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STAT-Mediated EGFR Signaling in Cancer

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Abstract The epidermal growth factor receptor (EGFR) and signal transducers and activators of transcription (STATs) are commonly expressed and activated in many malignancies. EGFR is an upstream activator of several pathways involved in tumor progression, and STATs activate selected genes involved in oncogenesis. There are several different mechanisms by which STAT proteins can mediate intracellular EGFR signaling, including direct activation of STATs by EGFR binding and indirect activation of STATs through Src-mediated EGFR signaling. EGFR likely activates STAT in a manner distinctive from other mechanisms of STAT activation; STAT5 can be phosphorylated in an EGF-dependent manner at unique sites, conferring novel functions. Cumulative evidence suggests that targeting EGFR signaling pathways at several levels may demonstrate synergistic therapeutic effects compared with targeting the upstream receptor alone. Thus, methods to inhibit EGFR in conjunction with oncogenic STATs may represent a novel therapeutic strategy for cancers characterized by upregulation of EGFR signaling. J. Cell. Biochem. 102: 311–319, 2007.

Key words: EGFR; STAT; cancer; pathway; review

Epidermal growth factor receptor (EGFR) is expressed in many types of cancer, including breast, lung, esophageal, and head and neck. The receptor signals downstream to effect cellular processes such as differentiation, proliferation, and invasion and is involved in both oncogenesis and tumor progression. Currently, there are several EGFR-targeted therapies approved by the food and drug administration (FDA) for use in selected cancers, although recent studies have shown the response to these drugs is modest unless they are administered concomitantly with other modalities such as chemotherapy or radiation [Baselga and Arteaga, 2005]. There are several possible mechanisms by which EGFR-targeted therapies are rendered inefficient as monotherapies, most likely due the activation of signaling pathways downstream of EGFR. Concurrent targeting of downstream mediators of EGFR may enhance the therapeutic efficacy of EGFR inhibitors.

Signal transducers and activators of transcription (STAT) proteins are a family of proteins downstream of tyrosine kinase receptors including EGFR that have been shown to mediate the transcription of several proteins whose upregulation leads to aberrant proliferation, cell cycle progression, and inhibition of apoptosis thereby enhancing tumor progression. STATs are activated in a wide variety of cancers. Currently no inhibitors specifically targeting STATs have reached the clinic, but studies have consistently demonstrated an essential role for STATs in tumor progression. Here we describe how STATs mediate EGFR signaling in cancer cells, discuss the role of STATs in EGFR-mediated oncogenesis, and introduce the concept of targeting STATs in combination with EGFR for cancer therapy.

STAT ACTIVATION IN CANCER

Expression of STAT proteins has been identified in many malignancies including cancers of the breast, lung, colon, and head and neck (Table I). The STAT family is comprised of seven proteins including STAT1, STAT2, STAT3,

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TABLE I. Activation of STATs in Solid Tumors Expressing EGFR

Solid tumor type	STAT activation	References
Breast	Stat1, Stat3, Stat5	Haura et al. [2005b], Bowman et al. [2000], Grandis and Sok [2004]
Head and neck	Stat1, Stat3, Stat6	Haura et al. [2005b], Bowman et al. [2000], Grandis and Sok [2004]
Lung	Stat3, Stat5	Haura et al. [2005b], Buettner et al. [2002], Grandis and Sok [2004]
Prostate	Stat3, Stat5	Haura et al. [2005b], Bowman et al. [2000], Grandis and Sok [2004]
Colon	Stat3	Corvinus et al. [2005] and Grandis and Sok [2004]
Glioma	Stat3	Schaefer et al. [2002] and Grandis and Sok [2004]
Melanoma	Stat3	Lewis et al. [2005] and Bowman et al. [2000]
Ovarian	Stat3	Bowman et al. [2000] and Grandis and Sok [2004]
Pancreatic	Stat3	Bowman et al. [2000] and Grandis and Sok [2004]
Renal	Stat3	Bowman et al. [2000] and Grandis and Sok [2004]
Liver	Stat3	Kannangai et al. [2006]

