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The Fibroblast Growth Factor Receptor Signaling Pathway as a Mediator of Intrinsic Resistance to EGFR-specific Tyrosine Kinase Inhibitors in Non-Small Cell Lung Cancer

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Abstract

The EGFR has been targeted through the development of selective tyrosine kinase inhibitors (TKIs) that have proven effective in a subset of non-small cell lung cancer (NSCLC) patients, many bearing gain-of-function EGFR mutations or *egfr* gene amplification. However, the majority (~80–90%) of NSCLC patients do not respond to EGFR-specific TKIs and a high rate of acquired resistance to these therapeutics is observed in those that do respond. Thus, EGFR-specific TKIs will not, as single agents, make a high impact on overall lung cancer survival. A number of studies support the activities of other receptor tyrosine kinase pathways including cMet, IGF-1R and FGFRs as mechanisms for both intrinsic and acquired resistance to EGFR TKIs. While the role of cMet and IGF-1R signaling systems as mechanisms of resistance to EGFR TKIs has been widely reviewed in recent years, the potential role of FGFR-dependent signaling as a mechanism for EGFR TKI resistance has more recently emerged and will be highlighted herein. Due to the high degree of homology of FGFRs with VEGFRs and PDGFRs, FGFR-active TKIs already exist via development of VEGFR-targeted TKIs as angiogenesis inhibitors. Thus, these agents could be rapidly advanced into clinical investigations as FGFR inhibitors, either alone or in combination with TKIs selective for EGFR, cMet or IGF-1R as a means to expand the spectrum of NSCLC patients that can be effectively targeted with TKI-directed therapies.

Keywords

FGF; FGFR; NSCLC; intrinsic resistance; receptor tyrosine kinase; tyrosine kinase inhibitors

1. Introduction

Lung cancer, the leading cause of cancer deaths in the United States and worldwide (Jemal et al., 2008), is divided into non-small cell lung cancer (NSCLC), accounting for ~85% lung cancers and small-cell lung cancer (SCLC), accounting for ~15% of all lung cancers. Despite

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advances in early detection and continued incremental improvement in standard cytotoxic therapy-based care, the five-year survival rate for lung cancer has changed minimally over the past twenty-five years from 13% for those diagnosed between 1975–1977 to 16% for those diagnosed between 1996–2003 (Jemal et al., 2008).

The failure of advancements in standard cytotoxic chemotherapy to markedly prolong lung cancer patient survival has provided an impetus to pursue novel targets in lung cancer. The six hallmarks of cancer as outlined by Hanahan and Weinberg present a useful structure for considering the dominant pathways and cell programs controlling cancer cell behavior (Hanahan and Weinberg, 2000). Among these hallmarks, “self-sufficiency in growth” is frequently driven by receptor tyrosine kinase-dependent growth factor pathways operating in an autocrine fashion. In this regard, the high frequency of epidermal growth factor receptor (EGFR) expression in NSCLC (Hirsch et al., 2003) highlighted this receptor tyrosine kinase (RTK) as an attractive target for the development of small-molecule tyrosine kinase inhibitors (TKIs), including gefitinib and erlotinib (Dancey, 2004). In 2005, a placebo-controlled phase III trial, the National Cancer Institute of Canada BR.21 (NCIC BR.21) evaluated erlotinib in stage IIIB/IV NSCLC patients with prior chemotherapy exposure. Erlotinib yielded an objective response rate of ~9% versus <1% for placebo, increased overall survival (6.7 vs. 4.7 months), and reduced cancer-related symptoms. Independent predictors of survival were Asian ethnicity, adenocarcinoma histology, and never smoker status (Shepherd et al., 2005). In light of these aforementioned independent survival predictors in the NCIC BR.21 trial, a slight benefit did exist in an unselected population. However, a significant number of patients who received erlotinib do not respond despite reassuring demographic factors (i.e. female, Asian, adenocarcinoma, never smokers) (Shepherd et al., 2005). Clearly, demographics alone are not sufficient to define who will respond to erlotinib. Moreover, EGFR expression is necessary for EGFR TKI responsiveness, but is also not sufficient to predict response as a single measurement. However, the presence of activating EGFR mutations (Lynch et al., 2004; Mitsudomi and Yatabe, 2007; Paez et al., 2004) or *egfr* gene amplification (Cappuzzo et al., 2005) are highly predictive of response to EGFR TKI therapy. Activating EGFR mutations are present in ~10% of lung adenocarcinomas in Western populations and 30–50% in Asian populations (Herbst et al., 2008). Importantly, response to gefitinib or erlotinib is not dictated simply by gain-of-function EGFR mutations as a significant number of EGFR TKI-responsive patients bear lung tumors with wild-type EGFR (Sequist et al., 2007). Combined, these studies demonstrate the necessity of using EGFR TKIs on selected patient populations. Also, as indicated in Figure 1, the majority of NSCLC patients do not respond to EGFR TKIs, indicating that additional target pathways mediating self-sufficiency in growth will need to be identified and appropriate inhibitors deployed to impact the outcome of these patients.

