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Molecular Target Approaches in Head and Neck Cancer: EGFR and Beyond

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Abstract

Approximately 50,000 new cases of head and neck squamous cell carcinoma (HNSCC) will be diagnosed in the United States (US) in 2009. Whereas the gradual decline in smoking rates in the US is a highly favorable trend, future global HNSCC incidence will likely reflect the increased marketing and penetration of tobacco products across several of our most populous countries. Although modern surgery, radiation and conventional chemotherapy strategies for HNSCC continue to provide gradual improvement in outcome, the first molecular targeting approach to demonstrate a survival advantage for HNSCC patients has recently emerged in the context of EGFR biology. The scientific background and current challenges accompanying this recent advance are described in this article as are several additional promising molecular targets for HNSCC. There is cautious anticipation that the logical advancement of molecular targeting agents in conjunction with radiation may afford increased cure rates and diminished normal tissue toxicity profiles for HNSCC patients over the years to come.

EGFR as a therapeutic target in head and neck cancer

The loss of growth control in head and neck squamous cell carcinoma (HNSCC) is characterized by acquisition of an autocrine regulatory pathway involving the epidermal growth factor receptor (EGFR) (1-3). Several studies have demonstrated that EGFR and its autocrine ligand transforming growth factor alpha (TGF-ą, are upregulated in HNSCC (4, 5). EGFR expression was first described in HNSCC cell lines in the early 1980's (Beguinot et al 1984). Soon after, Partridge et al reported that EGFR was expressed in HNSCC tumors. Elevated EGFR expression levels in the primary SCCHN tumor have consistently been

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correlated with decreased survival (6, 7). Increased expression of EGFR in HNSCC appears to be the result of gene amplification and transcriptional activation (8, 9). In addition to HNSCC, EGFR overexpression has also been described in premalignant dysplastic tissue that preceded the development of invasive cancer (10). The primary rationale for the design of EGFR targeting strategies has been based on the increased EGFR expression levels detected on tumor cells, although evidence suggests that constitutive EGFR activation can occur in the absence of increased expression (11). In addition to the importance of EGFR expression in human HNSCC, many studies have reported anti-tumor effects when EGFR targeting strategies were used in preclinical HNSCC models. Several therapeutic approaches have been developed including monoclonal antibodies, tyrosine kinase-specific inhibitors, ligand-linked immunotoxins, and antisense approaches (12).

EGFR inhibitors have been shown to abrogate the growth of HNSCC cell lines and xenografts when administered alone, or in combination with standard therapy such as chemotherapy and/or radiation (13). The EGFR monoclonal antibody cetuximab has been combined with cisplatin in platinum-refractory HNSCC patients in a phase III trial supported by the Eastern Cooperative Oncology Group (ECOG) that demonstrated enhanced response rates when subjects received the combined treatment regimen (14). The FDA approved the use of cetuximab for SCCHN in 2006 based on the results of a phase III trial showing prolonged survival when cetuximab was administered in conjunction with radiation (15). This was the first phase III trial to demonstrate a survival advantage using a molecular targeting agent combined with radiation. In addition, the combination of radiation and cetuximab did not significantly increase the toxicity profile or compromise the effective delivery of full course external beam radiation therapy. It is noteworthy that cetuximab was the first new drug approved for use in this cancer in 45 years. While the combination of cetuximab and radiation increased survival compared with radiation alone, cetuximab did not reduce the incidence of distant metastases nor did it completely prevent local-regional failure. These facts indicate the persistence of oncogenic signaling pathways.

EGFR-specific tyrosine kinase inhibitors such as erlotinib have also been explored as antitumor agents in SCCHN, although phase III data are lacking (16). Several ongoing US and international clinical trials are exploring the combination of chemoradiotherapy with EGFR targeting as a curative treatment strategy. Other questions that remain to be answered include the timing of radiation or chemotherapy delivery with EGFR targeted therapies and the role of other targets, in addition to EGFR. There is no evidence to date of an association between human papilloma virus (HPV) status of the tumor and response to EGFR targeting. An improved understanding of EGFR signaling interactions with other oncogenic pathways should facilitate the design of more effective targeting strategies by elucidating the critical proliferative and survival pathways that persist in the setting of EGFR blockade.