STAT4, STAT5a, STAT5b, and STAT6. Of those seven family members, STAT1, STAT3, STAT5a, and STAT5b are known play a role in cancer. Growth factor or cytokine signaling leads to activation of STATs and is initiated by ligand binding followed by receptor subunit aggregation, association with a member of the Janus kinase family (JAK) and transphosphorylation of tyrosine residues critical for STAT activation. Activated STATs form dimers and multiprotein complexes that translocate into the nucleus where target gene expression is initiated [Darnell et al., 1994]. A large amount of diversity exists with regard to the functions of STAT target genes. STAT3 and STAT5 stimulate cell cycle progression, angiogenesis, and inhibition of apoptosis through the expression of target genes, and are hence considered oncogenes. Conversely, STAT1 target genes activate cell cycle arrest and apoptosis, and many studies have demonstrated that STAT1 exhibits the properties consistent with a tumor suppressor [Watanabe et al., 2001; Widschwendter et al., 2002]. STAT2, STAT4, and STAT6 are activated exclusively by a small subset of cytokines [Silva, 2004] and do not appear to contribute to cancer formation or progression [Lim and Cao, 2006].

STAT target genes identified to date contribute to a broad scope of functions including proliferation, differentiation, and survival [Silva, 2004]. Of all the STAT proteins, STAT3 target genes have been most thoroughly investigated. STAT3 target genes involved in cell cycle regulation include CyclinD1, CyclinD3, c-Myc, $p21^{wafl}$, and p27. Vascular endothelial growth factor (VEGF) is a STAT3 target gene involved in angiogenesis, and MMP-2 and MMP-9 are STAT3 target genes that contribute to migration and invasion. STAT3 target genes involved in the inhibition of apoptosis include Survivin, Mcl-1, and Bcl-X_L [Leeman et al., 2006].

STAT Phosphorylation and Activation

All STAT proteins are involved in both signal transduction and activation of transcription, and likewise all STAT proteins share a very similar structure. STAT proteins contain three basic domains: (1) an oligomerization domain, (2) a DNA binding domain; and (3) a Src homology 2 domain (SH2) [Schrump and Nguyen, 2001]. STAT proteins can bind to and become activated by EGFR directly through the SH2 domain [Silvennoinen et al., 1993]. The SH2 domain of STAT is, in fact, required for activation by tyrosine phosphorylation most commonly at Y701, Y705, or Y694, respectively for STAT1, STAT3, and STAT5 [Rane and Reddy, 2002]. Upon activation by tyrosine phosphorylation, STAT proteins dimerize. STAT3 can homodimerize and form what is commonly referred to as the SIF-A complex, or STAT3 can heterodimerize with STAT1 and form the SIF-B complex [Song and Grandis, 2000]. The activated STAT complex translocates to the nucleus where it can bind to a variety of target genes or bind other transcription regulatory proteins like c-fos and c-jun to regulate transcription [Leeman et al., 2006]. However, STAT proteins can also be phosphorylated on their C-termini at serine residues, most commonly at S727. Serine phosphorylation serves to increase transcription factor activity of STAT, and cell lines with an S727A mutation have abrogated transcriptional activity. Serine phosphorylation may also serve to decrease tyrosine phosphorylation in the case of STAT3, either through inhibition or dephosphorylation, although the precise mechanism remains incompletely understood [Decker and Kovarik, 2000].

Activation of STATs can occur downstream of growth factor receptor signaling, cytokine receptor signaling, and/or non-receptor tyrosine kinase signaling. The cytokine IL-6 activates STAT1 and STAT3 by stimulating dimerization of GP130 receptor subunits that in turn activates JAKs, which results in STAT activation (Fig. 1). Inhibiting EGFR does not abrogate STAT3 activation in breast cancer cell lines indicating that in this tumor system, STAT3 activation is not primarily downstream of ErbB family receptors [Badache and Hynes, 2001]. JAK also plays a role in recruiting STAT proteins to Src, and it has been shown that many Src family kinases are capable of phosphorylating STAT3, including: Src, Gck, Lyn, Fyn, and Fgr [Silva, 2004]. In some cases, Src may even signal to STAT5a upstream of JAK as demonstrated by the finding that both Src inhibitors and a dominant negative c-Src ablated phosphorylation of STAT5a and JAK in breast cancer cell lines [Olayioye et al., 1999].

