Published in final edited form as:
Clin Cancer Res. 2010 May 1; 16(9): 2489–2495. doi:10.1158/1078-0432.CCR-09-2318.

New Strategies in Head and Neck Cancer: epidermal growth factor receptor inhibition in head and neck cancer

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Abstract

The epidermal growth factor receptor (EGFR) is a validated target in squamous cell carcinoma of the head and neck (HNSCC). However, despite high expression of EGFR in these cancers, EGFR inhibitor monotherapy has only had modest activity. Potential mechanisms of resistance to EGFR-targeted therapies involve EGFR and Ras mutations, epithelial-mesenchymal transition, and activation of alternative and downstream pathways. Strategies to optimize EGFR-targeted therapy in head and neck cancer involve not only the selection for patients most likely to benefit but also employing combination therapies to target the network of pathways involved in tumor growth, invasion, angiogenesis, and metastasis.

1. Background

Epidermal Growth Factor Receptors in Squamous Cell Carcinomas of the Head and Neck

Epidermal growth factor receptor (EGFR) is a ubiquitously expressed transmembrane glycoprotein in the ErbB/HER family of receptor tyrosine kinase. These receptors are composed of an extracellular ligand-binding domain, a hydrophobic transmembrane segment, and an intracellular tyrosine kinase domain. Binding of natural ligands (amphiregulin and transforming growth factor alpha (TGF-α) in head and neck cancer) to EGFR results in a conformational change in EGFR. This promotes homo- or heterodimerization with other ErbB/HER family of receptors with subsequent autophosphorylation and activation of the tyrosine kinase (1). This activation of EGFR leads to the initiation of intracellular signaling pathways which regulate the activation of cell proliferation, invasion, angiogenesis, and metastasis (1).

High expression of EGFR occurs in most epithelial malignancies including head and neck squamous cell carcinoma (HNSCC) (1). Elevated expression of EGFR in HNSCC correlates with poor prognosis (1). Two therapeutic strategies have been implemented in the inhibition of EGFR. The first utilizes monoclonal antibodies (mAb) to target the extracellular domain of EGFR and the second targets the intracellular EGFR domain with small molecule tyrosine kinase inhibitors (TKIs) (including gefitinib, erlotinib, and lapatinib). Despite near universal expression of EGFR in HNSCC, treatment with these anti-EGFR agents has only been modestly active in patients. Two FDA-approved monoclonal antibodies for targeting EGFR are cetuximab (a chimeric IgG1 mAb) and panitumumab (a fully human IgG2 mAb). Preclinical data from Bonner et al in 2000 showed that cetuximab and concurrent radiation resulted in a greater decrease in cell proliferation in a number of HNSCC cell lines (2). A multicenter phase III trial demonstrated an improvement in median overall survival in locoregionally advanced

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HNSCC patients treated with curative intent with definitive radiotherapy combined with weekly cetuximab versus the same radiotherapy regimen alone (3). There was an improvement in 3-year survival by 10% in patients receiving concurrent cetuximab and radiotherapy (3). However, the efficacy of cetuximab with radiotherapy compared with standard concomitant chemoradiotherapy remains under investigation. Preclinical data show that there is at least an additive effect of both classes of EGFR inhibitors when combined with cisplatin in the treatment of HNSCC (4).

Furthermore, cetuximab combined with platinum-fluorouracil chemotherapy improves survival compared with platinum-fluorouracil alone in patients with recurrent or metastatic HNSCC (5,6). Adding cetuximab increased median overall survival from 7.4 months in the platinum chemotherapy-alone group to 10.1 months in the group receiving chemotherapy plus cetuximab (7). In a phase II trial of gefitinib in patients with recurrent or metastatic HNSCC, the overall response rate with gefitinib was 11% (8). In a similar population of recurrent and/or metastatic HNSCC patients, erlotinib was shown by Soulieres et al to have a response rate of 4% (9). A phase I study of chemoradiotherapy combined with lapatinib, a dual inhibitor of EGFR and HER2, for locally advanced HNSCC reported an overall response of 81% (10). BIBW2992, an irreversible dual inhibitor of EGFR and HER2 tyrosine kinase, which binds to Cys773 of EGFR and Cys805 of HER2, is currently being evaluated in clinical trials for HNSCC (11). A feature of BIBW2992 is its broad activity against multiple receptors in the ErbB family making it theoretically more effectively against tumor cells containing several ErbB family members and heterodimerizations. In preclinical studies it has been shown to inhibit cellular proliferation of lung cancer cell lines resistant to erlotinib, and cause tumor regression in xenografts and transgenic lung cancer models (11).