Challenges of EGFR targeted therapy

Despite the nearly ubiquitous expression of EGFR in HNSCC tumors, there is no evidence to date that expression levels can predict an individual patient's response to EGFR targeted therapy. Therefore, we still do not know how to identify those HNSCC patients who will respond to EGFR targeted therapy. There are several potential explanations for the modest efficacy of EGFR inhibitors in patients whose tumors express EGFR including: 1) heterogeneous EGFR expression with tumorigenic potential residing in EGFR-null cells; 2) persistent signaling through pathways that crosstalk with EGFR despite upstream blockade; and/or 3) mutations that confer resistance to EGFR inhibition. EGFR is a member of the receptor tyrosine kinase family (Type I) of cell surface receptors that also include HER2, HER3 and HER4. While there is little evidence that HER4 is expressed in HNSCC, the role of EGFR dimerization and signaling through HER2 or HER3 remains incompletely

understood. Clinical studies to date using HER2 inhibitors including the monoclonal antibody trastuzumab or the small molecule inhibitor lapatinib in HNSCC patients have been disappointing (17). Further investigation is required to delineate the role of EGFR-HER2 heterodimers in oncogenic signaling pathways in HNSCC. Although HER3 upregulation has been implicated in the lack of response to EGFR tyrosine kinase inhibitors in lung cancer, the role of Her3 signaling in HNSCC is largely unexplored.

EGFR is bound by a series of ligands that trigger receptor dimerization, internalization and downstream signaling. These ligands include TGF-α, amphiregulin, and EGF. Both TGF-α and amphiregulin are produced at relatively high levels by HNSCC tumors leading to the stimulation of autocrine growth pathways. In addition to direct activation by EGFR autocrine ligand, EGFR can also be activated indirectly by G-protein-coupled receptors (GPCR), a process known as transactivation. Ligands for GPCRs including prostaglandin E2 (PGE2) bradykinin (BK), lysophosphatidic acid (LPA), and gastrin-releasing peptide (GRP) have been implicated in the pathophysiology of multiple tumor lineages (18). For example, PGE2 is a downstream product of cyclooxygenase 2 (COX-2), and this pathway is upregulated in SCCHN where it correlates with tumor progression and survival (19, 20). BK is known to induce calcium influx and cause phosphorylation of EGFR in HNSCC (21, 22). We recently found upregulation of bradykinin 2 receptor (B2R) in HNSCC tumors implicating an autocrine loop for this peptide hormone in HNSCC (unpublished observations). Likewise, increased PGE2 levels have been described in many tumors including HNSCC (23).

Activation of EGFR by direct ligand or transactivation by GPCR leads to stimulation of downstream signaling pathways including Src family kinases, PI3-Kinase/AKT, Signal Transducers and Activators of Transcription (STATs), phospholipase C-gamma (PLC-g) and mitogen activated protein kinase (MAPK). In general, activation of Src has been shown to mediate invasion, PI3-K/AKT stimulates growth and survival, STATs induce proliferation and survival, PLC-gmediates invasion and MAPK induces growth in HNSCC models (24) (**Figure 1**). The precise role of each pathway downstream of EGFR, or other receptors, in mediating the response to EGFR targeting is incompletely understood.

Unlike non-small cell lung cancer, there is little evidence of activating mutations of the EGFR tyrosine kinase domain in HNSCC (25). A genetic variant of EGFR, EGFRvIII, was originally described in glioblastoma where up to two thirds of patients express this variant. EGFRvIII is a somatic alteration in wtEGFR leading to deletion of exons 2-7 (26, 27). This deletion results in the loss of the ligand-binding domain, yet the protein remains constitutively activated in the absence of ligand (28, 29). Similarly, HER2 truncation molecules are also constitutively active and resistant to the action of trastuzumab (30). EGFRvIII is expressed by nearly 40% of all HNSCC tumors (31). EGFRvIII is capable of constitutively activating downstream signal transduction cascades leading to increased cell proliferation and survival *in vitro* and tumorigenicity *in vivo.* Expression of EGFRvIII, which lacks the ligand-binding domain recognized by therapeutic antibodies such as cetuximab, confers significant drug resistance to HNSCC and other tumor cells to cisplatin and erlotinib in addition to cetuximab (31-34). The contribution of EGFRvIII in mediating resistance to these agents in HNSCC is unknown.