Regulation of STATs

The activation of STAT is highly regulated and incompletely understood. Proteins that decrease STAT3 activation include Suppressors of Cytokine Signaling (SOCS) family members SOCS-1 and SOCS-3, Protein Inhibitor of STAT3 (PIAS3), and Genes Associated with Retinoid IFN Induced Mortality (GRIM) GRIM-19. SOCS proteins containing an SH2 domain can bind to JAK to suppress STAT3 activation [Endo et al., 1997] (Fig. 1), and both SOCS-1 and SOCS-3 may be methylated and silenced in cancer [Galm et al., 2003; He et al., 2003]. The mitogen-activated protein kinase (MAPK) pathway may negatively-regulate STAT protein signaling by activating specific JAK inhibitors

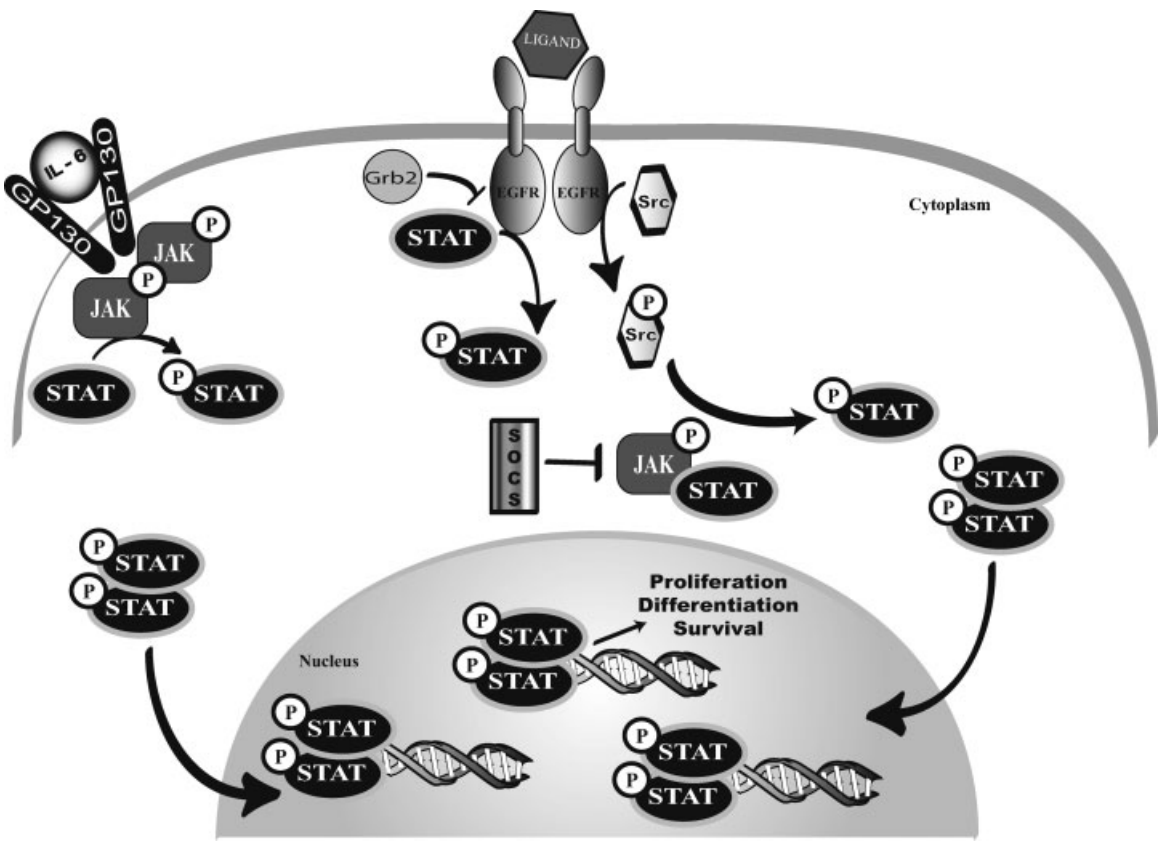

Fig. 1. Mechanisms of STAT-mediated EGFR signaling. STAT activation can occur as shown via cytokine signaling (IL-6), growth factor receptor signaling (EGFR), or non-receptor tyrosine kinase signaling (Src). JAK is not required when STATs bind directly to EGFR for activation (upper left), but JAK provides maximal activation of STATs phosphorylated by EGFR-activated Src (center). Grb2 and SOCS can inhibit STAT-mediated EGFR signaling, respectively, by either binding to the STAT activation site on EGFR (upper left) or by binding to JAK to suppress Src activation of STATs (center). Once activated, STATs dimerize and translocate to the nucleus (lower left and lower right) where they activate the transcription of genes involved in proliferation, differentiation, and survival.

that downregulate the activation of STAT3 [Zhang et al., 2003].

However, it should be noted that the MAPK pathway is also shown to positively regulate STAT signaling. Several components of the pathway including MAPK1, MAPK extracellular signal-regulated kinase 1 (MEK1), and Jun N-Terminal Kinase (JNK) have been shown to phosphorylate STAT3 on Ser727 not to activate the protein, but instead to increase its transcriptional activity [Zhang et al., 2003]. Further studies are needed to determine the contextual placement of these different MAPK pathway regulatory scenarios in vivo during tumor initiation and progression.