Mechanisms of Resistance to EGFR-Targeted Therapies

Even with high levels of EGFR expression within the tumor, clinical data demonstrate that many patients are refractory to EGFR inhibitor treatment underscoring that simple EGFR expression is not a reliable predictor of response to therapy. Primary resistance occurs in patients who either do not achieve stable disease or who progress within months after an initial clinical response while secondary or acquired resistance typically occurs after prolonged treatment. The majority of patients with HNSCC will be resistant to EGFR inhibitors and the mechanisms underlying this observation [Table 1] are beginning to be understood.

Among the first genetic alterations of the EGFR that have been identified, the type-III mutated variant (EGFRvIII) is characterized by an in-frame deletion from exons 2 through 7 in the extracellular domain which inhibits EGF and other EGFR ligands from binding and leads to constitutive activation of its tyrosine kinase domain (1). Structural changes in extracellular EGFR are hypothesized to affect the intracellular domain conformation and the ATP pocket leading to the constitutive activation by EGFRvIII and its resistance to EGFR-targeted therapy by monoclonal antibodies against the extracellular domain of EGFR (1). Irreversible EGFR inhibitors such as BIBW2992 have the added advantage in preclinical studies of being effective against EGFRvIII (12).

K-ras (v-KI-RAS2 Kirsten rat sarcoma viral oncogene homolog) mutations also predict resistance to EGFR inhibitors (Figure 1) (13). Retrospective analyses of clinical trials using TKIs have demonstrated a significant decrease in time to progression and survival in the presence of K-ras mutations in colorectal cancer (13). Data also suggest K-ras mutation status to be predictive of lack of response to mAbs cetuximab and panitumumab in metastatic colorectal cancer patients and is also associated with a worse prognosis (14,15). However, K-ras mutations infrequently occur in HNSCC. Of the Ras proteins, however, H-ras mutations in HNSCC are likely more common than K-ras mutations and may play an important role in resistance to EGFR-targeted therapies (16,17).

In NSCLC, both high EGFR gene copy number as determined by fluorescent in situ hybridization (FISH) and EGFR tyrosine kinase mutations that lead to increased protein activity after ligand binding appear to be correlated with improved response to TKI (18). Even so, the same correlation rarely exists in HNSCC. In fact, EGFR TK mutations appear to be rare events in HNSCC underscoring essential differences between the two diseases. Moreover, recent findings by Lictra, et al demonstrated no association between overall survival in HNSCC and EGFR gene copy number as determined by FISH (19). The same negative findings were observed in a randomized study of gefitinib vs. methotrexate in recurrent/metastatic HNSCC where EGFR gene copy number was not predictive of a survival benefit in subjects treated with the EGFR TKI. Interestingly, response to TKIs in a subset of HNSCC may in fact be due to mutations in ErbB2 rather than EGFR but this preliminary finding has yet to be validated. (20).

Secondary or acquired resistance typically occurs in the setting of prolonged treatment. Several mechanisms contribute to the development of resistance including epithelial-mesenchymal transition, the development of secondary mutations in EGFR, activation of alternative pathways, and constitutive activation of downstream pathways. Epithelial to mesenchymal transition (EMT) is characterized by a change in the morphology with loss of polarity and cell-cell contacts by the epithelial cells with increased vimentin expression, and decreased E-cadherin, claudins 4 and 7 expression (21). Preclinical models of NSCLC cell lines and xenografts demonstrated a correlation between a mesenchymal phenotype and erlotinib resistance (21). In addition, clinical data in NSCLC patients receiving erlotinib and chemotherapy showed time to progression was better in patients with E-cadherin positive staining (22). Recently, cortactin, a cytoskeletal protein that regulates actin assembly, receptor-mediated endocytosis, and epithelial to mesenchymal phenotypic conversion of cells, has been associated with gefitinib resistance and invasive phenotype in HNSCC (23,24). In addition, the E-cadherin repressor delta-crystallin enhancer binding factor 1 (deltaEF1) was recently identified as a regulator of mesenchymal phenotype and correlated with erlotinib resistance in HNSCC *in vitro* (24).

