<http://www.clinicaltrials.gov/>, Identifier NCT00617734). These studies will be critical for evaluating whether the use of anti-IGF-1R and EGFR-targeted treatments will be more effective than single-agent modalities for treating patients with HNSCC.

4. A Role for Met/HGF Signaling in Head and Neck Cancers

The Met/HGF signaling axis is frequently upregulated and functional in HNSCC. The Met receptor is a single pass transmembrane protein that upon binding its ligand HGF—also known as scatter factor—promotes increased cell proliferation, survival and motility (reviewed in [78, 79]). HGF is the only physiological ligand for Met and

is secreted as an inactive precursor polypeptide chain by mesenchymal cells. HGF is proteolytically cleaved to form an active α/β heterodimer by a number of serine proteases including urokinase plasminogen activator (uPA), tissue-type plasminogen activator (tPA), coagulation factors X, XI, and XII. Met is a disulphide-linked α/β heterodimer derived from the proteolytic cleavage of a 170 KDa precursor. The α chain is exclusively extracellular while the β chain spans the membrane once. The α chain and N-terminal region of the β -chain form α sema domain, a seven β -propeller structure in which blades 2 and 3 bind to HGF. The sema domain is flanked by a cysteine-rich region followed by four immunoglobulin repeats. It is proposed that the cysteine-rich region and immunoglobulin repeat domains undergo a conformational change following HGF binding allowing for Met dimerization [80, 81].

Binding of HGF to Met results in receptor autophosphorylation at key catalytic residues and subsequent recruitment of several cytosolic signaling molecules that are shared with the EGFR and IGF-1R signaling pathways, including the Grb2/Sos complex, the p85 regulatory subunit of PI3K, Gab1 and Jak/Stat3 (Figure 1). Subsequent activation of the MAPK and Jun-N-terminal Kinase (JNK) pathways is responsible for the mitogenic and motogenic properties of Met/HGF signaling resulting in “invasive growth”, depending on the physiological setting [79].

Increased Met signaling in human cancers can be the result of enhanced ligand-binding (autocrine and paracrine), Met overexpression or missense mutations that often induce constitutive kinase activity, failure of Met down regulation and interactions with other cell surface receptors such as EGFR (reviewed in [82–84]). Met is overexpressed in 84% of HNSCC patient samples [85]. Interestingly, amplification of the MET gene (>10 copies per cell) is present only in 3 of 23 (13%) tumor tissues. HGF overexpression is detected in 45% of HNSCCs, suggesting that HGF functions predominantly in a paracrine manner to drive Met signaling in these cancers. Moreover, high levels of HGF are detected in HNSCC patient plasma samples [86] supporting the idea that ligand availability is not a limiting factor for Met activation. Mutations in the Met ligand-binding domain (T230M/E168D), transmembrane or JM domain (R988C, T1010I) and the tyrosine kinase domain (T1275I, V14333I) have also been identified in HNSCC tumor samples [85], although their relative contribution to HNSCC progression remains to be determined. Two somatic Met mutations have been detected in HNSCC that result in constitutively active receptor signaling that confers an invasive phenotype when ectopically expressed in cell lines [87]. The Y1230C mutation confers anchorage-independent growth and an invasive phenotype in transfected cells, whereas the Y1235D Met mutation stimulates epithelial cells to invade reconstituted basement membrane in the absence of HGF. In the case of the MetY1235D mutation, genomic analyses of HNSCC patient samples detected the presence of this mutant allele in 50% of metastatic tumors versus 2–6% in primary tumors, raising the possibility that this could be a critical genetic lesion for the acquisition of a metastatic phenotype. Alternatively, increased Met signaling could afford HNSCC a selective

advantage for growth and/or survival in metastatic sites, such as the lymph node and lung. Indeed several studies indicate that Met overexpression correlates highly with lymph node metastasis, pathologic stage, and disease recurrence [88–91]. Moreover, patient survival was significantly reduced in biopsy samples with positive Met expression relative to negative Met expression, suggesting the association of Met with HNSCC disease progression. Consistent with these findings, treatment with the TKI PF-2341066 caused a significant reduction in tumor growth, a high level of apoptosis and cellular debris within the tumor using a xenograft animal model for HNSCC [91].