The precise signaling pathways induced by EGFRvIII are incompletely understood. Indirect evidence of preferential signaling of EGFRvIII through STATs was provided by a study demonstrating a correlation between EGFRvIII and pSTAT3 expression in a cohort of malignant gliomas (35). The potential role of Src in EGFRvIII signaling was demonstrated by the recent finding that the growth of EGFRvIII-expressing glioblastoma xenografts containing a dominant-negative (DN) mutant c-Src was inhibited to a greater degree

compared with tumors that did not contain DN Src, (36). In gliomas, EGFRvIII has been reported to signal through PI3-kinase/Akt (37). Several strategies to target EGFRvIII are in active clinical development including the immunotoxin MR1-1, the antibody 806 and several irreversible EGFR/HER2 tyrosine kinase inhibitors that have selective activity against EGFRvIII (38-41). The contribution of EGFRvIII in mediating resistance to EGFR targeting in HNSCC requires further investigation.

Intrinsic and Acquired Resistance to EGFR Inhibitors

As described above, targeting the EGFR has been intensely pursued in the last decade as a cancer treatment strategy. Clinical trials to investigate the activity of EGFR inhibitors commonly identify 10-20% major response rates. Although highly valuable for those patients that respond, approximately 80% of patients tumors show no response (intrinsic resistance) to EGFR inhibition strategies. Further, as increasing numbers of patients are treated with EGFR inhibitors, the emergence of acquired resistance following initial favorable response has been identified. Preclinical models of resistance to EGFR inhibitors have been recently established and are providing new insights regarding mechanisms of response to EGFR agents.

One approach to inhibit the activity of the EGFR involves the use of small molecule tyrosine kinase inhibitors (TKIs) that bind to the ATP-binding site in the tyrosine kinase domain (TKD) of the EGFR. These agents inhibit EGFR autophosphorylation and ultimately lead to blockade of downstream signaling and cellular proliferation. To date, three anti-EGFR TKIs have been approved by the FDA for use in oncology; erlotinib (OSI-774, Tarceva), gefitinib (ZD1839, Iressa) and lapatinib (GW572016, Tykerb). The identification of mutations in the TKD of the EGFR that predict response to EGFR-TKIs in selected lung cancer patients represents a landmark development in the EGFR field (42). Mutation in exon 21 of the EGFR TKD, L858R, may predict increased sensitivity to TKIs, whereas the T790M mutation in exon 20 is associated with acquired resistance to TKI therapy (43). These findings suggest that patient selection may be critical for successful therapies using EGFR TKIs (44). Although TKD mutations have not been identified with high frequency in HNSCC, there may well emerge mutations in other key signaling pathways that prove more pertinent to HNSCC growth behavior.

Other receptor tyrosine kinases (RTKs) with overlapping signal transduction pathways with the EGFR can promote resistance in EGFR driven cancers (45). cMET (HGFR) is a RTK that regulates cell cycle progression, migration, angiogenesis and cell survival. Increasing evidence identifies cMET as an attractive target for molecular-targeted cancer therapy (46). Previous studies identified cross-talk between the EGFR and cMET in transformed cells (47). However, until recently there has been no clear evidence demonstrating that cMET is involved in regulating acquired resistance to EGFR targeting agents. Engelman *et al* recently reported that NSCLC HCC827 cells chronically exposed to gefitinib *in vitro* led to the amplification of cMET. This increased activity of cMET resulted in the constitutive activation of the HER3-Akt signaling pathway in gefitinib-resistant cells and was abrogated by the selective cMET inhibitor PHA665752 thus restoring the sensitivity of resistant cells to gefitinib (45). Taken together, these data indicate that mutations in the EGFR or altered signaling can lead to acquired resistance to EGFR TKIs.

A second approach to inhibit the activity of the EGFR uses monoclonal antibodies (mAbs) that target the extracellular domain of the EGFR. Cetuximab (IMC-C225, Erbitux) blocks natural ligand binding (48), prevents receptor activation and dimerization and ultimately induces receptor internalization and downregulation (49). Cetuximab exhibits promising anti-tumor activity as monotherapy or in combination with chemotherapy and/or radiation

(50, 51). A series of clinical trials demonstrating clinical benefit led to the FDA approval of cetuximab for use in patients with HNSCC and in metastatic colorectal cancer (52, 53). Another anti-EGFR monoclonal antibody, panitumumab, has also gained recent FDA approval for use in the metastatic colorectal cancer setting (54). Although EGFR TKD mutations appear to correlate with response to the TKIs erlotinib and gefitinib, no such correlation exists for cetuximab response (55). This indicates that other molecular based mechanisms exist for resistance to cetuximab therapy.