Inherent cell signaling is a significant factor in response to therapy. Upregulation of cyclin D1 in HNSCC cell lines is specifically associated with resistance to gefitinib by hyperphosphorylation of retinoblastoma protein (pRb) by cyclin D1-cyclin dependent kinase 4 (CDK4) (25). Furthermore, mutations or decreased expression of PTEN, a phosphatase regulator of PI3K/AKT signaling, is also associated with EGFR inhibitor resistance [Figure 1] (26). In cells dependent on EGFR, loss of PTEN was shown to uncouple the EGFR from its downstream signaling pathway and the presence of constitutive activation of AKT leads to cell survival independently of EGFR (26).

Cumulative evidence suggests that activation of signaling pathways downstream of EGFR may contribute to resistance to upstream inhibition of EGFR. Cancer cells have been shown to selectively activate alternative signaling pathways in the setting of single pathway inhibition. Stommel et al reported that in glioblastoma cell lines, xenografts and primary tumors, various receptor tyrosine kinases are simultaneously activated resulting in the sustained activation of signaling pathways in the face of receptor TKI monotherapy (27). In fact, blockade of specific pathways have been shown to initiate feedback mechanisms that trigger pro-survival signaling cascades in cancer. For example, inhibition of the PI3K/Akt pathway stimulates the MAPK/ERK signaling cascade in different cancer models (28).

Activation of cytoplasmic signaling pathways in the setting of EGFR blockade can occur through several mechanisms including: 1) concomitant activation of other receptor and non-receptor kinases including c-Met, IGF-1R, and Src family kinases, among others; 2) G-protein-coupled receptor (GPCR)-mediated activation of EGFR-independent pathways; and/or 3)

induction of alternative oncogenic pathways by EGFR blockade. Signal transducer and activator of transcription-3 (STAT3) mediates proliferative, survival and invasion pathways in HNSCC induced by upstream activation of EGFR, Src and/or IL-6/gp130 (29,30). We previously reported that increased activation of signal transducer and STAT3 was associated with increased resistance to EGFR tyrosine kinase inhibition in HNSCC (31). Further investigation demonstrated that siRNA-mediated knockdown of EGFR or treatment with cetuximab induced oncogenic signaling through activation of p70S6 kinase in HNSCC, in the setting of GPCR stimulation (unpublished observations).

Single nucleotide polymorphisms (SNPs) play a role in drug pharmacokinetics and pharmacodynamic processes. They are not only a factor in drug efficacy but also contribute to drug toxicity. The first intron of the EGFR gene has an important regulatory function and contains a heritable polymorphic microsatellite sequence of 9–23 CA repeats (32). The number of CA repeats is inversely proportional to EGFR expression on both mRNA and protein level *in vitro* (32). Two SNPs associated with increased expression of EGFR are in the promoter (−216G>T and −191C>A) (33). Preclinical data show a nonsynonymous SNP (1808G>A) in the extracellular domain of EGFR is associated with a lower affinity for ligand (EGF and TGFα) binding and an abated growth response (34). Amador et al. reported higher sensitivity to erlotinib in cell lines with less than or equal to 35 CA repeats compared with cell lines with greater than 35 repeats. There was also increased incidence of skin toxicity in gefitinib treated colorectal patients with less than or equal to 35 CA repeats (32). However, in a single arm study in patients with HNSCC, NSCLC, and ovarian cancer treated with erlotinib, even though there was a correlation between diarrhea and two EGFR promoter SNPs, the same correlation was not seen with skin toxicity (35).