Selective inhibitors of Met/HGF signaling include humanized monoclonal antibodies for HGF and Met, and small-molecule tyrosine kinase inhibitors directed against Met (Figure 1). Although their efficacy for treating a variety of solid tumors is increasingly recognized, we await results of preclinical and clinical trials for head and neck cancer that are ongoing. The humanized antibody AMG 102 shows high potency towards the mature and processed form of HGF with no detected effects on proteolytic activation of proHGF. AMG 102 interferes with Met signaling, by competing with HGF for binding to the β chain of the Met receptor [92]. In phase I clinical studies in patients with advanced solid tumors, 70% of patients had a best response in terms of achieving stable disease [93, 94]. Drug-related toxicities included mild fatigue and gastrointestinal symptoms. Importantly, no antiAMG 102 antibodies were detected and circulating HGF levels were dose dependent [93]. Another promising clinical therapeutic is the one-armed 5D5 humanized antibody (OA5D5/MetMab) directed against Met. MetMab binds Met with high affinity, preventing HGF binding, Met phosphorylation, receptor internalization and downstream signaling events and has been shown to inhibit tumor growth in animal models by more than 95% [95, 96]. MetMab is currently in phase I/II human clinical trials in comparison with erlotinib in patients with NSCLC (<http://www.clinicaltrials.gov/>, Identifier NCT00854308). Future clinical trials will be required to determine the suitability of AMG102 and MetMab as either single agents or combinatorial therapeutics for treating HNSCC patients. Foretinib (formerly XL880) is a TKI whose primary targets include Met and VEGF, and to a lesser extent the platelet-derived growth factor (PDGF) receptor, Ron, Kit and TIE2 RTKs [97]. Foretinib recently completed phase II clinical trials in head and neck patients (<http://www.clinicaltrials.gov/>, Identifier NCT00725764). Interim results suggest that after 12 months, 12 of 18 patients had stable disease [83]. XL184 is another TKI agent entering phase III clinical trials. XL184 targets Met, VEGFR2, and Ret. A phase I dose-escalation study of the safety and pharmacokinetics of XL184 administered orally to patients with advanced malignancies (showed that, on average, patients survived for more than 3 months with several up to 6 months while on treatment) (reviewed in [84]). Due to encouraging data from this study, a randomized phase III trial of XL184 in HNSCC patients was initiated to investigate XL184 as a first-line treatment (compared with placebo) for survival benefit to

patients with HNSCC (<http://www.clinicaltrials.gov/>, Identifier NCT00704730). ARQ197 (ArQule) is a nonATP-site competitive, selective small molecule inhibitor of the Met intracellular region [98]. Although the mechanism of ARQ197 is presently unknown, the results of phase I trials suggest potential antiinvasive activity for this compound [99]. Overall, Met, and HGF-targeted therapies have been well tolerated in clinical trials with negligible toxicities. However, it remains to be determined whether Met is a better therapeutic target than HGF. Clearly, in patients where Met is activated by autocrine HGF secretion, both HGF and Met targeted therapies may prove to be more efficacious treatment options.