All patients with tumors which are initially sensitive to EGFR TKIs will relapse (reviewed in (Camp et al., 2005; Engelman et al., 2008; Morgillo et al., 2005)). Of the patients with acquired resistance to EGFR TKI treatments, approximately 50% bear the EGFR T790M mutation that resides within an analogous position to previously-defined acquired resistance mutations in Abl, PDGFRα and cKit. In addition, c-Met amplification is likely to account for approximately 20% of acquired resistance to EGFR TKIs (Engelman et. al, 2007, Bean et. al., 2007). The mechanism(s) that account for the remaining 30% of acquired resistance to EGFR TKIs remains ill-defined. If intrinsic resistance is defined as having an initial clinical response followed by disease progression within 6 months, or having no initial response to treatment, then the majority (~80–90%) of NSCLC patients from Western populations who harbor a tumor not bearing an activating EGFR mutation are likely to exhibit intrinsic resistance to EGFR TKI therapy (Figure 1). One established mechanism for intrinsic resistance is seen in lung tumors bearing gain-of-function K-Ras mutations leading to EGFR-independent activation of multiple effector pathways including the ERK MAP kinase signaling pathway (Cox and Der, 2003). K-Ras mutations are detected in 10–30% of lung adenocarcinomas (Herbst et al., 2008), but rarely

in squamous and large cell carcinomas, and present in a mutually exclusive pattern with regard to EGFR activating mutations (Eberhard et al., 2005; Pao et al., 2005).

The NSCLC tumors that exhibit intrinsic resistance to EGFR TKIs distinct from K-Ras mutation, in fact, represent the majority of lung cancers (Figure 1). Moreover, no specific targeted therapies presently exist for the treatment of this group. It is increasingly evident, from both clinical and biological perspectives, that EGFR is only one of many important growth factor pathways that function in lung cancer. We and others have considered the hypothesis that EGFR-independent receptor tyrosine kinase signaling pathways dominate in EGFR TKI-insensitive NSCLC (Marek et al., 2009;Morgillo and Lee, 2005;Thomson et al., 2008). Therefore, continued progress towards successful therapeutics of NSCLC will ultimately depend on identification and inhibition of additional receptor tyrosine kinases and their downstream signaling pathways dominant in individual NSCLC tumors. As proof-of-principle for this hypothesis, we will briefly consider the role of the HGF/c-Met and insulin-growth factor receptor (IGF-1R) signaling pathways in the intrinsic resistance of NSCLC to EGFR TKIs and more extensively discuss the role of the fibroblast growth factors (FGFs) and their receptors as components of a novel autocrine growth pathway in lung cancer.

2. Alternative receptor tyrosine kinase pathways as mechanisms for intrinsic resistance to EGFR TKIs

As indicated in Figure 1, 80 to 90 percent of NSCLC patients bear tumors that lack activating EGFR mutations and/or *egfr* gene amplification and less likely to be responsive to EGFR TKIs. Besides mutations in K-Ras that contribute to EGFR TKIs intrinsic resistance (Herbst et al., 2008), a simple explanation for the insensitivity of the majority of NSCLC to EGFR inhibitors is the dominant activity of alternative RTK systems distinct from EGFR. In this regard, it has been demonstrated that 33 of the 58 defined RTKs are expressed at the mRNA level in at least 25 percent of a panel of primary NSCLC tumors (Muller-Tidow et al. 2005). An independent phosphoproteomic approach confirms the extensive array of RTKs that are expressed and active in NSCLC cell lines and tumors (Rikova et al., 2007). Thus, there is no shortage of candidates for RTKs that may function as alternatives to EGFR in signal transduction of growth and transformation in NSCLC. Among these, the literature highlights roles for cMet (Engelman et al., 2007;Lutterbach et al., 2007), Axl (Shieh et al., 2005;Wimmel et al., 2001), IGF-1R (Morgillo and Lee, 2005) and PDGFR (Thomson et al., 2008;McDermott et al., 2009) as well as novel tyrosine kinases such as Alk and Ros (Rikova et al., 2007). Among these, a role for c-Met and IGF-1R has been most extensively explored (Camp et al., 2005;Engelman and Janne, 2008;Morgillo and Lee, 2005) (Figure 2).

The RTK c-Met, is expressed and active in 60% of NSCLC as measured by IHC staining (Ma et al., 2005). High levels of HGF, the c-Met ligand, have also been correlated with poor prognosis of NSCLC patients (Siegfried et al., 1998). Recent studies have demonstrated c-Met amplification and over-expression as a mechanism for both intrinsic and acquired gefitinib resistance in NSCLC (Bean et al., 2007). Autocrine signaling via the c-Met/HGF loop leads to intrinsic gefitinib resistance by restoring the PI3K/AKT signaling pathway independent of EGFR or ErbB3 activation in adenocarcinoma NSCLC lines harboring EGFR activating mutations (Yano et al., 2008). Furthermore c-Met gene amplification serves as a mechanism for acquired gefitinib resistance in both NSCLC lines and ~20% of NSCLC tumors (Engelman and Janne, 2008). In response to gefitinib treatment, NSCLC lines underwent amplification of chromosomal region 7q31.1–33.3 containing the c-*Met* gene, allowing c-Met activation of the PI3K/AKT pathway in an ErbB3-dependent manner, but independent of either EGFR or ErbB2 activation (Engelman et al., 2007). Importantly, inhibition of c-Met restored gefitinib sensitivity in these cells. Thus, c-Met signaling likely contributes to reduced EGFR-specific TKI response in NSCLC.