EGFR-targeted monoclonal antibodies (mAbs), but not tyrosine kinase inhibitors, are FDA-approved for use in HNSCC. This apparent increased activity of antibody-mediated therapeutic strategies suggests that the immune system may contribute to clinical responses to EGFR targeting. Currently, the two FDA-approved mAbs targeting EGFR are cetuximab and panitumumab. Monoclonal antibodies recognize determinants expressed on the extracellular domain of EGFR and antagonize normal ligand-receptor interactions thereby disrupting downstream signaling. The mechanism(s) underlying the clinical response to EGFR-specific mAb-based immunotherapy are poorly understood. Evidence to date suggests that mAbs may induce activation of cellular immunity, including natural killer and T cells, thereby contributing to clinical response. Monoclonal antibodies have been shown to mediate antibody-dependent cellular cytotoxicity, complement-dependent lysis, and activation of tumor antigen-specific T cells. Cell-mediated cytotoxicity of target cells triggered by EGFR-specific mAbs appears to play a role in the clinical outcome of colorectal carcinoma patients (36). The variables influencing the extent of lysis of HNSCC cells by NK cells and EGFR-specific mAbs have been characterized, and have shown to include the level of EGFR expression, the amount of mAb, and the genotype of the Fcγ receptor (FcγR) which mediates the interactions of NK cells with the mAbs bound to target cells, i.e. FcγR IIIa (37). In addition, mAbs may mediate NK cell-dependent lysis of HNSCC cells. The lysis of HNSCC cells by NK cells and the EGFR-specific mAb cetuximab may trigger a series of events, which lead to the generation of cytotoxic T lymphocytes (CTL) recognizing tumor antigens expressed on the HNSCC cells. A variety of factors such as polymorphisms in Fcγ receptors expressed by immune cells, activity of T-regulatory cells, and tumor escape through downregulation of antigen-processing machinery in tumor cells, may modulate the immune activation mediated by therapeutic mAbs. Understanding the interplay of these factors is likely to improve the selection of the most appropriate candidates for mAb-based immunotherapy, prediction of clinical response, and our understanding of mechanisms of tumor escape from therapeutic mAbs.

2. On the Horizon

Predictive Markers for Response to EGFR-Targeted Therapies

In the current era of targeted therapies, the identification of predictive markers remains a challenge. Predicting outcome in EGFR-targeted therapies is complex, involves genetic and clinical characteristics, and the interplay of a network of pathways. The human papilloma virus (HVP), i.e. HPV type 16, has recently been identified to be associated with a subset of HNSCC, especially those arising from the lingual or palatine tonsils (38). Studies in oropharyngeal cancers have shown an association between lower HPV titers and high EGFR expression with worse overall survival (39). Several studies report a positive association between EGFR gene amplification and response to EGFR-directed antibody treatment in NSCLC and metastatic colorectal cancers (40,41). Although recent reports suggest that EGFR gene copy number by FISH is not correlated with response in HNSCC, different methodologies and scoring methods are used which is compounded by the intra-tumor heterogeneity observed in EGFR gene copy in HNSCC. These factors account for the variation in EGFR gene amplification and copy number rates reported thus far in HNSCC and further investigation to characterize the role of EGFR gene amplification remains to be performed. The presence of EGFR gene amplification in a significant portion of HNSCC suggests that this still may be a potential predictive marker for response to EGFR-targeted therapies.

Other candidate predictive markers for EGFR-targeted therapies include K-ras/H-ras mutations, PI3k/Akt pathway mutations, and polymorphisms in EGFR, FcγRIIa and FcγRIIIa. K-ras mutations in NSCLC vary from 8–20% and approximately 30% in colon cancers (42). K-ras mutations are relatively rare (3–7%) in HNSCC; however, H-ras mutations occur at a higher rate (22% per one published study available) and may be a potential marker for decreased response to EGFR-targeted treatment (16,17). Interestingly, H-ras mutations frequently occur in HNSCC patients from India and Southeast Asia and the oral carcinogenesis is likely related to betel quid use (43,44). In metastatic colorectal cancer, mutations in the PI3K catalytic subunit (PIK3CA) have been reported to correlate with EGFR mAb resistance (45). Although PIK3CA mutations only occur in up to 8% of HNSCC, loss of the expression of the phosphatidylinositol phosphatase, PTEN, results in the loss of negative regulation on the Akt signaling pathway which leads to resultant activation of downstream survival mechanisms (26). Therefore, even though few data in HNSCC are available, the data from metastatic colorectal cancer suggest PTEN expression, PIK3CA mutation status, and Akt amplification may be potential predictive markers in HNSCC and warrant further investigation.