EGFR AS AN UPSTREAM RECEPTOR SIGNALING TO STATs

EGFR is a member of the ErbB family of human epidermal growth factor receptor (HER) tyrosine kinases consisting of EGFR (ErbB1), HER2 (ErbB2, Neu), HER3 (ErbB3), and HER4 (ErbB4). Each receptor has a unique extracellular region, a transmembrane domain, and an intracellular kinase domain that is highly conserved within the family. EGFR is activated by receptor overexpression, ligand-independent, and most commonly ligand-dependent mechanisms [Scaltriti and Baselga, 2006]. EGFR is involved in diverse cellular processes including growth, differentiation and survival [Grandis and Sok, 2004].

EGFR has six known ligands, including EGF, transforming growth factor alpha (TGFα), amphiregulin, betacellulin, heregulin, and heparin-binding EGF. Ligand-binding to EGFR causes a conformational change in the extracellular segment of the receptor, which leads to dimerization and autophosphorylation [Scaltriti and Baselga, 2006]. Autocrine signaling occurs when a cell produces both a ligand and its receptor, as is the case in cells that overexpress both EGFR and TGFα leading to uncontrolled cell growth [Song and Grandis, 2000]. TGFα stimulates proliferation of esophageal carcinoma cell lines via autocrine signaling through EGFR, and is shown to stimulate constitutive activation of STAT3 [Schrump and Nguyen, 2001]. Increased STAT3 activation increases proliferation in head and neck cancer cells and is caused, at least in part, by TGFα-mediated activation of EGFR [Kijima et al., 2002]. Treatment of head and neck squamous cell carcinoma (HNSCC) cell lines with TGFα has also been shown to increase activation of several Src family kinases [Leeman et al., 2006]. These results suggest that not only can EGFR signal to STATs directly by binding to its SH2 domain but also through EGFR-mediated activation of Src, upstream of STATs.