The IGF-IR and its ligands insulin, insulin-like growth factor I (IGF-I), and insulin-like growth factor II (IGF-II) have also been implicated as components of an active growth factor pathway in NSCLC. NSCLC cell lines and primary tumors express IGF-IR (Cappuzzo et al., 2006; Morgillo et al., 2007; Quinn, 1996), although one study suggests that IGF-1R protein, by itself, does not correlate with EGFR TKI resistance (Cappuzzo et al., 2006). Primary NSCLC tumors have been shown to express higher concentrations of circulating IGF-I than surrounding normal lung tissue (Minuto et al., 1986), suggesting a paracrine signaling mechanism between tumor stromal fibroblasts secreting IGF-I and neoplastic epithelial cells. Clearly NSCLC cell lines are responsive to exogenous IGF-I (Ankrapp and Bevan, 1993) and knockdown of IGF-IR in NSCLC cell lines with antisense or RNAi strategies inhibited cell proliferation and *in vitro* and *in vivo* estimates of transformed growth (Ankrapp and Bevan, 1993; Dong et al., 2007; Lee et al., 1996). Evidence also suggests that inhibition of IGF-IR in NSCLC cells lines may increase their sensitivity to gefitinib (Morgillo et al., 2007). Together, this evidence suggests that the IGF-I/IGF-IR and HGF/cMet signaling pathways may contribute to both intrinsic and acquired resistance to EGFR TKIs in NSCLC.

Despite an extensive literature demonstrating a role for FGF receptors (FGFRs) in human disease including cancer (see Section 3), these receptors have not been widely considered as a putative autocrine growth factor pathway in lung cancer. The literature supporting the contribution of specific FGFs and FGFRs to EGFR TKI resistance in NSCLC is reviewed in Section 4.

3. FGF and FGFR signaling in cancer

The mammalian fibroblast growth factors (FGFs) are comprised of 22 family members and mediate numerous developmental programs during embryogenesis as well as critical roles in adult tissue repair and maintenance (reviewed in (Eswarakumar et al., 2005; Grose and Dickson, 2005; Mohammadi et al., 2005)). FGFs initiate signal transduction by binding members of a family of RTKs (FGFR1-4), usually in the context of heparan sulfate proteoglycans (HSPG), inducing receptor dimerization and commencement of downstream signaling. FGFRs contain two or three extracellular Ig-like loops that comprise the FGF and HSPG binding sites. Particularly important, alternative splicing in the membrane-proximal Ig loop of FGFR1-3 dictates ligand specificity (Zhang et al., 2006). The third loop is encoded by an invariant N-terminal portion (exon IIIa) and either exon IIIb or IIIc for the C-terminal portion. FGFR2-IIIb and FGFR3-IIIb splice variants bind FGF7 and FGF10 with high affinity while FGFR1-3 IIIc splice variants bind FGF2 and FGF9. Generally, these isoforms are also cell type specific with FGFR-IIIb expression on epithelial cells and FGFR-IIIc expression on mesenchymal cells. In addition, FGF7 and FGF10, which bind to epithelial FGFR-IIIb, are expressed in mesenchymal cell types while FGF2 is expressed in epithelial cells and binds to FGFR-IIIc presented on mesenchymal cells, establishing paracrine signaling pathways (Grose and Dickson, 2005).

Aberrant signaling by FGFs and FGFRs has been implicated in diverse human cancers (Eswarakumar et al., 2005; Grose and Dickson, 2005). In many cancers, FGF and FGFR signaling is enhanced by acquisition of somatic gain-of-function mutations in specific FGFRs. In urothelial cancers, mutations in the ligand binding domain of FGFR3 cause ligand-independent dimerization or stabilization of the active conformation of the receptor while mutations in the FGFR3 kinase domain can render the receptor constitutively active (Grose and Dickson, 2005). Somatic mutations in FGFR2 conferring gain-of-function are observed in ~10% of primary endometrial cancers as well as endometrial tumor cell lines (Dutt et al., 2008; Pollock et al., 2007). Moreover, blocking FGFR2 activity inhibits transformation and survival of endometrial carcinoma lines (Dutt et al., 2008). Similar somatic mutations in FGFR2 and FGFR3 are also observed in gastric cancer and colorectal cancers, respectively

(Jang et al., 2001). Blocking FGFR2 activity with an FGFR-active TKI inhibited growth of gastric cancer cell lines (Takeda et al., 2007).

In the hematologic malignancy 8p11 myeloproliferative syndrome (EMS), genomic sequences encoding the kinase domain of FGFR1 are fused with one of several donor genes causing constitutive activation of the FGFR1 kinase domain (reviewed in (Grose and Dickson, 2005)). Also, a similar gene translocation event occurs with FGFR3 in some multiple myelomas (Grose and Dickson, 2005).

FGFRs 1, 2, and 4 are frequently over-expressed in breast cancers and a selective inhibitor of FGFR activity caused G1 growth arrest in breast cancer cell lines (Koziczak et al., 2004). FGFRs have also been shown to be physically associated with N-cadherin in various cancer cells, resulting in cell survival and motility (Cavallaro and Christofori, 2004; Suyama et al., 2002). It is hypothesized that N-cadherin facilitates FGF2 binding while preventing ligand-induced internalization, thereby yielding sustained signaling (Cavallaro and Christofori, 2004; Suyama et al., 2002). Finally, tumorigenesis via FGFs and FGFRs does not always depend on the accumulation of receptor mutations or marked over-expression. In the mouse, FGF3, FGF4 and FGF8 were initially identified as mouse mammary tumor virus-activated genes leading to mouse mammary gland tumorigenesis, presumably through an autocrine mechanism mediated by the deregulated FGFs (Payson et al., 1996; van Leeuwen and Nusse, 1995). Similarly, FGF2 and FGF9 are expressed by stromal cells within the prostate as well as by epithelial carcinoma cells. These growth factors can then signal in a paracrine and autocrine fashion by binding to FGFR1-IIIc and FGFR2-IIIc detected on prostate epithelial cancer cells (Kwabi-Addo et al., 2004; Kwabi-Addo et al., 2001). These studies provide examples where FGF/FGFR-mediated transformation does not require somatic mutation, but can occur simply through inappropriate expression of a specific FGF.