5. Understanding Resistance to EGFR-Targeted Therapies in HNSCC

Acquired resistance is likely the result of several mechanisms including (1) EGFR mutations initially present as well as those acquired during therapy, (2) receptor independent activation of downstream signaling cascades, (3) cross-talk with other RTKs and converging signaling pathways and (4) environmental factors including inflammatory agents and viral infection. Resistance to cetuximab has been associated with the coexpression of the truncated EGFR mutant, EGFRvIII with wild-type EGFR. EGFRvIII is the result of an in frame deletion of exons 2–7 spanning the extracellular ligand-binding domain. The deletion results in a truncated EGFR receptor that signals in a ligand-independent manner [100]. EGFRvIII expression has been detected in 42% of HNSCC patient samples, and closely correlates with increased HNSCC cell proliferation *in vitro* and increased tumor growth using *in vivo* xenograft models. EGFRvIII preferentially activates the PI3K pathway instead of the Ras/Raf/MEK pathway, which is activated by wild-type EGFR [101]. Of particular interest to the therapeutic treatment of HNSCC, EGFRvIII expression decreases the proliferative response of EGFR expressing tumor cells to cetuximab treatment relative to vector control cells. In a recent study, EGFRvIII cells were shown to be resistant to the antiinvasive effects of cetuximab due to an increase in phosphorylation of STAT3 rather than increased PI3K signaling. EGF-induced expression of the STAT3 target gene HIF1 α was abolished by cetuximab in HNSCC cells expressing wild-type EGFR under hypoxic conditions, but not in EGFRvIII-expressing HNSCC cells [102, 103]. These data suggest a role for EGFRvIII in mediating HNSCC resistance to cetuximab.

Despite EGFRs critical role in the development of HNSCC, clinical data indicate modest clinical benefits for locoregional control and survival of head and neck cancer patients treated with EGFR-targeted therapies. HNSCC patients resistant to cetuximab, often succumb to local tumor recurrence as well as regional and distant metastasis. The addition of cetuximab to radiation therapy was reported to show improved locoregional disease control, progression-free survival, and overall survival in patients with locally advanced HNSCC [56]. However the data revealed a disproportionate benefit of cetuximab with radiotherapy to

oropharyngeal cancer patients when compared to patients treated with hyperfractionated radiotherapy [57]. Accumulating evidence suggests that human papilloma virus (hpv) 16 status (Hpv+) is an important prognostic factor associated with a favorable outcome in a subset of head and neck cancers, including oropharyngeal and tonsillar cancers [104]. Hpv+ tumors tend to have unique genetic aberrations including decreased EGFR expression, whereas increased IGF-1R levels characteristic of HNSCC appear to be independent of hpv status. Clinically, hpv+ tumors are characterized by more favorable patient prognosis regarding disease-free survival as well as overall survival [104, 105], possibly as a result of increased genomic stability associated with global gene hypermethylation in hpv+ tumors [106]. Thus it will be interesting to determine whether hpv+ status explains some of the benefits derived from the addition of cetuximab to radiotherapy in this subset of HNSCC patients. At present, there are few clinical indicators of which HNSCC patients will most likely respond to EGFR-targeted therapies. Accordingly, strategies to optimize EGFR-targeted therapy remain an active area of research.

Additional mechanisms that result in EGFR activation include activating mutations in downstream signaling components or cross-talk between different RTK pathways. Activating mutations in the PI3KA oncogene occurs in 10% of HNSCC tumors [107] whereas elevated levels of phosphorylated STAT3 correlates with lymph node metastasis and poor patient prognosis [108–110]. Conversely, H-Ras mutations are infrequent in HNSCC cases (less than 5%), although a higher incidence has been detected in Asian populations and correlates with Areca nut chewing [111, 112].

Met signaling has been shown to contribute to resistance in cell lines derived from multiple tumor types including breast, gastric and lung. In one key study, NSCLC with activating mutations in the EGFR acquire resistance to the TKI gefitinib and erlotinib, by amplification of the Met gene to maintain Akt and Her3 signaling [113]. These studies underscore the role of cross-talk between RTKs to preferentially signal through the PI3K-Akt survival pathway as a mechanism for acquired drug resistance. The relevance of Met as a mechanism for escape from EGFR-targeted therapy in head and neck cancers remains to be determined. Hypoxia results in the transcriptional upregulation of Met gene expression via HIF1 α in a number of tumors including head and neck [114], often downstream of EGFR signaling [115]. In normoxia, hydroxylation of 2 prolines in HIF1 α enables its binding to the von Hippel-Lindau tumor suppressor protein (pVHL) linking HIF1 α to a ubiquitin ligase complex. During hypoxia, minimal or no hydroxylation occurs enabling HIF1 α to avoid proteasomal degradation and dimerize to other HIF family members such as HIF1 β and coactivators, to form an active transcriptional HIF complex on the hypoxia response element (HRE) of target genes such as MET [116]. The ubiquitin ligase catalyzes polyubiquitination of HIF1 α targeting it for proteasomal degradation [117]. Under hypoxic conditions, increased Met signaling directs the invasive growth program, enabling cells to invade more oxygenated tissues [118]. Since Met has been

reported to promote invasive and angiogenic effects in the tumor microenvironment, the use of HGF/Met inhibitors may afford a means of impairing tissue colonization as well as tumor vascularization in head and neck cancer patients.

