

Anti-Epidermal Growth Factor Receptor Therapy in Head and Neck Squamous Cell Carcinoma: Focus on Potential Molecular Mechanisms of Drug Resistance

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Key Words. Head and neck squamous cell carcinoma • Anti-EGFR therapy • Cetuximab • Intrinsic and acquired resistance

ABSTRACT

Targeted therapy against the epidermal growth factor receptor (EGFR) is one of the most promising molecular therapeutics for head and neck squamous cell carcinoma (HNSCC). EGFR is overexpressed in a wide range of malignancies, including HNSCC, and initiates important signal transduction pathways in HNSCC carcinogenesis. However, primary and acquired resistance are serious problems and are responsible for low single-agent response rate and tumor recurrence. Therefore, an improved understanding of the molecular mechanisms of resistance to EGFR inhibitors may provide

valuable indications to identify biomarkers that can be used clinically to predict response to EGFR blockade and to establish new treatment options to overcome resistance. To date, no predictive biomarker for HNSCC is available in the clinic. Therapeutic resistance to anti-EGFR therapy may arise from mechanisms that can compensate for reduced EGFR signaling and/or mechanisms that can modulate EGFR-dependent signaling. In this review, we will summarize some of these molecular mechanisms and describe strategies to overcome that resistance. *The Oncologist* 2013;18:850–864

Implications for Practice: The introduction of epidermal growth factor receptor (EGFR) targeted therapeutic agents in the treatment of head and neck squamous cell carcinoma (HNSCC) has led to a new therapeutic challenge, namely resistance. Resistance can either be intrinsic or acquired during treatment. The knowledge we have gained from the underlying molecular mechanisms of resistance in HNSCC as well as other cancer types will be helpful in learning both how to predict resistance, and, if possible, how to overcome this resistance. Eventually, this will lead to personalized therapy for cancer patients where the right drug will be selected for the right patient.

INTRODUCTION

Most cancers originating from the squamous epithelium of the upper aerodigestive tract, including lip, oral cavity, pharynx (oropharynx, hypopharynx, and nasopharynx), larynx, and paranasal sinuses, are grouped as head and neck squamous cell carcinoma (HNSCC). Overall, HNSCC comprise 90% of all head and neck cancers and represent the sixth most common form of cancer worldwide [1]. In only 50% of HNSCC patients, the current conventional treatment strategies, including surgery, chemotherapy, and radiation, are effective, underscoring the need for new approaches to treat this malignancy [2, 3]. The existing cytotoxic therapies are nonselective and associated with considerable toxicity in HNSCC patients. Therefore, the need for additional treatment options that improve clinical outcome and have a better toxicity profile is pressing. As our understanding of the molecular biology of HNSCC continues to improve, this may provide the opportunity to develop targeted therapy for HNSCC treatment. Ideally, targeted agents are directed against unique molecular features of cancer cells, which cause, promote, or maintain the malignant be-

havior of these cells. To maximally exploit these features, characterization of the tumor at the molecular level, understanding the biological heterogeneity of human cancer, and insight into the inter-individual variation in the human genome are essential [4–6]. Thus, identifying biological markers, or biomarkers, that allow prediction of response to therapy has become increasingly important [7–9]. Eventually, this will lead to a personalized therapy for cancer patients where the right drug will be selected for the right patient.

Currently, HNSCCs are classified according to the TNM system, based on morphology and anatomic distribution. However, it is obvious that this classification lacks biological and molecular markers [10], leading to the same treatment for malignancies with a different biology [9].

The epidermal growth factor receptor (EGFR) and its ligands play an essential role in proliferation, differentiation, antiapoptotic signaling, and the processes of angiogenesis and metastasis, thereby driving the malignant behavior of the tumor [11, 12]. The

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oncoprotein EGFR belongs to the ErbB family of cell surface receptors and is also known as ErbB1 or HER1 [11]. Expression of EGFR is found in up to 90% of HNSCC cases, and it is an independent prognostic marker, as high expression is associated with increased tumor size, decreased radiation sensitivity, and increased risk of recurrence [2, 13]. As a result, EGFR overexpression is related to decreased overall survival [14].

Because EGFR is overexpressed in a wide range of malignancies, and initiates important signal transduction pathways in carcinogenesis, it has emerged as a promising therapeutic target (Fig. 1). Considering EGFR targeting therapies, two main categories of molecules are of key importance: monoclonal antibodies (mABs, such as cetuximab and panitumumab) and tyrosine kinase inhibitors (TKIs, such as gefitinib and erlotinib). The therapeutic effect of the mABs is exerted by binding to the extracellular domain of EGFR, thereby preventing ligands to activate EGFR, while promoting EGFR internalization and some by antibody-dependent cell-mediated cytotoxicity (ADCC) [15, 16]. In contrast, the quinazoline-derived TKIs directly inhibit the kinase function by blocking ATP binding to the intracellular tyrosine kinase domain of EGFR, thereby preventing downstream signaling [17]. Currently, the EGFR monoclonal antibody cetuximab (Erbix) is the only FDA-approved EGFR targeting strategy for HNSCC, in three specific settings: either as a single agent for metastatic/recurrent disease (after failure of platinum-based chemotherapy), in combination with radiation for locally or regionally advanced HNSCC, or in combination with platinum/5-FU in the first-line metastatic/recurrent disease setting [18–20].

Nevertheless, one main challenge in the targeted therapy of HNSCC remains, namely (intrinsic and acquired) drug resistance. Many HNSCC tumors remain nonresponsive to EGFR targeting agents, as the response rate with such agents, as for instance cetuximab as a single agent, is consistently lower than 15% [21]. Nevertheless, EGFR inhibition has shown to be promising also in the clinical setting, when combined with conventional cytotoxic approaches [18, 22]. Therefore, an improved understanding of the molecular mechanisms of resistance to EGFR inhibitors might allow identification of biomarkers that can be used clinically to predict response to EGFR blockade and/or to establish new treatment options to overcome resistance [7–9].

This applies not only to HNSCC, but also to other forms of cancers where anti-EGFR targeting agents are used, such as non-small cell lung cancer (NSCLC) and colorectal cancer (CRC). In fact, in these types of cancer, biomarkers of response have already been identified (*EGFR* tyrosine kinase and *K-Ras* mutations). However, as not all unresponsive CRC and NSCLC cases could be clarified by these mutations, other genes must be involved too.

Because cetuximab has been most successful in improving clinical outcomes in HNSCC and is approved by the FDA and EMEA for the treatment of HNSCC, this review focuses on mechanisms of resistance to monoclonal-based anti-EGFR therapy, mainly cetuximab.