Thus, FGFRs can be activated in the setting of human cancer through somatic mutations, gene fusion events or induction of ligands and/or FGFRs, thereby resulting in autocrine activation.

4. FGF and FGFR autocrine signaling in non-small cell lung cancer

Accumulating evidence implicates specific FGFs and FGFRs as components of an autocrine signaling pathway in NSCLC tumors and cell lines. While rare somatic mutations in different FGFRs have been detected in NSCLC ((Ding et al., 2008), http://www.sanger.ac.uk/genetics/CGP/cosmic/), numerous *in vitro* studies employing NSCLC cell lines reveal that specific FGFs (FGF2 and FGF9) as well as FGFR1 and FGFR2 are frequently co-expressed (Berger et al., 1999; Chandler et al., 1999; Fischer et al., 2008; Kuhn et al., 2004; Marek et al., 2009; Thomson et al., 2008). Several independent studies also demonstrated frequent expression of FGF2, FGFR1 and FGFR2 mRNA and protein in primary NSCLC specimens as well (Behrens et al., 2008; Donnem et al., 2009; Muller-Tidow et al., 2005; Volm et al., 1997). Molecular analysis of the FGFR1 and FGFR2 splice variant status in NSCLC cell lines revealed that the relevant FGFR-IIIc forms are expressed (Fischer et al., 2008; Marek et al., 2009), a requirement for serving as a receptor for FGF2 and FGF9 (Figure 3). Moreover, application of anti-sense RNA or RNAi approaches (Kuhn et al., 2004; Marek et al., 2009), neutralizing FGF2 antibodies (Kuhn et al., 2004) or FGFR-active TKIs (Fischer et al., 2008; Marek et al., 2009; Thomson et al., 2008) caused inhibition of cell proliferation or tumor growth, suggesting that the co-expressed FGF2 and FGFRs function as an autocrine growth pathway in some NSCLC cell lines. The FGF-FGFR pathway has also been suggested to mediate EGFR TKI resistance (Marek et al., 2009; Thomson et al., 2008). In general, these studies show that an FGF-FGFR autocrine pathway dominates in NSCLC cell lines of squamous cell (SCC) and large cell carcinoma (LC) histologies that frequently exhibit a more mesenchymal differentiation state (Marek et al., 2009; Thomson et al., 2008). This contrasts

with the enrichment of adenocarcinoma (AC) and bronchoalveolar histologies observed in primary tumors and NSCLC cell lines sensitive to EGFR-specific TKIs ((Coldren et al., 2006; Herbst et al., 2008) and Figure 3). Moreover, Thomson *et al* suggest that a switch to FGFR and PDGFR signaling occurs in concert with an epithelial to mesenchymal transition (EMT) (Thomson et al., 2008), a malignant phenotype (Sabbah et al., 2008) that has also been correlated with EGFR-specific TKI resistance (Coldren et al., 2006).

5. FGFR inhibitors as therapeutics in NSCLC

5.1. Receptor TKIs

Both small-molecule TKIs and inhibitory FGFR1 monoclonal antibodies have been developed as potential therapeutics to disrupt FGFR signaling in cancer cells. Due to extensive sequence homology, FGFRs, VEGFRs and PDGFRs constitute a sub-family of receptor tyrosine kinases (Manning et al., 2002). As a result, many TKIs developed as inhibitors for VEGFRs will also inhibit PDGFR and FGFR family members at similar potencies (see Table 1). In our recent study demonstrating an autocrine role for FGFR1 and FGFR2 in NSCLC cells, RO4383596 served as an effective FGFR inhibitor (Marek et al., 2009). Likewise, AZD2171 (cediranib) was developed as a VEGFR inhibitor (Nikolinakos and Heymach, 2008), but exhibits good potency for FGFRs (Table 1) and has been employed as an effective inhibitor of growth of FGFR2-driven gastric cancer cell lines (Takeda et al., 2007). Additionally, a multi-kinase targeted TKI, TKI-258, has been used to inhibit activated FGFR3 in multiple myeloma (Xin et al., 2006). It is possible that this class of TKIs with broad-spectrum activity against multiple members of the FGFR/VEGFR/PDGFR family will serve as superior anti-cancer agents. However, it may also be possible to develop TKIs with higher selectivity for the FGFR family relative to VEGFRs and PDGFRs. An advantage of such inhibitors might be the lack of various side-effects caused by VEGFR or PDGFR inhibition.