One of the first reports on acquired-resistance to cetuximab suggested that altered control of angiogenesis could be one mechanism responsible for resistance to EGFR targeting agents. Ciardiello *et al* found a 5- to 10-fold increase in VEGF production and secretion in cetuximab-resistant cell lines established from GEO colon cancer xenograft. Growth of EGFR inhibitor-resistant tumors could be inhibited by ZD6474, a dual EGFR/VEGFR TKI. (56). In addition, using A431 xenograft, Viloria-Petit *et al* generated six variant cell lines resistant to anti-EGFR antibody and found that these cells produced significant amounts of VEGF when compared to parental cells (57). Collectively these findings indicate that receptor ligands play a critical role in EGFR inhibitor resistance.

Another pre-clinical study addressing acquired-resistance to cetuximab *in vitro* utilized high-throughput screening to examine the activity of RTKs in cetuximab-resistant tumor cells following chronic exposure to cetuximab (58). The findings suggested that cells developing acquired-resistance to cetuximab exhibited increased steady-state EGFR expression secondary to alterations in trafficking and degradation. EGFR upregulation promoted increased dimerization with HER2 and HER3 leading to their transactivation and subsequent activation of the PI(3)K/Akt pathway. Blockade of EGFR and HER2 led to loss of HER3 and PI(3)K/Akt activity. These data suggest that acquired-resistance to cetuximab is accompanied by dysregulation of EGFR internalization/degradation and subsequent EGFR-dependent activation of HER3. These findings suggest a rationale for the clinical evaluation of combinatorial anti-HER targeting approaches in tumors manifesting acquired resistance to cetuximab (58). Further investigations of this model of acquired resistance to cetuximab indicated that Src family kinases (SFKs) are highly activated in cetuximab-resistant cells and enhance EGFR activation of HER3 and PI(3)K/Akt. Studies using the FDA approved Src kinase inhibitor dasatinib decreased HER3 activity followed by loss of PI(3)K/Akt (unpublished data). In addition, dasatinib therapy re-sensitized cetuximab resistant cells to cetuximab therapy (**Figure 2**). These results indicate that SFKs and the EGFR cooperate in acquired-resistance to cetuximab and suggest that dasatinib therapy in combination with cetuximab may have strong clinical benefit (**Figure 3**).

Promising molecular targets for HNSCC

Looking beyond EGFR, many promising molecular targets are emerging in HNSCC. One highly promising molecule that resides within the HER family is HER3. Themes have emerged in the last two years that escape from therapies targeting the EGFR involve equilibrium shifts in HER family signaling via HER3 (45, 58, 59). Several laboratories are designing antibodies that will block HER3 hetero-dimerization with EGFR or HER2 to prevent signals transduced to PI(3)K/Akt.

The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, and transcription. Further, it has recently been implicated in the pathogenesis of HNSCC and therefore serves as a promising molecular target (60). mTOR is activated by Akt and ultimately blocks apoptosis and increases proliferative potential of cancer cells. Several mTOR inhibitors are being investigated (Rapamycin, CI-779, RAD001 and AP23753).

Signaling through the insulin-like growth factor-I receptor (IGF-IR) leads to cellular proliferation, apoptosis, metastasis, and resistance to cytotoxic cancer therapies. Recent work combining the fully humanized anti–IGF-IR monoclonal antibody A12 (ImClone Systems, Inc., New York, NY) with radiation resulted in pronounced anti-tumor growth than either agent alone (61). In addition, TKIs directed against the IGF-IR are now in clinical development. These results suggest IGF-IR signal transduction blockade as a promising strategy to improve radiation therapy efficacy in human tumors, forming a basis for future clinical trials in HNSCC.

Aurora kinases are serine/threonine kinases that are essential for cell proliferation by playing a crucial role in cell division by controlling spindle formation during mitosis. Studies investigation the relationship between Aurora kinases and HNSCC found a strong correlation between the up-regulation of aurora kinase (A) mRNA and distant metastases, poor disease-free and overall survival (62). Several aurora kinase A inhibitors (MP529, MLN8054) and aurora kinase B (AZD1152) are in clinical development.

Conclusions

HNSCC provides a rich environment for preclinical and clinical study in light of the relative accessibility of tumor and normal tissue for controlled investigation. The recent incorporation of EGFR inhibitors into the available armamentarium for HNSCC therapy is a landmark development for a highly complex cancer. Several additional molecular targets of potential interest for HNSCC therapy are described in this article. Strategies that combine radiation with molecular targeting agents offer high promise for achieving meaningful improvements in HNSCC therapy. The radiation/EGFR story represents only the beginning of many successful future steps.