POTENTIAL PREDICTIVE MARKERS FOR ANTI-EGFR THERAPY IN HNSCC

Until now, the only clinical marker for response to cetuximab therapy is the severity of skin rash, which is correlated with outcome in HNSCC patients [22]. However, in the literature,

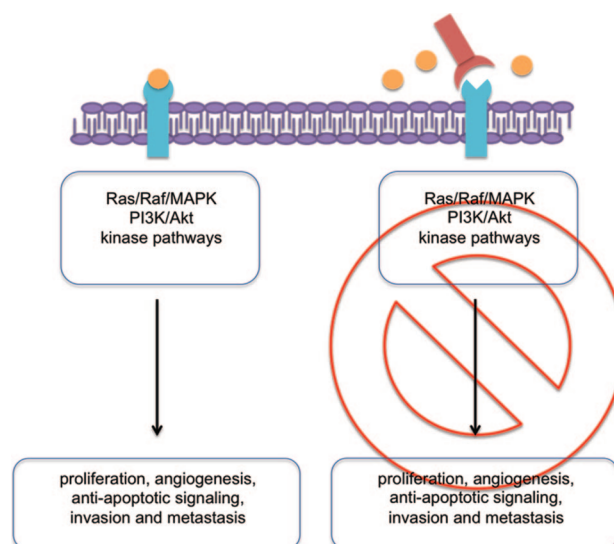


Figure 1. EGFR signaling and anti-EGFR mAB. Left: Activation of EGFR by binding of a natural ligand to the receptor and subsequent activation of signaling pathways. Right: Blockade of the EGFR receptor by an anti-EGFR mAB preventing the subsequent activation of the signaling pathway.

Abbreviation: EGFR, epidermal growth factor receptor.

several possible causes for altered responses to anti-EGFR therapy in HNSCC have been described, and will be discussed below. Therapeutic resistance to anti-EGFR therapy may arise from mechanisms that either compensate for reduced EGFR signaling and/or modulate EGFR-dependent signaling (Fig. 2).

The genes and proteins discussed below are involved in altered response to anti-EGFR therapy in HNSCC patients, and can be considered potential predictive biomarkers for anti-EGFR therapy. However, their role has not been crystalized yet and more studies are warranted to identify new reliable predictive biomarkers and effective therapeutic combinations that overcome treatment resistance and improve clinical outcome in HNSCC patients.

Altered Response Elicited at the Level of EGFR

Sustained EGFR signaling can be elicited at the level of the target itself by ligand or receptor overexpression, amplification, or mutation. Moreover, EGFR can escape lysosomal degradation routes, and subsequently functions as a transcription factor in the nucleus, thereby inducing prolonged EGFR signaling [23, 24].

Ligand Overexpression

Binding of ligands to EGFR drives homodimerization or heterodimerization with ErbB family members, resulting in the initiation of downstream signaling pathways. Therefore, overexpression of its ligands may contribute to cetuximab resistance.

Hatakeyama et al. showed that cetuximab-sensitive HNSCC cell lines become resistant to cetuximab when stimulated with the ligand heparin binding EGF (HB-EGF), whereas knockdown of HB-EGF reverses resistance to cetuximab in the resistant HNSCC cell lines [25]. Additionally, activated EGFR was evoked by three ligands, amphiregulin, HB-EGF, and TGF- α even in the presence of cetuximab [25]. Transactivation

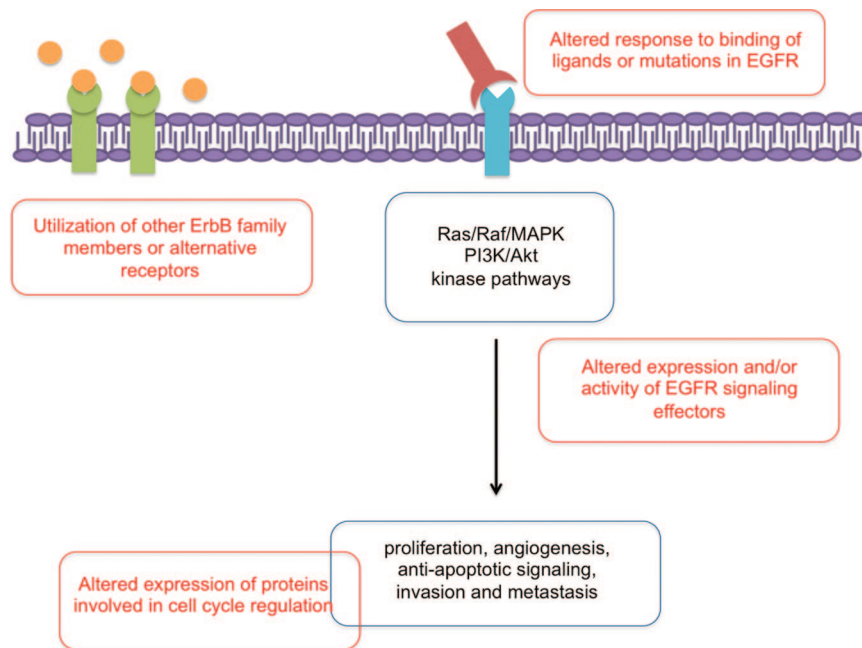


Figure 2. Despite mAB-mediated anti-EGFR treatment, the signaling cascades induced by EGFR activation may still be active because of molecular resistance mechanisms at different levels, leading to proliferation, angiogenesis, antiapoptotic signaling, invasion, and metastasis. Abbreviation: EGFR, epidermal growth factor receptor.

of EGFR and ERK signaling can be blocked by neutralization of TGF- α [26]. Furthermore, an *in vivo* study showed that HNSCC xenografts grown in the presence of cetuximab resulted in the development of resistant tumor cells that expressed relatively higher levels of TGF- β compared with untreated tumor-bearing mice [27]. Combination therapy with cetuximab and a TGF- β blocking antibody prevented the development of such resistant tumor cells and induced complete regression [27].

A correlation with enhanced response to cetuximab therapy and overexpression of the EGFR ligands amphiregulin and epiregulin in K-Ras wild-type metastatic colorectal tumors has been reported [28].

In HNSCC patients receiving cetuximab-docetaxel treatment, high amphiregulin levels were detected in 45% of the patients. A significant correlation was found between high amphiregulin levels and shortened overall survival and progression-free survival compared with patients with low amphiregulin expression [29].

Activating Mutations in the EGFR Gene

Until now, neither the expression level of the EGFR protein nor the amplification status of the *EGFR* gene could be linked to therapeutic response [30, 31].

Activating mutations have been observed in the tyrosine kinase domain or in the extracellular ligand-binding domain of EGFR [32]. The most common tyrosine kinase *EGFR* mutations include deletion of four conserved amino acids residues (leucine-arginine-glutamic acid-alanine) in exon 19 and a point mutation, L858R, in exon 21, which account for 90% of all *EGFR* tyrosine kinase mutations in NSCLC [33–35]. These *EGFR* tyrosine kinase mutations are associated with an improved clinical response to TKIs (gefitinib or erlotinib) in NSCLC patients but they are rarely found in HNSCC. Literature data suggest that the incidence of such activating mutations in HNSCC patients range from 0 to 15.7% (Table 1) [33–48]. In these studies, a total of 889 HNSCC

Table 1. Frequency of *EGFR* tyrosine kinase mutations in HNSCC patients

No. of samples	Origin of HNSCC samples	<i>EGFR</i> -TK mutation frequency	References
24	Tonsil	0.0%	Van Damme et al. [33]
24	Various	8.3%	Willmore-Payne et al. [34]
100	Various	1.0%	Loeffler-Ragg et al. [35]
19	Various	5.3%	Murray et al. [36]
82	Various	7.3%	Hama et al. [37]
31	Various	3.2%	Hama et al. [38]
71	Various	0.0%	Szabo et al. [39]
82	Various	0.0%	Cohen et al. [40]
82	Various	0.0%	Chung et al. [41]
41	Various	7.3%	Lee et al. [42]
31	Various	0.0%	Lemos-Gonzalez et al. [43]
172	Oral cavity	0.0%	Huang et al. [44]
127	Various	2.4%	Schwentner et al. [45]
91	Various	0.0%	Sheikh Ali et al. [46]
56	Oral cavity	3.6%	Hsieh et al. [47]
108	Various	15.7%	Na et al. [48]

Abbreviations: EGFR, epidermal growth factor receptor; HNSCC, head and neck squamous cell carcinoma.

samples were screened for *EGFR* tyrosine kinase mutations, of which 34 (3.8%) contained a mutation.