5.2. Monoclonal antibodies

Based on the effectiveness of inhibitory monoclonal antibody reagents targeting EGFR or ErbB2 (Croce, 2008), similar antibody-base reagents targeting members of the FGFR family may serve as excellent therapeutics for cancers such as NSCLC where these receptors may function in autocrine growth signaling. An advantage of monoclonal antibody-based agents is their exquisite specificity for distinct FGFR proteins as well as avoiding activity on VEGFR and PDGFR family members. In this regard, fully-human FGFR1 inhibitory monoclonal antibodies have been developed with specificity for either FGFR1-IIIb or FGFR1-IIIc (Sun et al., 2007). These monoclonal antibodies potently inhibited FGF signaling mediated by the respective FGFR1 isoforms. Based on the observation that FGFR2-IIIc appears to be a dominant FGFR2 isoform active in NSCLC cell lines (Marek et al., 2009), inhibitory FGFR2-IIIc antibodies could also be developed and assessed as inhibitors of FGFR signaling. Human single-chain Fv antibodies to FGFR3-IIIc have been shown to inhibit growth of bladder cancer cells *in vitro* (Martinez-Torrecuadrada et al., 2005). Finally, as an alternative approach, soluble FGFR1 reagents have been developed as ligand traps that exhibit potent inhibitory actions on human NSCLC cells grown as xenografts in immunodeficient mice (Ogawa et al., 2002).

5.3. Identification of lung tumors bearing an activate FGFR pathway

Based on the clinical experiences with gefitinib and erlotinib, FGFR TKIs and/or FGFR monoclonal antibodies should be targeted to lung cancer patients whose tumors bear active FGFR signaling pathways. While gain-of-function FGFR mutations have been identified in certain malignancies, studies to date have failed to reveal frequent mutation or amplification of FGFRs in lung cancer. Rather, it is likely that the FGFR pathway is activated through autocrine production of the ligands including FGF2 and FGF9 (Fischer et al., 2008; Marek et al., 2009; Thomson et al., 2008). Our own study revealed enrichment of the squamous cell and

large cell lung carcinoma histologies in the FGFR-dependent NSCLC cell lines (Marek et al., 2009) although not all squamous cell and large cell carcinoma cell lines were sensitive to FGFR inhibition. Another potential identifier of FGFR-dependent NSCLC cell lines is the ligand, FGF2. In this regard, elevated FGF2 levels are noted in serum and urine from diverse cancer patients (Nguyen et al., 1994; Ueno et al., 2001) as well as NSCLC patients (Brattstrom et al., 2002; Brattstrom et al., 2004; Ueno et al., 2001). Secreted FGF2 levels have not yet been directly linked to FGFR-dependence in the primary tumor. However, if high serum or urinary levels of FGF2 accurately predict FGFR dependence, then the growth factor itself may provide a simple biomarker. Yet, our recent experience with NSCLC cell lines reveals a number of lines that express FGF2, but not FGFR1 or FGFR2 (Marek et al., 2009 and unpublished observations). Thus, measurements that relate tumor co-expression of FGF2 and FGFRs are likely to be a more reliable measure of functional activity of an FGF-FGFR pathway in NSCLC.

5.4. Re-evaluation of VEGFR TKI clinical trial results

As previously noted, many VEGFR TKIs also inhibit FGFRs at similar potency (Table 1) due to the inherent homology of FGFRs and VEGFRs (Manning et al., 2002). A relatively high potency for FGFRs is especially apparent with TKI-258, AZD2171 (cediranib), BMS-540215 (brivanib) and pazopanib. All four of these TKIs have entered clinical trial for evaluation and tumor responses have been noted (Kumar et al., 2007;Nikolinakos and Heymach, 2008;Platero et al., 2008;Sarker et al., 2008). While the results from these trials are interpreted in the context of the activity of the TKIs on VEGFRs, it is possible that both tumor responses and side-effects may be mediated, in part, through FGFR antagonism. In fact, evaluation of tumor response in a brivanib trial revealed that FGF2-positive tumors showed increased response to brivanib relative to FGF2-negative tumors (Platero et al., 2008). This study, in particular, provides impetus for reevaluating tumor responsiveness to VEGFR TKIs to determine if an active FGF and FGFR pathway in the tumor is better correlated with response.

6. Conclusions and future perspective: therapeutic approaches involving simultaneous inhibition of multiple receptor tyrosine kinases

Ultimately, effective treatment of NSCLC will likely require therapeutic strategies involving the combined use of two or more TKIs. Despite dramatic tumor responses to erlotinib and gefitinib in NSCLC patients whose tumors bear activating EGFR mutations, all sensitive tumors eventually undergo one or more mechanisms of acquired resistance (reviewed in (Engelman and Janne, 2008; Morgillo and Lee, 2005)). In addition to the ability of FGFR signaling to mediate intrinsic resistance to EGFR inhibitors as discussed above, our preliminary findings reveal that expression of FGFR2 and FGFR3 are induced at the mRNA and protein level following gefitinib treatment of EGFR-dominant NSCLC cell lines and head-and-neck cancer cell lines (Ware, Marshall and Heasley, unpublished observations), thus providing yet another mechanism for acquired resistance to EGFR TKIs. If induction of FGFRs in response to treatment with EGFR TKIs is proven to be a significant mechanism for acquired resistance to this class of TKIs in patients, then combined treatment with EGFR and FGFR-specific TKIs could represent a strategy to prolong or enhance the action of drugs like gefitinib and erlotinib. Moreover, a recent study (Fischer et al., 2008) as well as our own unpublished observations demonstrate that many NSCLC cell lines appear to employ baseline autocrine signaling through both EGFRs and FGFRs without pretreatment as evidenced by additive or synergistic growth inhibition by combinations of TKIs inhibiting EGFR and FGFRs. In a similar fashion, Stommel *et al* demonstrated coactivation of multiple RTKs in glioblastoma cell lines and showed that combinations of RTK inhibitors are required to decrease signaling, cell survival and anchorage-independent growth (Stommel et al., 2007). It is likely, therefore, that multiple RTKs including EGFR, c-Met, IGF-1R and FGFRs will be engaged simultaneously in specific NSCLCs such that specific combinations of selective TKIs will be required to completely inhibit signaling