Signaling Through Src, JAK, and MAPK Pathways

Ligand binding to EGFR has been shown to activate STAT1, STAT3, and STAT5 via Src, not JAK in a breast cancer cell line [Olayioye et al., 1999]. The role for JAKs in ligand-induced EGFR activation of STAT3 is cell-type dependent. JAKs provide maximal activation of STAT proteins in an EGF-dependent signaling scenario. Inhibiting JAKs in breast cancer cell lines, however, only partially blocks EGF-dependent STAT protein activation, further supporting the role of Src in STAT-mediated EGFR signaling. In the absence of EGF stimulation, Src and JAK cooperate to mediate STAT3 signaling in breast cancer cell lines. However, in the presence of EGF stimulation, STAT3 is activated via EGFR and Src kinase activity is cooperative but not required [Garcia et al., 2001] (Fig. 1).

Proline-rich Tyrosine Kinase 2 (PYK2) has been implicated as a co-mediator of STAT protein activation with c-Src in response to EGF stimulation. Treatment of breast cancer cells with EGF induced STAT3-mediated cell proliferation by recruiting c-Src, PYK2 and STAT3 to EGFR where STAT3 is phosphorylated at Y705 [Shi and Kehrl, 2004]. Forced expression of EGFR did not, however, increase phosphorylation of STAT3 at Y705, suggesting that EGFR is required for phosphorylation at this specific residue, but is not a sufficient catalyst for this event. This could also be due to negative regulation in the MAPK pathway, where MAPK1 activation caused by EGFR activation inhibited STAT3 phosphorylation at Y705 [Quadros et al., 2004].

Ligand-activated EGFR commonly signals downstream to the Ras/Raf/MAPK pathway mediating cell proliferation and survival. In this pathway, growth factor receptor-bound protein 2 (GRB2) and SOS complex and bind to EGFR, where they phosphorylate Ras [Scaltriti and Baselga, 2006]. In breast cancer cell lines, GRB2 and STAT3 bind to the same tyrosine phosphorylation sites on EGFR (Y1086 and Y1068), which enables negative regulation to

occur when GRB2 binds to EGFR with a higher affinity than STAT3. GRB2 has a similar regulatory effect on STAT1, but not STAT5a [Zhang et al., 2003] (Fig. 1). This is not the only scenario in which competitive binding to EGFR is shown to negatively regulate STAT protein activation. Physical interaction of STAT1 and STAT3 with EGFR occurs across multiple domains in the carboxyl terminus of EGFR. SOCS-1 and SOCS-3 also interact with the cytoplasmic domain of EGFR, likely inducing ubiquitination and degradation of ligand-bound EGFR and resulting in a decrease of STAT1 and STAT3 activation [Xia et al., 2002].

Alternative Modes of STAT-Mediated EGFR Signaling

EGF-dependent phosphorylation of STAT5 occurs at a novel site, which implies that EGFR stimulates STAT in a manner unique from other STAT phosphorylation events. EGFR-dependent, c-Src mediated activation of STAT5 occurs via Y694, a novel site compared to the previously described JAK phosphorylation site of Y699 (Fig. 2). Phosphorylation at Y694 causes a different activated conformation of STAT5 than phosphorylation at Y699, as well as different nuclear localization and an impaired ability to bind DNA. Further research identified several other EGF-induced STAT5 phosphorylation sites (Y725, Y740, Y743) in breast cancer cell lines co-overexpressing STAT5 and EGFR [Silva, 2004]. Additionally, EGFR has been shown to be phosphorylated by c-Src at the Y845 residue, and STAT5b may be activated downstream of this pathway [Boerner et al., 2005], providing a means by which c-Src indirectly activates STAT5b through EGFR. These results imply that signaling to STAT proteins via EGFR may elucidate new roles for STAT proteins in oncogenesis, and are hence important areas for further investigation [Silva, 2004].