The *EGFRvIII* Mutation

Next to the above-discussed activating mutation, the EGFR variant III (*EGFRvIII*) is a truncated form of EGFR. The causing

mutation consists of an in frame deletion of 801 base pairs (exon 2–7) in the coding sequence of the extracellular domain, resulting in ligand-independent tyrosine kinase activity [49, 50]. Interestingly, the deletion creates a novel glycine epitope, which might be used as a target for specific antibody-based vaccines in the future [51]. Moreover, in contrast to wild-type EGFR, EGFRvIII seems to preferentially activate the phosphatidylinositol-3 kinase (PI3K) pathway. The mutant EGFRvIII form is associated with increased proliferation, tumor growth, cell motility and invasion in vitro, and resistance to anti-EGFR therapy [50, 51]. Studies in glioma cells have shown that cetuximab binds to EGFRvIII, attenuating its expression and reducing its phosphorylation [52]. Furthermore, cetuximab induces ADCC against EGFRvIII expressing glioma cells [53]. However, treatment with cetuximab does not inhibit the activation of EGFRvIII expressing cells, nor its downstream Akt and MAPK signaling pathways [53, 54].

The mutation frequency of EGFRvIII in HNSCC ranges from 0 to 48%. However, this mutation frequency was reported in only seven studies, of which five studies reported an EGFRvIII mutation frequency ranging from 17% to 48% [29, 39, 51, 55, 56]. In contrast, the two other studies detected a lower frequency of EGFRvIII in HNSCC patients [37,

The presence of nuclear EGFR is not only associated with poor prognosis, but also with treatment resistance. For example, it has been reported that nuclear EGFR expression plays a role in the therapeutic response to cisplatin and radiation by modulation of DNA repair kinetics and may have implications for EGFR-targeted combination therapies. Moreover, cells with acquired resistance to cetuximab have up-regulated HER family ligands and this enhances the translocation of EGFR to the nucleus.

57]. In these latter studies, the EGFRvIII could not be found at all [37] or only in 2% of cases of oral squamous cell carcinoma [57]. It has been suggested that the EGFRvIII might be more available in the recurrent/metastatic disease setting and be responsible for the lack of response to EGFR-targeted therapies [57]. However, most of the time only archival formalin-fixed paraffin-embedded primary tumor biopsy material is available, as was the case in the study reported by Tinhofer et al. Tinhofer et al. analyzed tumor biopsies from 47 recurrent or metastatic HNSCC patients relapsing after platinum-containing chemoradiotherapy or after platinum-containing first-line chemotherapy who were treated in a single-arm phase II multicenter study with cetuximab and docetaxel. High expression of EGFRvIII was found in eight patients (17%) and was significantly associated with reduced disease control rate and shortened progression-free survival (HR: 3.3, $p = .005$), but not with overall survival [29]. Consequently, more studies involving the mutation frequency of EGFRvIII in HNSCC and its association with response to anti-EGFR therapy are warranted. The clinical implications of the presence of EGFRvIII in HNSCC patients have not been studied in prospective clinical trials.

Nuclear EGFR

Occasionally, a part of the EGFR receptor escapes the internalization and lysosomal degradation route and translocates to the nucleus [23, 24]. Nuclear EGFR functions either as a transcription factor of cyclin D1, iNOS, b-myb, and COX-2, or as a tyrosine kinase phosphorylating and stabilizing proliferating cell nuclear antigen (PCNA), resulting in an activation of the nitric oxide pathway and increased G₁/S progression of the cell cycle [58–62]. Consequently, the proliferative potential of the cancer cells is thereby enhanced. The presence of nuclear EGFR is not only associated with poor prognosis, but also with treatment resistance [13, 63, 64]. For example, it has been reported that nuclear EGFR expression plays a role in the therapeutic response to cisplatin and radiation by modulation of DNA repair kinetics and may have implications for EGFR-targeted combination therapies [24]. Moreover, cells with acquired resistance to cetuximab have upregulated HER family ligands and this enhances the translocation of EGFR to the nucleus [65]. Perinuclear and nuclear EGFR have been found in gefitinib-resistant cancer cells [66, 67]. Furthermore, A341 epidermal carcinoma cells with acquired gefitinib resistance also show increased levels of nuclear EGFR [68].

Besides its potential involvement in resistance mechanisms, nuclear EGFR is also associated with local recurrence [13]. In oral squamous cancers, nuclear EGFR was observed in 24.3% of patients [63].

The Protein Tyrosine Phosphatase Receptor S

The protein tyrosine phosphatase receptor S (PTPRS) directly interacts with EGFR and phosphorylates and inactivates EGFR. Loss of PTPRS has been reported to enhance EGFR-induced transformation [69, 70]. Therefore, inactivating mutations or other mechanisms responsible for loss of function could contribute to anti-EGFR therapy resistance. One study detected intragenic PTPRS deletion in 26% of HNSCC tumors, resulting in loss of mRNA expression and promoting EGFR/PI3K pathway activation [71]. Moreover, in vitro results showed that PTPRS expression could predict response to cetuximab in HNSCC cell lines [71]. Evidently, more studies are warranted to define the relationship between loss of PTPRS expression and resistance to anti-EGFR therapy.

Molecular Alterations in Effectors Downstream of EGFR

Stimulation of EGFR leads to activation of different signaling pathways, which are probably among the best-studied pathways in cancer biology. Aberrant EGFR signaling can be provoked by molecular changes in downstream effectors of EGFR; particularly the K-Ras, PIK3CA, PTEN, and signal transducer and activator of transcription (STAT) proteins have been shown to contribute to resistance to EGFR-targeted therapies in other malignancies.

K-Ras

K-Ras is a protein located downstream of EGFR in the Ras-MAPK pathway. Somatic point mutations in *K-Ras* occur in a variety of human malignancies, most frequently in pancreatic cancer, NSCLC, and colon cancers [72, 73]. A mutation in codon 12 or 13 in this gene leads to constitutive activation of the protein, regardless of upstream activating signals. In colorectal tumors, these mutations confer resistance to therapy

Table 2. Studies involving *K-Ras* mutations in HNSCC patients

No. of samples	Origin of HNSCC samples	<i>K-Ras</i> mutation frequency	References
22	Tonsil	9.1%	Van Damme et al. [33]
71	Various	2.8%	Szabo et al. [39]
89	Various	5.6%	Weber et al. [80]
37	Sinonasal	0.0%	Lopez et al. [82]
26	Larynx	0.0%	Ruiz-Godoy et al. [83]
20	Various	0.0%	Anderson et al. [84]
51	Various	0.0%	Yarbrough et al. [85]
41	Larynx	4.8%	Rizos et al. [86]
37	Oral cavity	0.0%	Cohen et al. [87]
183	Larynx, oropharynx, hypopharynx	0.0%	Fujii et al. [88]
47	Oral cavity	0.0%	Wang et al. [89]

Abbreviation: HNSCC, head and neck squamous cell carcinoma.

with the EGFR targeting monoclonal antibodies cetuximab and/or panitumumab [74–77]. Approximately 30%–40% of colorectal tumors harbor a *K-Ras* mutation [78, 79].