and cell transformation. As the repertoire of TKIs and antibody-based inhibitors of RTKs continues to expand, it will be important to also generate gene expression signatures (Coldren et al., 2006), genotype signatures (McDermott et al., 2007) or phosphoproteomic signatures (Rikova et al., 2007) for human cancers such as NSCLC that delineate the activated and dominant pathways in a given tumor so that appropriate combinations of inhibitors can be deployed.

In conclusion, the high frequency of intrinsic resistance to EGFR-specific TKIs as well as the occurrence of acquired-resistance to these drugs indicates that additional receptor tyrosine kinase-dependent pathways must be targeted to bring about effective inhibition of tumor growth. The literature reviewed here indicates that FGFs and FGFRs function in an autocrine fashion in NSCLC. Because of the inherent homology between FGFRs and VEGFRs, a number of TKIs with activity on FGFRs are already available and can be quickly tested as therapeutics, either alone or in combination with EGFR TKIs.

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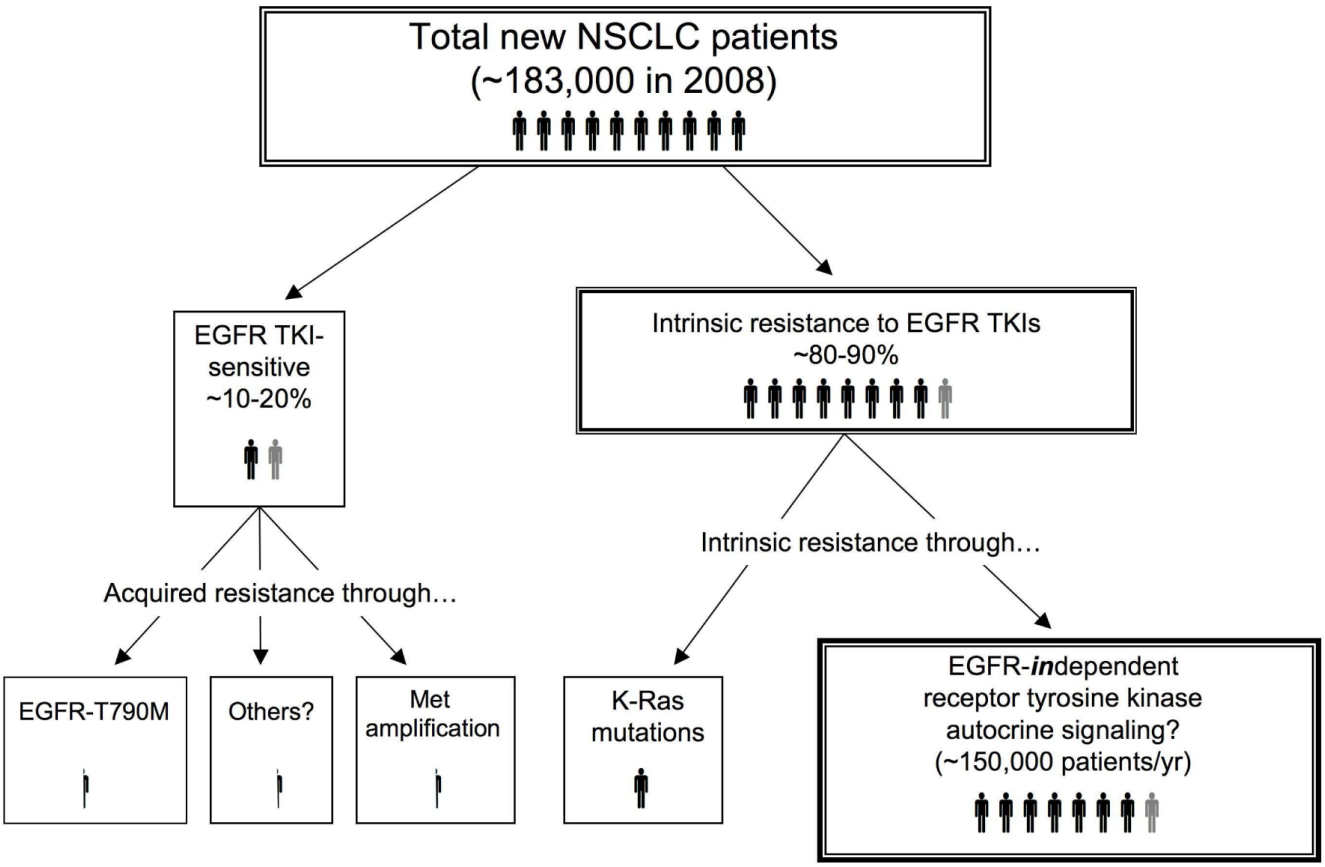

Figure 1. Frequencies of intrinsic resistance to EGFR-specific TKIs relative to sensitivity/acquired resistance in NSCLC

The diagram indicates the relative frequencies of EGFR TKI sensitivity (10–20% in the United States) versus intrinsic resistance (80–90%). Mutation of K-Ras occurs in ~10–30% of adenocarcinomas (but rarely in squamous and large cell carcinoma) and accounts for a known resistance mechanism to EGFR TKIs (Herbst et al., 2008). We hypothesize that autocrine signaling through EGFR-independent receptor tyrosine kinases functions as a mechanism of intrinsic resistance to EGFR TKIs in NSCLC not bearing EGFR or K-Ras mutations.