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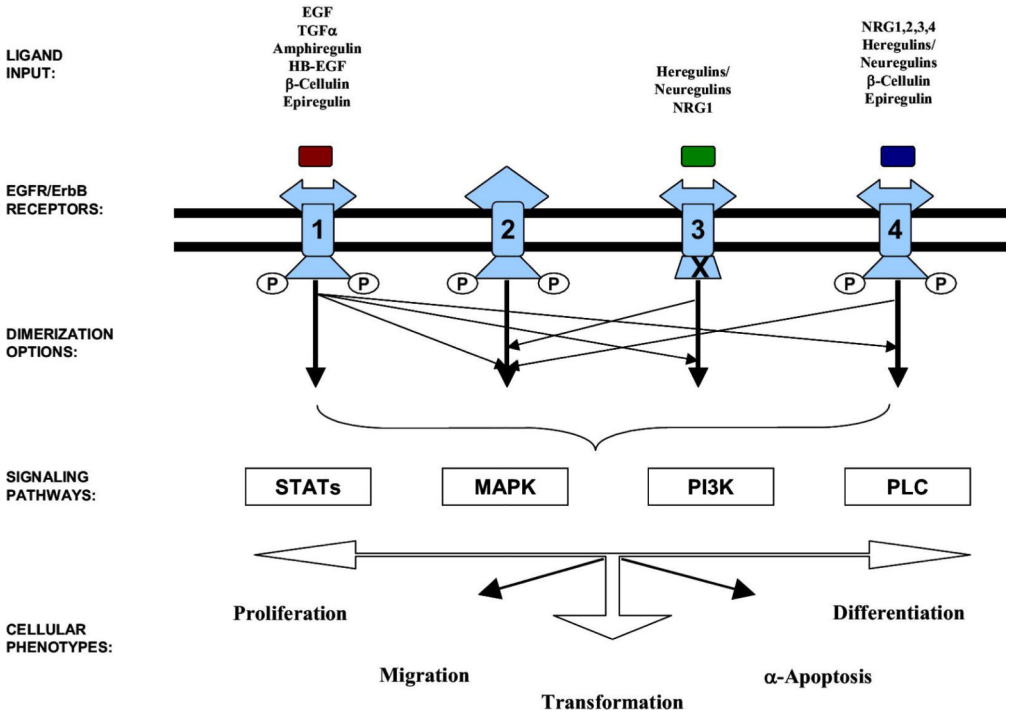

Figure 1. The diversity of the epidermal growth factor receptor-signaling network

The EGFR transduction cascade is a highly complex network, consisting of signaling options based on multiple layers. The ligand input and receptor engagement occurs in the extracellular layer. Receptor-specific ligands for ErbB1,ErbB3, and ErbB4 have been identified as shown. Note that no direct ligands for ErbB2 had been isolated to date. At the cell surface, receptor engagement leads to tyrosine phosphorylation and several receptor dimerization options (depicted by arrows: thick arrow denotes homodimerization and thin arrow denotes heterodimerization; the "X" in ErbB3 represents absence of the intrinsic tyrosine kinase activity). The selective activation of well-characterized signaling transduction pathways (shown in boxes), depends on the various arrangements of ligand-ErbB engagement, tyrosine phosphorylation, and subsequent receptor dimerization combinations beneath the cell surface. Finally, the output layer includes a variety of cell responses (shown in bold). J.R. Grandis, J.C. Sok / Pharmacology & Therapeutics 38 102 (2004) 37–46