In contrast, in HNSCC, these *K-Ras* mutations are infrequent; in different reports, the frequency of *K-Ras* mutations in HNSCC is ranging from 0 to 9.1% (Table 2) [33, 39, 80, 82–89]. In areas with a betel quid chewing habit, a higher *K-Ras* mutation incidence ($\pm 20\%$) has been noticed [81]. Overall, these mutations are rare, and therefore little is known about the predictive value of *K-Ras* mutations in HNSCC patients.

K-Ras expression could also be regulated by alterations in binding of microRNAs (miRNAs). These are short, noncoding RNAs, which bind the evolutionarily highly conserved 3' untranslated regions (3'-UTR) of mRNAs, thereby preventing translation of the mRNA. The family of let-7 miRNAs downregulates the Ras gene family, including *K-Ras*, after binding to specific sites in the 3'-UTR of Ras mRNA. Recently, a functional single nucleotide polymorphism (T > G) has been identified in the let-7 complementary site (LCS6) in the *K-Ras* 3'-UTR, which alters let-7 binding, resulting in increased *K-Ras* expression and decreased let-7 exposure. Several studies have investigated the role of this variant as a prognostic or predictive biomarker for anti-EGFR therapy. However, the results were contradictory. In NSCLC, Chin et al. reported an association between the G variant and increased risk for NSCLC among moderate smokers [90], whereas Nelson et al. could not find any association between the G variant and survival [91]. In HNSCC, the variant is associated with poor prognosis and the prognosis was worst among cases of oral cancer [92]. Moreover, in HNSCC patients, the G allele variant may be associated with tumor progression rather than initiation [92]. Furthermore, knockdown of let-7d promotes epithelial-to-mesenchymal transition (EMT) traits and migratory/invasive capabilities in oral SCC cells, whereas lentiviral-mediated let-7a overexpression significantly inhibited the stemness signature and the chemoresistant abilities of head and neck cancer cells [93, 94].

In CRC, LCS6 G-allele variant and response to cetuximab have been reported in three studies. In 2010, it was shown that G allele carriers had a worse overall survival and a shorter progression-free survival [95]. Strikingly, early stage CRC patients with the LCS6 variant had a better prognosis, whereas the opposite was observed in advanced disease [96]. Additionally, LCS6 allele G carriers seemed to have a better response rate than wild-type carriers regardless of stage [97].

H-Ras

Another family member of the Ras proto-oncogenes is *H-Ras*. Mutations in *H-Ras* have been reported in the literature and vary between 0 and 22% [85, 98–101]. Rampias et al. showed that silencing of *H-Ras* in *H-Ras* mutant HNSCC cell lines restored sensitivity to cetuximab and caused a direct downregulation of pERK1/2 levels [101]. Furthermore, treatment of a *H-Ras* mutated BB49 cell line with cetuximab and PI3K inhibitor LY294002 led to a marked reduction of their viability, whereas these same HNSCC cells were found to be resistant to cetuximab [100]. Collectively, these data suggest that *H-Ras* mutations might play a role in cetuximab resistance and therefore further studies are warranted.

Dual-Specificity Phosphatase (DUSP)

Further downstream of *K-Ras* in the MAPK signaling pathway, a member of the dual-specificity phosphatase (DUSP) family is located. DUSP proteins are involved in a negative feedback mechanism of the MAPK signaling pathway by dephosphorylation of the threonine-glutamic acid-tyrosine motif on MAP kinases [102]. Therefore, DUSP proteins can be seen as tumor suppressor proteins, and loss of their expression may promote constitutive activation of ERK and uncontrolled cell growth. Moreover, inhibition of the MAPK pathway can be compensated by suppression of the DUSP enzymes [103]. Both the cytoplasmic DUSP5 and the nuclear DUSP6 can dephosphorylate ERK1/2, thereby blocking the MAPK signal transduction cascade [104].

A recent study investigated DUSP6 expression in esophageal squamous cell carcinoma (ESCC) and nasopharyngeal carcinoma (NPC) tumor tissue. Reduced expression was observed in 40% and 75% of ESCC and NPC tumor tissue, respectively [105]. Reduced expression of DUSP6 can be accomplished either by loss of heterozygosity of the DUSP6 locus or by promoter methylation [106]. Moreover, regulation of DUSP6 is mediated at the promoter level by Ets1, a nuclear target of activated ERK [107]. Furthermore, suppressive effects of DUSP6 in tumor formation and cancer cell mobility were seen in vitro and in vivo, and DUSP6 overexpression impairs EMT-associated properties [105]. Finally, using microarray analysis, Oliveras-Ferraros et al. reported that molecular functioning of cetuximab in A431 epidermoid cancer cells was dependent on EGFR ligands, reduced expression of DUSP6, and EMT-associated proteins [108].

Taken together, the exact function of the DUSP family proteins in relation to cetuximab resistance in HNSCC needs to be further elucidated.

The PI3K/Akt Pathway

Besides activation of the Ras/Raf/MAPK signaling pathway, EGFR can also mediate activation of the PI3K/Akt pathway. Consequently, alterations in proteins involved in the PI3K/Akt

pathway might also play a role in resistance to anti-EGFR therapy.

Mutations in the PIK3CA protein occur in 6%–11% of HNSCC patients [71, 109, 110] and are associated with activation of the Akt signaling pathway [111]. Treatment of cetuximab-resistant HNSCC cells with cetuximab did not result in the decreased levels of Akt phosphorylation that were seen in cetuximab-sensitive HNSCC cells. A mutation in exon 20 of the *PIK3CA* gene was identified in a HNSCC cell line, leading to persistent Akt activation [112]. Furthermore, persistent activation of either MAPK or Akt, or both, was observed in HNSCC and colon cell lines showing limited efficacy of cetuximab therapy [113].

In addition, loss of the tumor suppressor protein PTEN also resulted in persistent activation of the PI3K/Akt pathway. Reintroducing PTEN in PTEN null prostate cancer cells was associated with restoration of cetuximab-induced cell growth inhibition and apoptosis induction. Strikingly, in HNSCC cells, it was reported that treatment of *PTEN*-silenced Cal27 cells with cetuximab led to decreased pAkt and pERK1/2 levels [114]. Although screening of 16 HNSCC cell lines for mutations in *PIK3CA* and *PTEN* identified two mutations for each gene (12.5%), no correlation was found between these mutations and the response to cetuximab therapy [115].

To define the exact role of mutations in the PI3K/Akt pathway, regarding response to anti-EGFR therapy, more in-depth studies are needed.

Src Kinases

Src kinases are upstream as well as downstream activators of EGFR and other receptor tyrosine kinases. Upon EGFR stimulation, Src kinases are activated and associate with EGFR. As such, they can affect cellular proliferation and survival by activation of STAT family of transcription factors, especially STAT3 and STAT5 [116, 117].

In vitro studies showed reduced activity of Src kinases following EGFR inhibition [118]. Elevated Src levels and/or kinase activity have been shown in HNSCC and other malignancies [117, 119]. Therefore, activation of Src kinases by EGFR upstream or downstream signaling might result in resistance to anti-EGFR therapy.