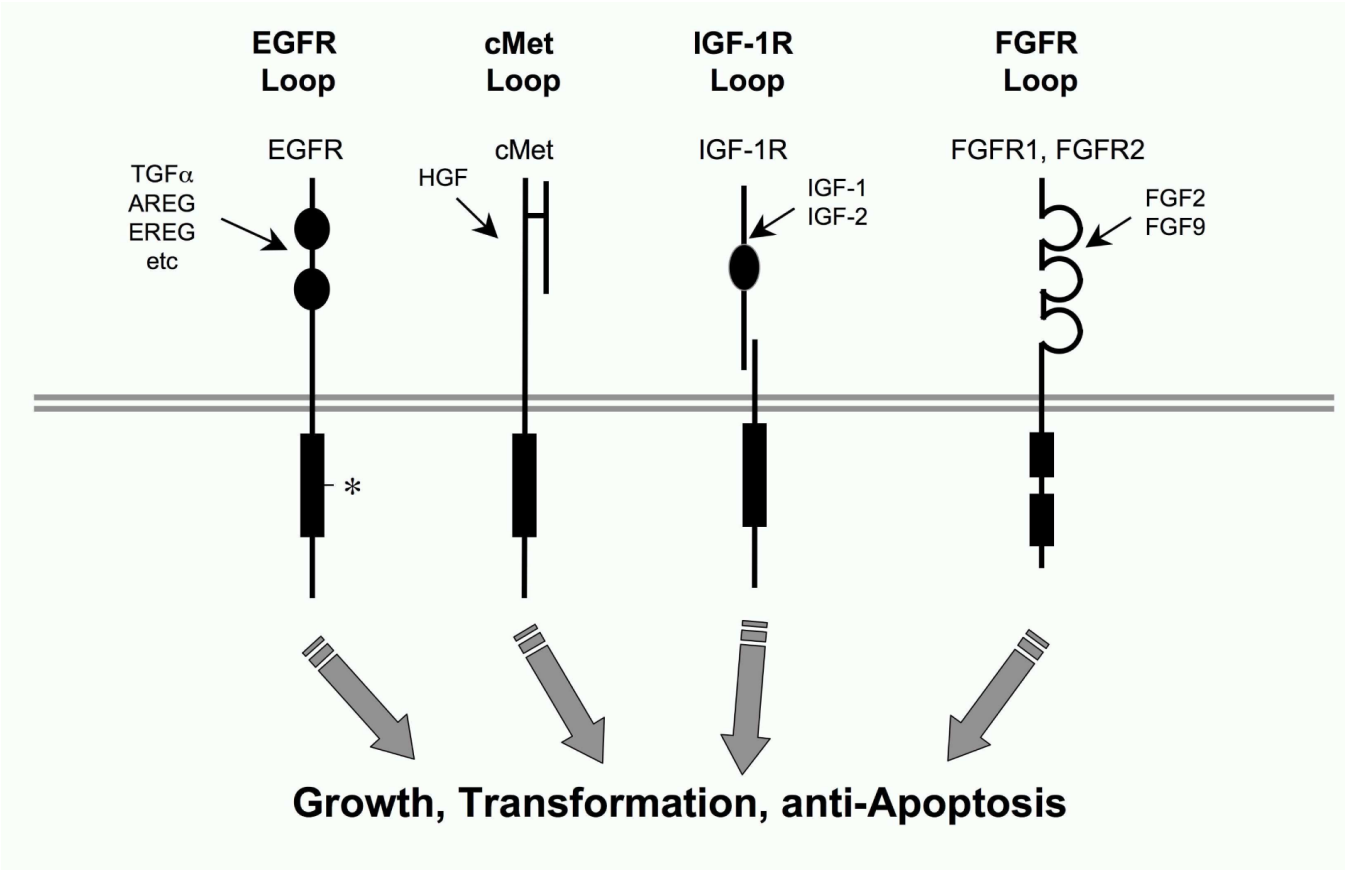

Figure 2. Autocrine and paracrine growth loops that may contribute to growth and transformation in NSCLC independent of EGFR

The literature (see accompanying text) supports the involvement of cMet, IGF-1R and FGFR receptor tyrosine kinases in the autocrine and paracrine-stimulated growth of NSCLC, thereby conferring intrinsic resistance to EGFR TKIs. In addition to autocrine signaling through EGFR via expression and release of distinct EGF family ligands (TGFα, amphiregulin (AREG), epiregulin (EREG)), the asterisk indicates the presence of activating somatic mutations that occur in EGFR.