EGFR can also be activated through several ligand-independent mechanisms, although the subsequent signaling to STAT in these instances is not well understood. Ligand-independent activation of EGFR activation can occur via mutations that render it constitutively active, overexpression of urokinase plasminogen activator receptor via integrin α5B, or cellular stress such as radiation which silences phosphotases and shifts basal levels of phosphorylation to the activated state [Scaltriti and Baselga, 2006]. Crosstalk with G-protein-coupled receptors (GPCRs) is another potential mechanism of ligand-independent EGFR activation [Kalyankrishna and Grandis, 2006]. Little is known about the intracellular pathway of STAT-mediated, ligand-independent EGFR

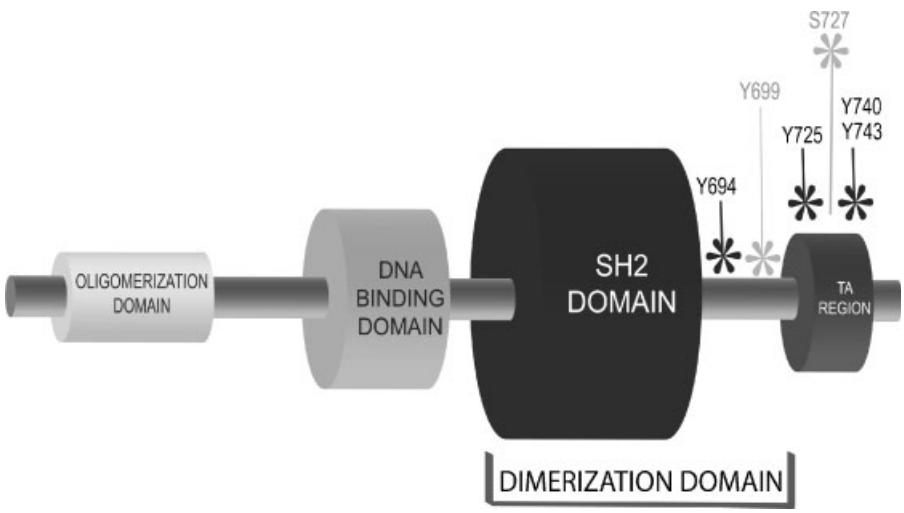

Fig. 2. Phosphorylation sites on STAT5. All STAT proteins contain an oligomerization domain, a DNA binding domain, and an SH2 domain. The SH2 domain of STAT5 is required for binding to proteins like EGFR prior to activation, which occurs when STAT5 is phosphorylated at tyrosine residues just outside of the SH2 domain in the region that is required for STAT5 dimerization. Phosphorylation of certain residues, both serine and tyrosine, within a transactivating region of STAT5 can also occur. Serine phosphorylations in this region enhance the transcriptional activity of STAT5, and tyrosine phosphorylations here may also activate STAT5 in addition to increasing its transcriptional activity. Several novel EGF-dependent phosphorylation sites on STAT5 have been identified (black stars) in addition to other well-described phosphorylation sites that are not EGF-dependent (gray stars). SH2, Src homology 2; TA, transactivating; Y, tyrosine residue; S, serine residue.

activation and signaling. Further investigation is warranted in light of recent correlations between EGFR activating mutations and EGFR-targeted chemotherapeutic sensitivity, and the likely role STAT proteins may play in this apoptotic process [Rosell et al., 2006].

EGFR AND STAT EXPRESSION IN HUMAN CANCER

EGFR and STAT3

Expression levels of both TGFα and EGFR are increased in tumor cells of the head and neck as well as in the adjacent normal mucosa, indicating that the upregulation of these genes is an early event in carcinogenesis. Further, expression levels of both TGFα and EGFR are associated with adverse outcome in head and neck cancers [Song and Grandis, 2000], which suggests that not only is this an early event in carcinogenesis but also a persistent event in tumor progression. STAT3, meanwhile, is found only in the basal epithelium of normal head and neck cells but is widely expressed in both tumor cells and the adjacent normal tissue in head and neck cancer [Leeman et al., 2006]. These observations support the concept of field cancerization and suggest that the expression of STAT3, like the expression of EGFR and TGFα, is an early event in carcinogenesis.