Src-specific inhibitors resulted in decreased activation of STAT3 and STAT5 and reduced growth rates in vitro [117]. However, sustained Src inhibition resulted in only a transient inhibition, because of a compensatory mechanism leading to altered JAK-STAT3 binding and JAK kinase activity [116]. Furthermore, Koppikar et al. reported that mutual inhibition of Src by AZD0530 and EGFR by gefitinib resulted in an increased inhibition of invasion and growth, compared with nonmutual blockade of either tyrosine kinase alone [120]. The effect of saracatinib, a Src inhibitor, was examined in vitro as well as in vivo. These results showed inhibition of growth, cell cycle progression, and transwell Matrigel invasion using HNSCC cell lines. However, this drug had no significant growth inhibitory effect in an orthotopic mouse model [121].

As mentioned earlier, nuclear translocation of EGFR is a possible mechanism of resistance to therapy and this has been observed in patients treated with cetuximab and radiotherapy. Phosphorylation of EGFR on tyrosine 845 by the Src kinases enhanced EGFR-mediated mitogenesis by binding and

phosphorylating the STAT5b transcription factor, and this has been described as the underlying mechanism responsible for nuclear translocation of EGFR [122, 123]. Indeed, dasatinib, an Src inhibitor, blocked EGFR translocation to the nucleus in HNSCC cell lines and, therefore, might be a potential way to evade resistance to anti-EGFR therapy [124]. Moreover, dasatinib enhanced radiosensitivity of the HN5 HNSCC cell line by interfering with nuclear localization of EGFR and by blocking DNA repair pathways [125]. In cetuximab-resistant NSCLC cells, EGFR was shown to be responsible for activation of Src kinases, and the cells were highly dependent on this activity for proliferation and survival. Accordingly, dasatinib decreased HER3 and PI3K/Akt activity and resensitized these cells to cetuximab therapy [126].

Collectively, these results indicate that Src inhibitors may be useful in overcoming anti-EGFR resistance by decreasing activated STAT3 and STAT5.

Signal Transducer and Activator of Transcription (STAT) Proteins

When considering resistance to anti-EGFR therapy, the family of STAT proteins are also important downstream EGFR effectors. This family plays an important role in transmitting survival signals and antiapoptotic signals that are initiated through activation of EGFR; especially activation of STAT3 and STAT5 has been linked to phosphorylation of EGFR [117, 127, 128]. Therefore, dysregulation of the STAT signaling pathway has been proposed to be implicated in malignant transformation.

Activation of STAT3 leads to the activation of several survival proteins, including bcl-xl, bcl-2, and survivin [129]. In HNSCC, STAT3 activation can be mediated by JAK and Src signaling, and partially by EGFR signaling [124, 130]. Consequently, STAT3 can be inhibited via EGFR blocking in vitro as well as in vivo [131]. However, recent work reported that the mutant EGFRvIII increased STAT3 activation in vitro [50].

It has been shown that the antiproliferative effects of cetuximab, as well as cetuximab-induced apoptosis, are more pronounced in *STAT3* knockdown cells compared with control cells [129]. These antitumor effects were also seen in HNSCC cells in vitro and in vivo using erlotinib in combination with a *STAT3* transcription factor decoy [3]. Likewise, upon addition of erlotinib, less growth inhibition induced by erlotinib was detected in HNSCC cells expressing constitutive *STAT5* compared with empty vector-transfected control cells [132]. Moreover, the natural *STAT3* inhibitor guggulsterone enhanced the efficacy of erlotinib, cetuximab, and cisplatin treatment in HNSCC cell lines by inducing apoptosis, cell cycle arrest, and inhibition of invasion [133]. Additionally, similar results were found in vivo [133]. However, guggulsterone failed to confer protection against oral-induced carcinogenesis in a murine model, whereas erlotinib was able to decrease the incidence of preneoplastic and neoplastic lesions by 69% [134]. On the basis of these results, targeting *STAT3* and EGFR together seems promising in HNSCC carcinogenesis.

STAT1 is activated by interferon γ (INF- γ), independent of *STAT3* overexpression [135]. The INF- γ -phospho *STAT1* signaling pathway is able to downregulate components of the antigen-processing machinery, involved in tumor antigen presentation [135]. This pathway has been identified in chronically adapted cetuximab-resistant vulvar squamous carci-

noma cell clones, inducing prosurvival signals instead of apoptosis [136]. In addition, another study showed that STAT1 is required to promote the tumor-killing effects of STAT3 inhibition when cells were treated with JAK inhibitors [137].

Mechanism of Resistance Through Crosstalk with Other Receptor Tyrosine Kinases

Selective stress of anti-EGFR therapy may lead to activation of alternative parallel signaling pathways to compensate for the reduced EGFR signaling, thereby promoting cell survival. Other receptor pathways involving other ErbB family members, insulin growth factor type 1 receptor, MET, and so forth can activate common downstream EGFR effectors and therefore might also contribute to anti-EGFR resistance in HNSCC.

The ErbB Receptor Family

As mentioned earlier, EGFR is a family member of the ErbB receptor family, and activation of other members of this family might result in resistance to anti-EGFR therapy. In the literature, activation of HER2 signaling has been associated with cetuximab resistance because its signaling occurs through many of the same downstream effectors of EGFR. With use of an *in vitro* model of acquired cetuximab resistance, a marked increase in the phosphorylation status of the C-terminal fragment of HER2, 611-CTF, was observed. Combination therapy of afatinib, an irreversible dual EGFR/HER2 inhibitor, and cetuximab resulted in a dramatic reduction in cetuximab-resistant tumor volumes compared with either agent alone in monotherapy [138]. Therefore, it was suggested that dual inhibition of EGFR and HER2 could be an effective approach to enhance the efficacy of cetuximab, to prevent and/or overcome cetuximab resistance. Likewise, a study by Yonesaka et al. has shown that cetuximab resistance could be induced by activation of ErbB2 signaling [139]. The underlying mechanism involved amplification of ErbB2 or upregulation of heregulin, both leading to persistent ERK1/2 activation. Moreover, restoring cetuximab sensitivity was accomplished by inhibition of ErbB2 or by disruption of ErbB2/ErbB3 heterodimerization *in vitro* as well as *in vivo*.

Only one study investigated and reported the link between HER2 activation and response to TKI in HNSCC. Of four evaluable cases of TKI-responsive HNSCC patients, one harbored a heterozygous V773A mutation in HER2 and this mutation was not present in surrounding normal stromal tissue [40]. Given the very small sample population, caution is needed with regard to the association between HER2 mutations and EGFR-TKI sensitivity. More studies are warranted to determine the frequency of HER2 mutations in HNSCC and their role in the response to TKIs.

The Insulin Growth Factor Type 1 Receptor

Activation of the insulin growth factor type 1 receptor (IGF-1R) leads to downstream activation of the Ras/Raf/MAPK and PI3K/Akt pathway and enhances survivin expression, all contributing to cell proliferation, altered cell adhesion, enhanced motility properties, and impaired apoptosis [140, 141]. Furthermore, IGF-1R/EGFR heterodimerization has been reported in multiple epithelial cancers as well as in several HNSCC cell lines after stimulation of these cells with IGF or EGF [142].