Studies on other solid tumor types, most notably glioblastoma, indicate a role for IGF-1R upregulation in resistance to EGFR-targeted therapies [73]. IGF-1R mediates resistance to anti-EGFR therapy in primary glioblastoma through the continued activation of the PI3K/AKT survival pathway [119]. The apparent cooperation between IGF-1R and EGFR in promoting HNSCC pathogenesis as well as resistance to EGFR-targeted therapy, suggests an advantage to cotargeting these signaling axes for the treatment of head and neck cancers. To date, the effect of blocking oncogenic IGF-1R and EGFR signaling have been studied more extensively in breast cancer lines. Treatment with gefitinib and AG1024, a TKI for IGF-1R reduced cell proliferation when used as single agents and showed an additive effect when used in combination [76, 77]. Targeting IGF-1R and EGFR signaling is currently under evaluation in hormone-sensitive metastatic breast cancer using the IGF-1R inhibitor OSI-906 and the EGFR TKI erlotinib, although results are not yet available (<http://www.clinicaltrials.gov/>, Identifier NCT01205685). Similarly, an exploratory study to assess the modulation of biomarkers in HNSCC patients treated preoperatively with cetuximab and/or IMC-A12, a humanized antiIGF-1R monoclonal antibody is currently underway (<http://www.clinicaltrials.gov/>, Identifier NCT00617734). These studies will be critical for evaluating whether the use of antiIGF-1R and EGFR-targeted treatments will be more effective than single-agent modalities for treating patients with HNSCC.

6. Conclusions

Targeted therapies that block EGFR, Met, and IGF-1R signaling in head and neck cancers continue to show promising results in preclinical studies and clinical trials. However, it is difficult to predict which patients are most likely to benefit from these therapeutics and potential side effects during long-term *in vivo* use. Given the interplay between these RTK signaling pathways and the mediocre results obtained with monotherapy regimens thus far, clinical trials will be required to determine how EGFR-, Met-, and IGF-1R-targeted therapies can be used in combination in order to definitively abrogate their common downstream oncogenic signaling networks. Although gaps in our knowledge concerning the role of Met and IGF-1R in head and neck tumorigenesis, as well as acquired resistance to antiEGFR therapies remain to be addressed, efforts to translate current information towards clinical applications continue to be impressive.

Abbreviations

EGF: Epidermal growth factor
 EGFR: EGF receptor
 ErbBs: Epidermal growth factor receptor family of receptor tyrosine kinases

HGF: Hepatocyte growth factor
 HIF1 α : Hypoxia-inducible factor 1 alpha subunit
 HNSCC: Squamous cell carcinoma of the head and neck
 Hpv: Human papilloma virus
 HRE: Hypoxia response element
 IGF: Insulin-like growth factor
 IGF-1R: Type 1 insulin-like growth factor receptor
 mTOR: Mammalian target of rapamycin
 MAPK: p42/p44 Mitogen Activated Protein Kinase
 Mek: MAPK kinase
 Met: HGF receptor
 NSCLC: Nonsmall cell lung carcinoma
 PI3K: Phosphatidylinositol-3-kinase
 PDGF: Platelet-derived growth factor
 RTK: Receptor tyrosine kinase
 STAT: Signal transducer and activator of transcription
 TKI: Tyrosine kinase inhibitor
 VEGF: Vascular endothelial growth factor
 VEGFR: VEGF receptor
 uPA: Urokinase plasminogen activator
 tPA: Tissue-type plasminogen activator.

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