Non-small cell lung carcinoma (NSCLC) cells sensitive to gefitinib, a small molecule tyrosine kinase inhibitor used to inhibit EGFR, often contain a constitutively-activating EGFR mutation. These cells normally survive by signaling to Akt and STAT proteins to activate anti-apoptotic pathways, and gefitinib is capable of eliminating this survival technique in EGFR-dependent cells [Sordella et al., 2004]. Further, STAT3 is known to regulate multiple pathways in tumor progression and to promote survival in NSCLC cell lines, so phosphorylation levels of STAT3 may be a helpful marker in selecting patients for which tyrosine kinase inhibitors would be a successful mode of therapy [Haura et al., 2005a].

Poorly differentiated tumors of the oral cavity express higher levels of EGFR mRNA that tumors with a more differentiated phenotype [Arany et al., 2003], and STAT3 has also been shown to be phosphorylated at higher levels in poorly differentiated HNSCC tumors [Arany et al., 2003; Leeman et al., 2006]. Likewise, a trend toward co-expression of activated EGFR and STAT3 was observed in NSCLC [Cortas et al., 2007]. EGF-induced CyclinD1 was also expressed at greater levels in poorly differentiated HNSCCs, suggesting a role for STAT3 mediation of EGF-induced CyclinD1 expression [Arany et al., 2003]. STAT3 levels also correlate with nodal metastasis in head and neck cancer [Leeman et al., 2006], and STAT3 but not STAT1 is required for EGFR-mediated cell growth [Arany et al., 2003], consistent with their respective roles as an oncogene and a tumor suppressor gene.

EGFR and STAT1

Patients with esophageal carcinomas that demonstrate an activated EGF-STAT1 pathway have a better prognosis than those patients lacking activation of the pathway [Watanabe et al., 2001]. Breast cancer patients with STAT1 activation, as determined by either DNA binding or tyrosine phosphorylation, have prolonged survival and relapse less frequently, while the activation of STAT3 or STAT5 did not predict patient outcome [Widschwendter et al., 2002]. STAT1 is also activated at higher levels in well differentiated HNSCCs [Arany et al., 2003; Leeman et al., 2006], which tend to have a better prognosis than more poorly differentiated HNSCCs [Eriksen et al., 2005]. These results indicate that STAT1 expression may confer a better patient outcome, and therefore targeting STAT1 in conjunction with EGFR may not produce any synergistic or additive antitumor effects.

EGFR and STAT5

Because of the role of STAT5 as an alternative survival pathway for cells targeted with EGFR-directed therapies STAT5 is a potential target for combination with EGFR therapies. STAT5b is overexpressed and activated in HNSCC, and STAT5b antisense mRNA inhibits tumor growth. STAT5 is also constitutively active in HNSCC, but when EGFR is inhibited there is only a partial abrogation in corresponding STAT5 levels [Kalyankrishna and Grandis, 2006], which suggests that there are other mechanisms of STAT5 activation independent of EGFR. However, it should be noted that due to a large overlap in target gene profiles between STAT3 and STAT5, inhibiting only STAT5 may not be sufficient for antitumor effects if STAT3 remains overexpressed.

COMBINING EGFR AND STAT THERAPIES

To date, no STAT inhibitor has been tested in cancer patients. Possible methods to selectively target STATs have been reported previously in the context of STAT3 targeting, including: (1) blocking DNA-binding sites, (2) interrupting STAT3 dimerization through the SH2 domain, and/or (3) inhibiting translation of STAT3 mRNA [Leeman et al., 2006]. Interruption of STAT binding to DNA can be achieved using a transcription factor or a peptide aptamer, and the latter may also be used to block the SH2 domain and interrupt dimerization [Nagel-Wolfrum et al., 2004; Xi et al., 2005]. The SH2 domain of STAT can also be blocked using a G-quartet oligodeoxynuclotide [Jing et al., 2003] or a phosphotyrosyl peptidomimetric [Turkson et al., 2001]. Inhibition of STAT mRNA translation can occur via an antisense oligonucleotide [Chiarle et al., 2005] or STAT siRNA [Zamo et al., 2002; Gao et al., 2005]. Non-selective targeting of STATs is also feasible using natural compounds or by targeting other molecules upstream of STATs such as EGFR, Src, JAK, IL-6, GP130, and other cytokines [Leeman et al., 2006].