In nasopharyngeal carcinoma cell lines, cetuximab resistance was associated with gene amplification and overexpres-

sion of H-Ras, which was probably associated with increased activity of the IGF-1R signaling pathway [143]. Moreover, treatment with the anti-IGF-1R antibody A12 in combination with cetuximab was more effective at reducing cell proliferation and migration of HNSCC cell lines than either agent alone [142]. In addition, complete regression of tongue cancer cell xenografts was more frequent in the combination group than in the monotherapy group [142]. Similar results were observed in cutaneous squamous cell carcinoma cell lines and tumor xenografts [144]. Concerning radiation therapy, this anti-IGF-1R antibody was reported to enhance the radiosensitivity of HNSCC cell lines and the radioresponse of FaDu xenografts [145]. The IGF-1R pathway has also been proposed to be involved in resistance to gefitinib and radiation [146, 147]. *In vitro* results have shown that dual inhibition of EGFR and IGF-1R by tyrosine kinase inhibitors is more efficacious compared with single agents [148].

MET

The *MET* proto-oncogene encodes a transmembrane receptor tyrosine kinase MET, also known as c-MET or hepatocyte growth factor receptor (HGFR). The *MET* pathway can be deregulated in two different ways: on the one hand, by mutation and/or amplification of *MET*, and on the other hand, by increased ligand expression and/or activity, both resulting in persistent activation of the PI3K/Akt signaling pathway [149]. Approximately 80% of primary HNSCC tumors express the ligand hepatocyte growth factor (HGF), MET, or both, thus activating important downstream signals, which overlap with EGFR signaling [55, 150]. Moreover, *MET* mutations or amplifications have been observed in 13.5% and 13% of HNSCC tumors, respectively [151]. As high *MET* expression could be observed in 58% of patients with recurrent/metastatic HNSCC [55], the role of *MET* in resistance to anti-EGFR therapy has been investigated in a number of studies. Chau et al. did not detect any association between response to erlotinib and time to progression or overall survival in recurrent/metastatic HNSCC patients with high *MET* expression [55]. In contrast, in metastatic colorectal cancer, a significant correlation has been reported between high *MET* expression and both shorter median progression-free survival and median overall survival in patients treated with cetuximab [152]. Likewise, *MET* activation has been associated with cetuximab resistance in gastric cell lines [153]. Interestingly, the *MET* mutation frequency increases from 4% to 7% in untreated lung tumors to 20% in tumors with acquired resistance to EGFR inhibition [154–159]. Moreover, *in vitro* results demonstrated an additive effect of cetuximab plus a *MET* inhibitor in cetuximab-resistant lung cancer cell lines [160]. Similar results were observed in colorectal cancer cells [161]. Furthermore, the inhibitory effect of cetuximab could be compensated by overexpression of the *MET* ligand HGF in colorectal cancer cells [161]. In a HNSCC xenograft model, a delay in tumor growth was observed after administration of crizotinib, a *MET* TKI [150]. Collectively, these data suggest that high *MET* expression might play a role in cetuximab resistance.

Other Potential Mechanisms of Resistance

Not only alterations in proteins linked to the EGFR signaling pathway but also proteins involved in more general cancer characteristics, such as proliferation, apoptosis, invasion, and

metastasis, might confer resistance to anti-EGFR therapeutics. Moreover, alterations in these genes/proteins may also lead to resistance to other anticancer agents.

Aurora Kinase

The Aurora kinases A and B are highly conserved serine/threonine kinases that play an essential and distinct role in mitosis [162, 163]. Overexpression of both kinases is frequently present in many types of malignant tumors, and in the case of HNSCC, overexpression of Aurora kinase A is found in up to 90% of tumors [163–165]. Overexpression of Aurora kinase A is correlated with tumor progression, a metastatic phenotype, and shortened survival, and is therefore regarded as a negative prognostic marker [162, 164, 165]. High expression levels of Aurora kinase B are found in glioblastoma, ovarian carcinoma, and hepatocellular carcinoma and are associated with poor prognosis [166].

EGFR can elicit overexpression of Aurora kinase A at two different levels, by increasing the translational efficiency of Aurora kinase A, or by binding to the *Aurora kinase A* promoter and thereby increasing its transcription, both resulting in chromosome instability and tumorigenesis [61, 167].

Next to its role as a prognostic factor, recent studies indicated evidence for a role of Aurora kinase A in the response to therapy. Overexpression of Aurora kinase A triggered the activation of two important molecules involved in the regulation of drug resistance, Akt and NF- κ B [168]. Interestingly, knock-down of *Aurora kinase A* in HeLa cells resulted in sensitization to cisplatin, and Aurora kinase A overexpression could overcome cell death induced by paclitaxel [168]. Furthermore, treatment of HNSCC cells with cetuximab and a pan-Aurora kinase inhibitor R763 resulted in a rapid and efficient decrease in the level of the Aurora kinase substrate S10HH3. These results could not be confirmed by using a specific Aurora kinase A inhibitor, and therefore it was concluded that the effects of the pan-Aurora kinase inhibitor were most likely mediated by its blockage of Aurora kinase B activity [162].

Collectively, these results indicate that the Aurora kinases may be an interesting target for HNSCC tumors resistant to anti-EGFR therapy.

Cyclin D1

The G₁/S-specific cyclin D1 forms a complex with CDK4 and CDK6 and functions as a regulatory subunit of CDK4 and CDK6, the activity of which is required for cell cycle G₁/S transition. As previously mentioned, nuclear EGFR functions as a transcription factor for cyclin D1. Moreover, constitutive activation of STAT3 is required for EGFR-mediated cell growth and results in elevated levels of STAT3 target genes, including cyclin D1 [128, 169]. These observations make cyclin D1 an interesting potential marker for predicting anti-EGFR therapy.

The cyclin D1 A870G polymorphism was evaluated in 58 advanced colorectal cancer patients treated with a combination therapy of cetuximab and irinotecan. This study identified the G allele as being associated with a shorter time to progression in wild-type *K-Ras* patients and a shorter overall survival in all patients [170]. On the basis of these results, the authors concluded that the cyclin D1 A870G polymorphism might be used as an additional marker for predicting cetuximab efficacy. In addition, cotargeting EGFR and cyclin D1 seemed promising in lung cancer [171, 172], as combination therapy

with erlotinib and the cyclin-dependent kinase inhibitor seliciclib resulted in a synergistic effect in both in vitro and in vivo studies [173]. With use of HNSCC cell lines, gefitinib resistance was shown to be associated with deregulated cyclin D1 overexpression [174]. However, more studies are warranted to reveal the exact role of cyclin D1 in anti-EGFR therapy resistance.

p53

The tumor suppressor protein p53 has a critical role in controlling cell cycle progression, and consequently, loss of its function is linked to the carcinogenic process. In response to a variety of cellular stimuli, p53 can induce cell cycle arrest, apoptosis, or senescence.