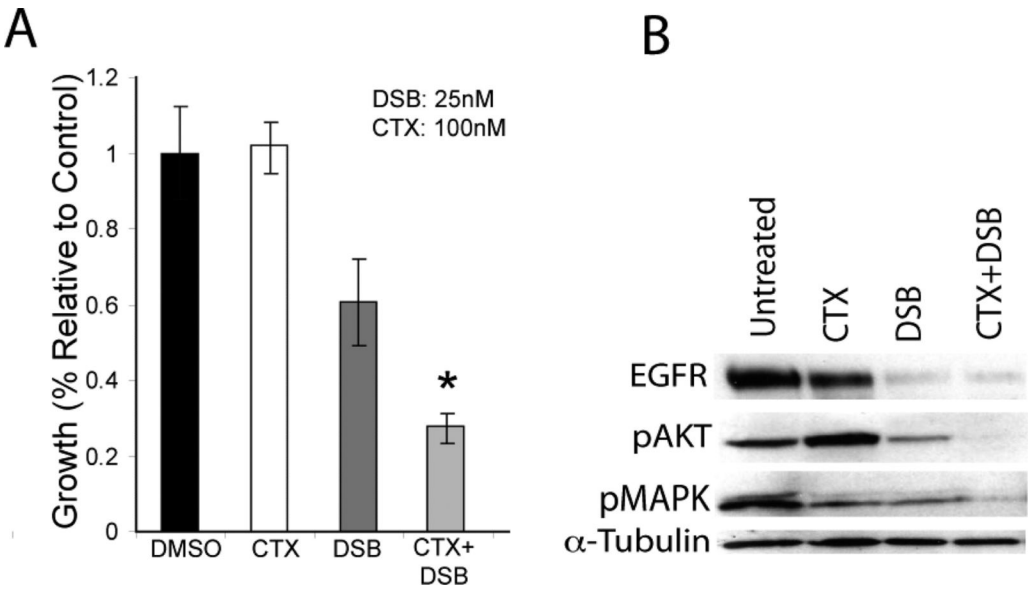

Figure 2. Dasatinib re-sensitizes cetuximab-resistant cells to cetuximab therapy

A: *Dasatinib re-sensitizes cetuximab-resistant cells to cetuximab therapy*. Cetuximab-resistant cells were treated with DMSO, cetuximab (100nM, CTX), dasatinib (25nM, DSB) or the combination for 72 hours. Growth was measured at 72 hours after drug treatment using the growth proliferation assay as described in the experimental procedures and plotted as a percentage of growth relative to the untreated control cells. Data points are represented as mean ±SEM (n=3). *, $P<0.05$

B: *Dual blockade of SFKs and EGFR have additive effects on Akt and MAPK activity*. HC4 cells were plated and treated with the vehicle DMSO (control), 100nM cetuximab, 25nM dasatinib or the combination for 48 hours. Cells were harvested and protein was collected, fractionated by SDS-PAGE and immunoblotted for the indicated proteins. a-tubulin was used as a loading control.

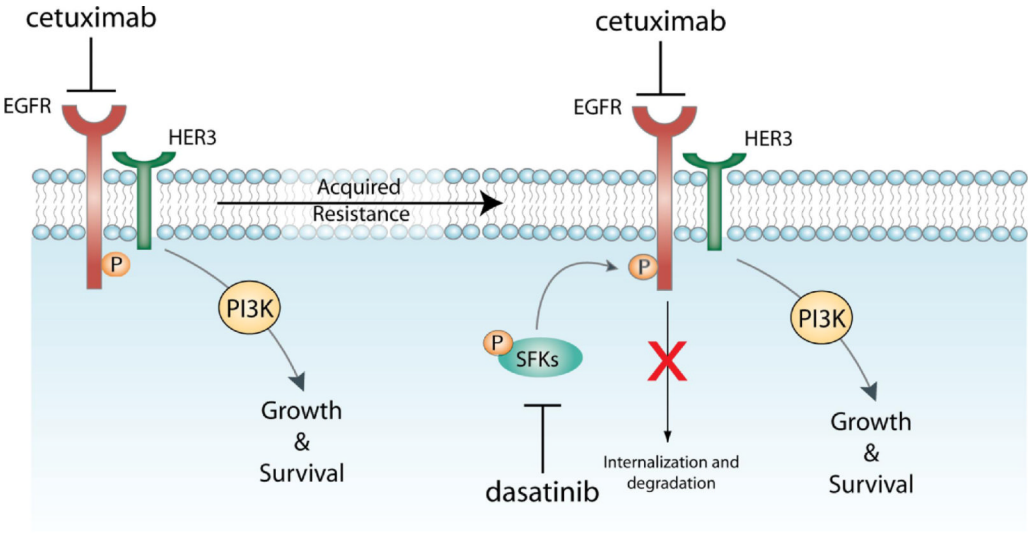

Figure 3. Potential mechanisms of acquired-resistance to cetuximab

Cells that acquire resistance to chronic cetuximab therapy dysregulate the Cbl/ubiquitination of the EGFR(58). This modulation of internalization and degradation of the receptor results in increased steady-expression of EGFR followed by increased cooperation and activation of Src Family Kinases (SFKs). This cooperation between SFKs and the EGFR results in resistance to cetuximab therapy. Blockade using the SFK inhibitor dasatinib re-sensitizes cetuximab-resistant cells to cetuximab therapy.