EGFR-targeted therapies, on the other hand, are approved for use by the FDA and are used routinely in the clinic. There are two classes of EGFR inhibitors, including monoclonal antibodies (mAbs) that bind to EGFR to inhibit ligand activation, and small-molecule tyrosine kinase inhibitors (TKIs) that block the EGFR tyrosine kinase domain. TKIs are FDA approved for use in pancreatic cancer and NSCLC, and mAbs are FDA approved for use in colorectal cancer and cancers of the head and neck [Scaltriti and Baselga, 2006] (Table II). Most patients treated with TKIs or mAbs to date have demonstrated only modest clinical responses [Baselga and Arteaga, 2005], suggesting that other pathways may also contribute to cancer progression.

One potential way to improve EGFR-targeted therapies may be to administer an EGFR inhibitor in conjunction with another pathway inhibitor. EGFR-inhibition only partially reduces STAT activity, making STATs ideal molecules to be targeted in conjunction with EGFR. STATs can be activated by several EGFR-independent mechanisms including cytokines, non-receptor tyrosine kinases, and even other growth factor receptors. Therefore, in order to completely abolish STAT activity, it may be necessary to target molecules both upstream and downstream in the EGFR-STAT pathway. Preliminary in vitro evidence exists suggesting that combining inhibition of EGFR and STAT3 in breast cancer cell lines results in synergistic inhibition of tumor growth [Dowlati et al., 2004].

It was also reported that lung cancer cell lines resistant to EGFR-targeted therapies induced cell cycle arrest and reduced invasion in response to dasatinib, a Src inhibitor [Song et al., 2006]. This research suggests that dasatinib may have an additive or synergistic effect when administered concurrently with EGFR-targeted therapies, perhaps due to EGF-independent mechanisms of Src-mediated STAT activation. Another pathway inhibitor that has been shown to work in conjunction with EGFR therapies to target STAT is rapamycin, an antibiotic that targets mTOR [Rajan et al., 2003]. Combining these therapies resulted in synergistic antitumor effects in glioblastoma multiforme cell lines, again demonstrating that inhibiting multiple signaling pathways is a more efficacious mode of treatment than targeting a single pathway alone.

FUTURE DIRECTIONS

While the role of ligand-dependent, STAT-mediated EGFR signaling through Src and JAK is reasonably well described, the role of cross-talk with this pathway requires further investigation. EGFR generally signals through several different pathways including MAPK, and Akt, which suggests that increased understanding of the dynamics of these interacting

TABLE II. EGFR-Targeted Therapies Approved for Use by the Food and Drug Administration

Drug	Trade name	Manufacturer	Class	Site approved for use
Erlotinib	Tarceva	OSI, Genetech	TKI	NSCLC, pancreatic cancer
Cetuximab	Erbitux	Imclone, Bristol-Myers Squibb	mAb	Colorectal carcinoma, HNSCC
Gefitinib	Iressa	AstraZeneca	TKI	NSCLC

mAb, monoclonal antibodies; TKI, tyrosine kinase inhibitors; NSCLC, non-small cell lung carcinoma; HNSCC, head and neck squamous cell carcinoma.

pathways is necessary to elucidate the precise role of STATs. Ligand-independent EGFR activation and signaling to STATs should also be explored further in order to elucidate how STATs contribute to EGFR-dependence in cells with activating EGFR mutations, and the role of STATs in EGFR-targeted therapy sensitivity or resistance. Finally, increased understanding of the role of specific phosphorylation residues, both tyrosine and serine, is needed to fully understand STAT-mediated EGFR signaling, as EGFR has been shown to phosphorylate STAT5 in novel sites which correspond to specific. Elucidation of both intracellular crosstalk with, and molecular activation of STAT-mediated EGFR signaling pathways is essential to rationally design the most effective molecularly targeted cancer therapies.

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