A study investigating the difference between cetuximab-resistant and their sensitive parental lung cancer cells identified p53 as the most downregulated and pERK1/2 as the most upregulated cellular signaling protein. Downregulation of p53 was also observed in erlotinib-resistant cells. Furthermore, silencing of p53 in cetuximab-sensitive cells resulted in reduced sensitivity to the drug, whereas restoring p53 function in resistant cells resulted in enhanced cetuximab sensitivity [175]. In vivo experiments, using a stable cetuximab-resistant clone with tetracycline-inducible p53, showed that repair of p53 restored cetuximab sensitivity in tumor xenografts resistant to cetuximab [175]. Another study, investigating the role of p53 in the response to gefitinib, suggested that, in human lung cancer A549 cells, gefitinib-induced apoptosis was at least partly mediated by phosphorylation of p53 at Ser15, resulting in activation of p53 [176]. A study investigating the p53 mutation status of 31 colorectal cancer patients observed that loss of functional p53 limited the response to gefitinib and chemotherapy, particularly in tumors with intact p21 [177]. In addition, cetuximab was able to inhibit cell growth in p53 wild-type cells, but not in p53-mutated cells [178]. In general, there is insufficient experimental evidence to unequivocally state that loss of functional p53 can be predictive of resistance to anti-EGFR therapy.

Epithelial-to-Mesenchymal Transition

Epithelial-to-mesenchymal transition (EMT) is characterized by loss of epithelial cell characteristics and acquisition of mesenchymal phenotypic traits, causing tumor cells to detach from neighboring cells and to migrate into adjacent tissue [179–181].

TGF- β is, together with the Ras pathway, a potent inducer of EMT [180]. Haddad et al. showed that erlotinib-resistant cell lines exhibited a greater migratory capacity and invasive potential compared with erlotinib-sensitive cell lines, and response to erlotinib was correlated with an “epithelial” molecular phenotype [182]. Furthermore, Skvortsova et al. suggested that c-myc, E-cadherin, and vimentin might be considered to be predictive biomarkers for HNSCC patients treated with cetuximab in combination with radiotherapy [183]. Moreover, upregulation of vimentin and downregulation of the EMT markers E-cadherin, claudin 4, and claudin 7 were associated with gefitinib resistance in HNSCC and NSCLC cell lines [184]. Similar results were found in other studies investigating the effect of erlotinib in NSCLC [185, 186]. In addition, pancreatic and colorectal tumor cell lines, resistant to EGFR inhibition, showed features of EMT [187]. HNSCC cells with a mesenchymal-like morphology and elevated migratory potential were found to be less sensitive to irradiation and cetuximab [188].

However, when these treatment modalities were combined, the authors observed an increased sensitivity [188].

Hypoxia and Hypoxia-Inducible Factors

Regions within solid tumors often experience mild to severe oxygen deprivation (hypoxia) and it has been well-documented that poor oxygenation is a pathophysiological property of the majority of human solid tumors, including HNSCC [189]. Importantly, oxygen deficiency has a major impact on clinical responses to cancer treatment, and it was shown that hypoxic tumor regions often contain viable cells that are intrinsically more resistant to treatment with radiotherapy and/or chemotherapy [190, 191]. Interestingly, preclinical and clinical studies support an important link between hypoxia and upregulation of EGFR in cancers that do not display genetic alteration of the receptor [192]. For example, in a fraction of aggressive human HNSCC, immunohistochemistry data showed that hypoxia induced EGFR activation [193]. These results were supported by a recent study, reporting colocalization of EGFR and the hypoxia marker pimonidazole in patients with HNSCC, predominantly at increasing distances from blood vessels [194]. Subsequent EGFR signaling stimulates hypoxia-inducible factor (HIF) signaling. As the HIF transcription factors play a pivotal role in the cellular adaptation to hypoxic stress, EGFR-induced HIF signaling thus augments the induction of proteins that promote cellular survival in a hostile microenvironment. Consequently, the presence of tumor hypoxia may contribute to resistance to EGFR inhibitors. HNSCC patients with high levels of hypoxia-associated factors indeed were more likely to relapse, following induction therapy that included cetuximab [195].

Interestingly, recent data suggest that the lack of clinical responses to EGFR-directed therapy may be circumvented by supplementation of the anti-EGFR therapy with additional approaches targeting HIF. For example, downregulation of HIF-1 by siRNA or a small molecule inhibitor enhanced responses of cetuximab-resistant HNSCC cells to cetuximab plus radiation [196]. Findings from such studies may provide important guidance for designing novel therapeutic strategies in which EGFR inhibitors are combined with approaches targeting tumor hypoxia to enhance the tumor response.

CLINICAL STUDIES TARGETING EGFR RESISTANCE NETWORKS IN HNSCC

Overcoming mechanisms of intrinsic and acquired resistance to current ErbB-targeted therapies is a critical area of investigation. In general, more preclinical *in vitro* and *in vivo* studies, evaluating the role of the above-described genes/proteins, are needed to associate one or more of these mechanisms to sensitivity or resistance to anti-EGFR therapy. As such, specific inhibitors of these signaling cascades, showing promising results in preclinical studies, can be evaluated in clinical trials with HNSCC patients.

First, both monoclonal antibodies and tyrosine kinase inhibitors, targeting IGF-1R, have entered the clinic. The efficacy and toxicity of the anti-IGF-1R antibody figitumumab was evaluated in 17 palliative HNSCC patients. No significant clinical activity was detected using figitumumab as a single agent in unselected palliative HNSCC patients. In contrast, even an acceleration in progression seemed to occur. Moreover, main relevant grade 3–4 toxicities (hyperglycemia, asthenia, anorexia, infection, anemia, and gastrointestinal bleeding) were observed.

Contrary to a downregulation of IGF-1R, the EGFR pathway was found activated, leading to upregulation of pEGFR and an increase in the plasma level of TGF- α [197]. Similarly, no antitumor activity of the anti-IGF-1R antibody A12 as single agent was observed in colorectal cancer patients. Additionally, the combination of cetuximab and A12 was investigated in a randomized phase II study and did not show a significant benefit in colorectal cancer patients refractory to EGFR inhibitors [198]. Currently, this combination is evaluated in a phase II clinical trial in HNSCC patients (ClinicalTrials.gov identifier: NCT00957853). In contrast, treatment of cetuximab-refractory HNSCC patients with the IGF-1R inhibitor AMG-479 did result in conversion of a gene-expression profile associated with cetuximab resistance to a profile associated with cetuximab sensitivity [199].

Human carcinomas frequently express one or more members of the ErbB family, and therefore, cross-activation of EGFR downstream signaling might be present. Agents that block other members of the ErbB family simultaneously or that bind multiple ErbB receptors irreversibly are promising and are currently investigated in clinical trials for HNSCC patients.

A recently started phase II study will assess the progression-free survival of HNSCC patients, treated with cetuximab plus the dual kinase inhibitor of both IGF-1R and insulin receptor, OSI-906, and this treatment schedule will be compared with cetuximab plus placebo (ClinicalTrials.gov identifier: NCT01427205).

Second, another promising molecular target in HNSCC treatment is the family of Src kinases. The Src inhibitor dasatinib was evaluated in a phase II trial in advanced HNSCC patients. Despite Src inhibition, no significant clinical activity was seen in single-agent therapy [200]. Similar results were obtained with single-agent saracatinib, another Src inhibitor, in a phase II study of patients with recurrent or metastatic HNSCC [201]. At the moment, clinical trials are ongoing with a combination therapy of dasatinib and EGFR inhibitors. A phase II study will evaluate the objective response rate with this combination in recurrent HNSCC patients who have recurred after cetuximab-containing therapy (ClinicalTrials.gov identifier: NCT01488318). Furthermore, activity and toxicity of dasatinib will be assessed when given in combination with cetuximab and radiotherapy or in combination with cetuximab and cisplatin and radiotherapy (ClinicalTrials.gov identifier: NCT00882583).