Finally, matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) has been shown to predict survival after treatment of lung cancer patients with EGFR-targeted therapy and recent data suggest MALDI-MS also has predictive value in HNSCC (46). A MALDI-MS profile was previously determined from over four hundred NSCLC patient serum or plasma samples to predict overall survival after EGFR TKI treatment (47). More than 300 samples from 5 HNSCC treatment groups (gefitinib, erlotinib and bevacizumab, cetuximab, surgery, and palliative chemotherapy) were then stratified into good or poor prognostic groups using the same MALDI-MS algorithm generated from NSCLC patients. Results demonstrated 98% success in classifying the HNSCC samples and predicting survival benefit in the EGFR-inhibitor treated groups (46).

The observation that cetuximab, but not EGFR TKI, prolong HNSCC survival when combined with standard therapeutic approaches suggests that the mechanism of EGFR targeting may be important (48). Based on improved anti-tumor effects in HNSCC preclinical models when EGFR expression was downregulated, a phase I antisense gene therapy trial was carried out (49). In this study, EGFR antisense therapy decreased EGFR protein expression in nearly all 17 patients treated where higher baseline levels of EGFR in the tumor were associated with an

enhanced clinical response (49). Studies are underway to develop an antisense strategy to target EGFR that can be safely and effectively delivered systemically to HNSCC patients. In addition to antisense, RNA interference (RNAi) approaches are being developed to suppress EGFR expression as a potential clinical strategy (50).

Combination Therapy to Overcome Resistance to EGFR-Targeted Therapy

One way to overcome resistance to EGFR-targeted therapy is to use a combination of monotherapeutic agents with different mechanisms of action to target the network of pathways involved in HNSCC pathogenesis. Approaches to combination therapy can involve adding STAT or SRC inhibitors, c-MET or IGFR inhibitors, mammalian target of rapamycin (mTOR) inhibitors, and other receptor TKIs, to anti-EGFR agents. Preclinical studies of HNSCC involving both EGFR and STAT3 inhibition have been effective in increasing apoptosis (51). EGFR and IGF-IR provide compensatory activation of similar downstream pathways when either is inhibited. AKT is controlled by both EGFR and IGF-IR. Buck et al demonstrated that inhibition of EGFR or IGFR activated the reciprocal receptor with a shift of EGFR inhibition of AKT from EGFR to IGF-IR (52). Guix et al reported the association between loss of IGF-binding protein as a mechanism of acquired EGFR TKI resistance and suggests the combination of EGFR and IGFIR inhibitors may be effective in abrogating this resistance(53). Similarly, gastric cancer and breast cancer cells treated with the combination of anti-EGFR and a c-MET inhibitor resulted in decreased cell proliferation warranting preclinical evaluation of this combination in HNSCC (54,55).

mTOR inhibitors block activation of PI3K and AKT pathway signals involved in cellular proliferation and angiogenesis (56). Studies involving mTOR inhibitors and TKIs (erlotinib and gefitinib) have demonstrated an additive antitumor effect in HNSCC, colon and pancreatic cancer cell lines forming the basis for ongoing phase I trials of the mTOR inhibitor RAD001 in combination with cetuximab (56,57). The vast majority of HNSCC express VEGF or VEGFR and therapy combining bevacizumab (a monoclonal antibody against VEGF-A) and erlotinib are promising with a response rate of 15% (58). Preclinical studies of human A431 squamous cell cancer xenografts show acquired resistance to mAb can develop via increased expression of VEGF further underscoring the multiple growth controlling pathways involved in tumorigenesis (59). Targeting other ErbB family receptors may also have synergistic effect in the treatment of HNSCC. Overexpression of ErbB2 is associated with gefitinib resistance and combination of pertuzumab (a monoclonal antibody against ErbB2) with gefitinib in HNSCC cell lines resistant to gefinitib monotherapy resulted in increased inhibition of cell growth (60). The role of ErbB3 and ErbB4 in HNSCC remains under investigation.

3. Summary

HNSCC is a heterogeneous disease and despite high expression of EGFR, resistance to EGFR-targeted therapies, especially as monotherapy, is common. Because EGFR signaling involves an interplay of other oncogenic pathways, improving response to EGFR-targeted therapies will not only involve using different genomic and proteomic biomarkers to select for improved patient response but also utilizing combination therapies to target the multiple pathways involved in neoplastic transformation. Currently, no biomarker has proven to predict response to EGFR-targeted therapy. Future studies will lead to improved optimization of the current challenges in anti-EGFR therapy.