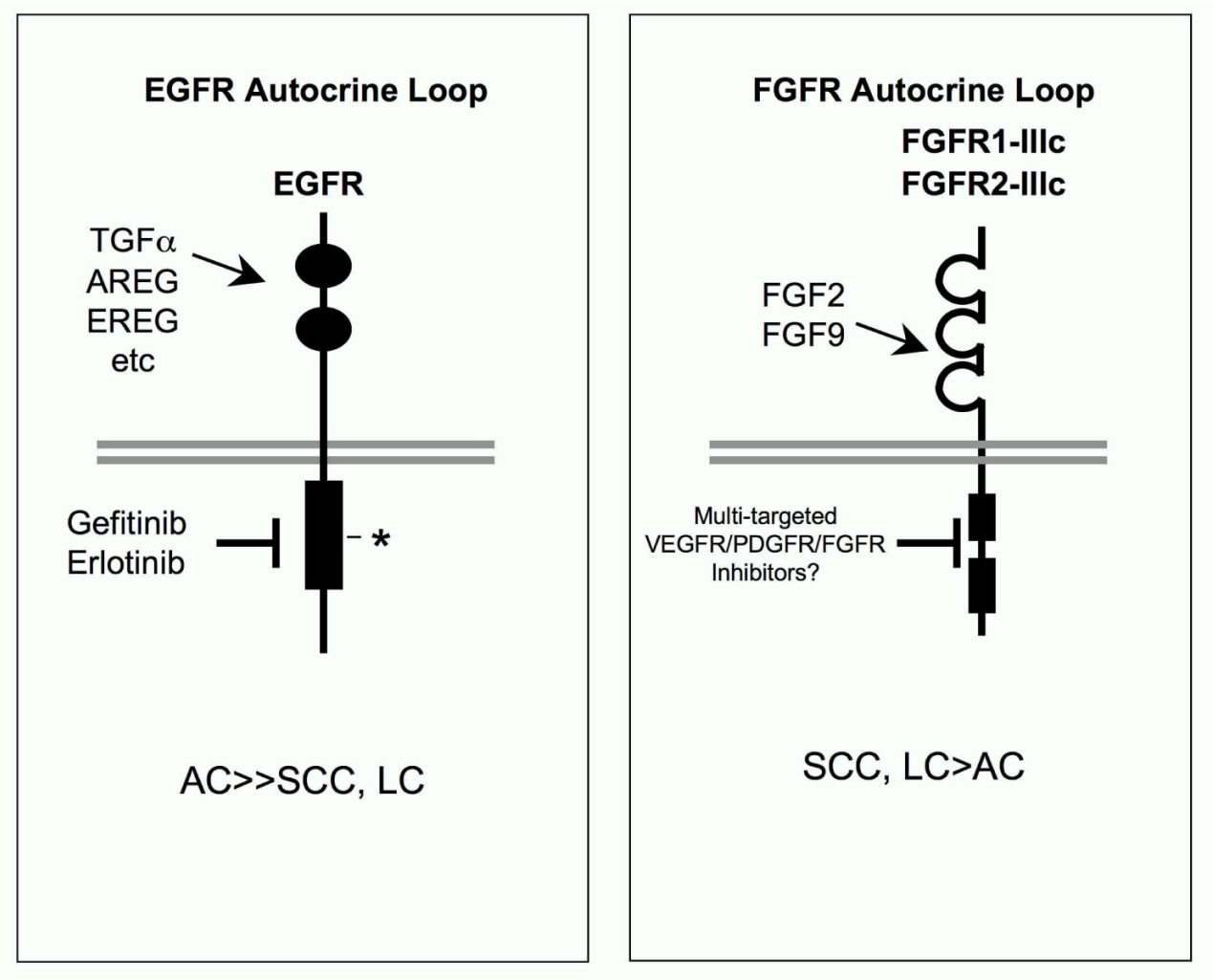

Figure 3. Specific FGFs and FGFRs comprise an autocrine growth loop in NSCLC cell lines

While adenocarcinoma is the dominant NSCLC histology sensitive to EGFR TKIs (Herbst et al., 2008), studies with NSCLC cell lines indicate more frequent autocrine signaling in squamous and large cell carcinoma-derived cells through FGF2, FGF9, FGFR1-IIIc and FGFR2-IIIc (Marek et al., 2009; Thomson et al., 2008). Importantly, the homology of FGFRs with VEGFRs and PDGFRs results in frequent activity of VEGFR-targeted TKIs on FGFRs as well (see Table 1). Thus, TKIs in this class target multiple RTKs including VEGFRs, PDGFRs and FGFRs.

Table 1

Receptor Tyrosine Kinase Targets for Anti-Angiogenic TKIs

	IC_{50}, nM						
TKI	**VEGFR-1**	**VEGFR-2**	**PDGFR-β**	**cKit**	**FGFR1**	**Others**	**Reference:**
RO4383596	-	44	33	-	29		(McDermott et al., 2005)
PD173074		100–200	18	-	22	cSrc, 20 nM	(Mohammadi et al., 1998)
TKI-258	10	13	27	2	8		(Lee et al., 2005)
Cediranib (AZD2171)	5	1	5	2	26		(Wedge et al., 2005)
Sorafenib	-	90	57	68	580	Raf-1, 6 nM	(Wilhelm et al., 2004)
Sunitinib (SU11248)	-	80	2	-	2900		(Sun et al., 2003)
Vandetanib ZD6474)	1600	40	-	-	3600	EGFR, 500 nM	(Hennequin et al., 2002)
Pazopanib	10	30	84	74	140		(Kumar et al., 2007)
Brivanib (BMS540215)	380	25	>6000	-	148		(Bhide et al., 2006)