Third, human carcinomas frequently express one or more members of the ErbB family, and therefore, cross-activation of EGFR downstream signaling might be present. Agents that block other members of the ErbB family simultaneously or that bind multiple ErbB receptors irreversibly are promising and are currently investigated in clinical trials for HNSCC patients. The efficacy and safety of afatinib, an aniline-quinazoline compound binding irreversibly to EGFR and HER2, is being assessed in different phase II and phase III trials. The objective of one of these trials is to explore different molecular pathways to identify tumor response and resistance mechanisms (ClinicalTrials.gov identifier: NCT01538381). Additionally, a phase II trial (ClinicalTrials.gov identifier: NCT00514943) has explored the efficacy of afatinib versus cetuximab in patients with metastatic or recurrent HNSCC

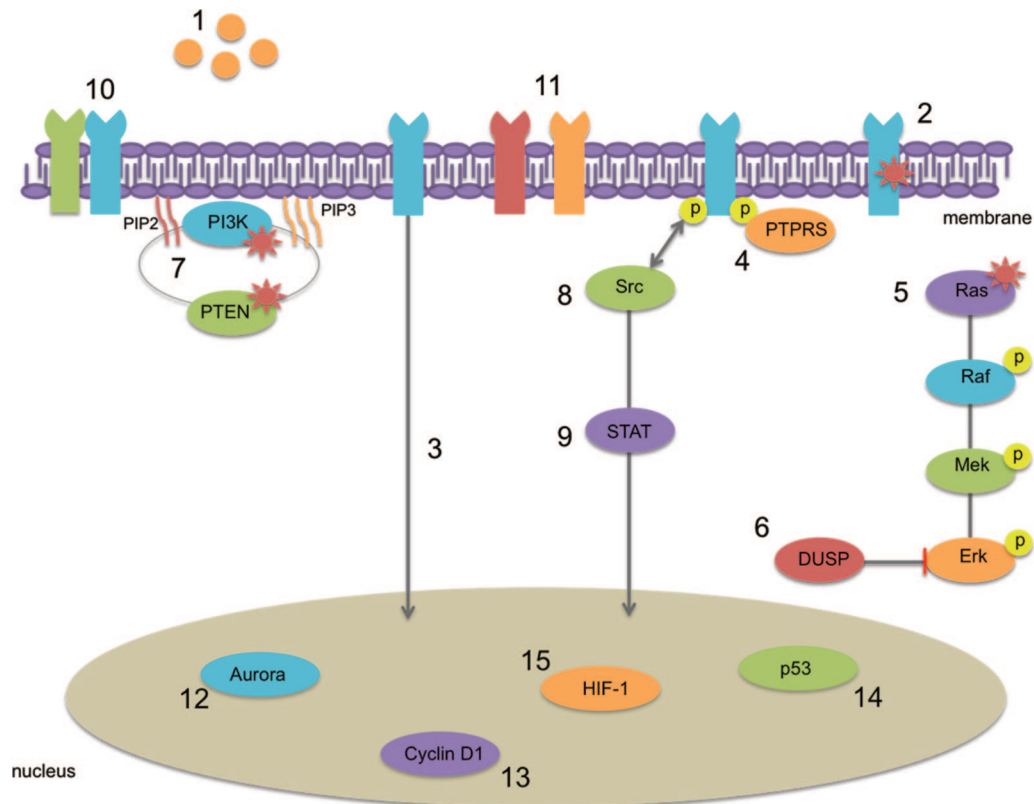


Figure 3. Possible mechanisms of resistance to EGFR-targeted therapy in HNSCC described in this review: (1) overexpression of ligands, (2) activating mutations in EGFR, (3) translocation of EGFR to the nucleus, (4) loss of PTPRS, (5) activating Ras mutations, (6) downregulation of DUSP, (7) activating mutations in PI3KCA or inactivating mutations in PTEN, (8) activation of Src kinases, (9) dysregulation of the STAT pathway, (10) heterodimerization with other ErbB family members, (11) parallel signaling of other receptors, IGF-1R or MET, (12) overexpression of the Aurora kinase A and B, (13) deregulation of cyclin D1 or the A870G polymorphism, (14) loss of functional p53, and (15) activation of HIF-1 signaling.

Abbreviations: EGFR, epidermal growth factor receptor; HNSCC, head and neck squamous cell carcinoma; STAT, signal transducer and activator of transcription.

and concluded that afatinib has at least comparable antitumor activity to cetuximab in HNSCC that failed on platinum-based therapy. Moreover, disease control rates were particularly notable for afatinib in stage 2 (crossover between treatment arms after disease progression or toxicity), 38.9% (afatinib as second treatment) versus 18.8% (cetuximab second) by investigator review, and 33.3% (afatinib as second treatment) versus 18.8% (cetuximab second) by independent central review. Patients on afatinib had more diarrhea and less rash/acne than those on the cetuximab arm.

Lapatinib, another dual inhibitor of EGFR and HER2, has been investigated in HNSCC also. In a phase II study, lapatinib in monotherapy did not result in complete or partial responses, but only in stable disease [202]. However, in a randomized phase II study, the activity and safety of concurrent chemoradiotherapy and lapatinib followed by maintenance treatment in locally advanced unresected advanced HNSCC was assessed. This combination proved to be well-tolerated with a numeric increase in complete response at 6 months post-CRT and median progression-free survival in p16-negative disease [203]. However, very recently, the combination of lapatinib with full dose docetaxel, cisplatin, and 5-fluorouracil showed unexpected renal toxicity and needs further evaluation [204]. The combination of lapatinib with cetuximab is under evaluation in an ongoing phase I trial to determine the maximum dosages patients can tolerate when these two

drugs are given at the same time (ClinicalTrials.gov identifier: NCT01184482).

Finally, although the STAT family of proteins has attracted interest as therapeutic targets in HNSCC, no STAT inhibitors have been tested in the clinic yet. Especially the combination of STAT3 inhibitors with EGFR inhibitors might be an attractive therapeutic strategy. Currently, four phase I clinical trials are ongoing in patients with advanced cancers to evaluate the safety profile and recommended dose for use in subsequent studies and to investigate the pharmacokinetics and antitumor effect of the compound (ClinicalTrials.gov identifier: NCT01184807, NCT00657176, NCT01423903, and NCT01563302).

CONCLUSION

Most HNSCC tumors rely on activation of the EGFR pathway for their proliferation, differentiation, antiapoptotic signaling, angiogenesis, and metastasis. Therefore, targeted therapies inhibiting this pathway are promising. However, intrinsic and acquired resistance to anti-EGFR therapies is a serious problem in the treatment of HNSCC. Although in other cancer types certain mechanisms of resistance have already been identified, it is becoming clear that what is relevant in one cancer type may not necessarily apply to other forms of cancer. In HNSCC cancer, mechanisms of resistance at the level of EGFR or its downstream effectors are potential causes, but also activa-

tion of alternative parallel signaling pathways might result in resistance to anti-EGFR therapeutics. Figure 3 gives an overview of all described mechanisms of resistance in this review.

In addition, inhibiting the EGFR pathway at multiple steps or in parallel signaling pathways might overcome intrinsic and/or acquired resistance. The findings discussed illustrate the complexity of the EGFR cascade. Further unraveling of this cascade will undoubtedly reveal markers for anti-EGFR therapy resistance and combinations of targeted therapies able to overcome this resistance.

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