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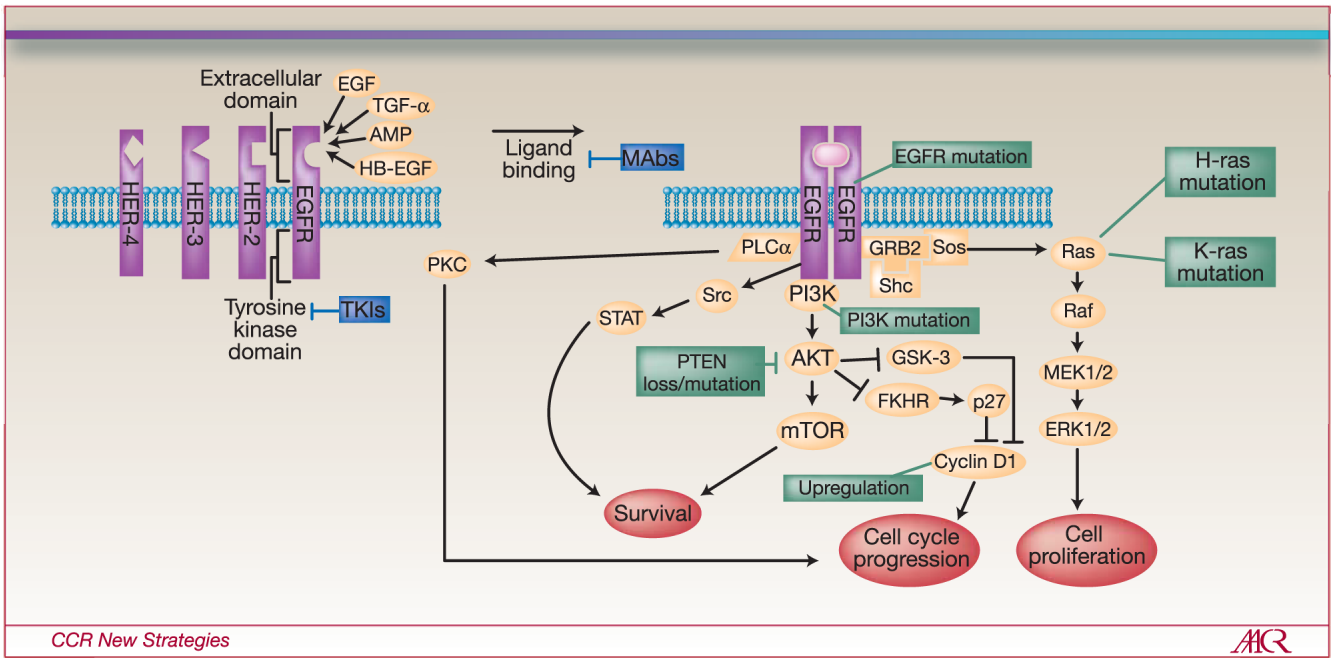

Figure 1.

EGFR Signaling Pathway and Several Mechanisms of Resistance to EGFR-Targeted Therapies

Abbreviations:

EGFR: epidermal growth factor receptor

K-Ras: v-KI-RAS2 Kirsten rat sarcoma viral oncogene homolog

H-Ras: v-Ha-ras Harvey rat sarcoma viral oncogene homolog

PTEN: phosphatidylinositol phosphatase

mTOR: mammalian target of rapamycin

mAbs: monoclonal antibodies

TKIs: tyrosine kinase inhibitors

Table 1

Mechanisms of Resistance to EGFR-Targeted Therapies

EGFR Mutations	• Extracellular domain (EGFRvIII) • Tyrosine kinase domain (T790M)
Ras Mutations	• K-ras mutations • H-ras mutations
Epithelial-Mesenchymal Transition	• Increased vimentin expression • Decreased E-Cadherin expression • Decreased Claudins 4 & 7 expression
Activation of Alternative/Downstream Pathways	• Cyclin D1 upregulation • PTEN mutations • PI3KCA mutations • Akt Amplification