IN THE SPOTLIGHT

Paths of Resistance to EGFR Inhibitors: Is NF Enough?

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Summary: Although the majority of patients with *EGFR*-mutant lung cancer respond well to EGF receptor (EGFR) tyrosine kinase inhibitors (TKI), all patients eventually develop resistance. The mechanism of acquired resistance is still unknown for a considerable subset of cases. This study reveals the *NF1* tumor suppressor gene as a new mediator of resistance to EGFR TKIs and provides a mechanistic rationale for developing combination therapies. *Cancer Discov; 4(5); 519–21. ©2014 AACR.*

See related article by de Bruin et al., p. 606 (4).

Activating mutations in *EGFR* occur in 10% to 15% of human non–small cell lung cancers (NSCLC) and are the most prevalent genetic drivers of lung cancer in never-smokers. Accordingly, the EGF receptor (EGFR) tyrosine kinase inhibitors (TKI) erlotinib and gefitinib have been approved for use in this molecular subtype of NSCLC (1). The majority (~60%) of patients with lung cancer who harbor activating *EGFR* mutations respond to these TKIs, which have been shown to be superior to conventional chemotherapy with respect to response rates and progression-free survival. However, in nearly all cases, tumors ultimately acquire resistance (2). Thus, although the relative success of EGFR inhibitors represents a paradigm for developing "effective" targeted therapies, the overall median survival of patients with *EGFR*-mutant lung cancer is still only a little over 2 years. As such, identifying mechanisms of resistance to inform the development of second-line therapies or more durable first-line treatments is of paramount importance.

The most common mechanism of resistance to EGFR TKIs is a secondary mutation in *EGFR* itself (T790M), which renders the activated kinase insensitive to these agents (1). T790M mutations account for 60% of cases of acquired resistance to erlotinib and gefitinib. Accordingly, agents that target the T790M-mutant kinase have been developed and are currently being evaluated in the clinic (2). However, several additional mechanisms of resistance have been reported, many of which impinge on the MEK–ERK or PI3K pathways (reviewed in refs. 1 and 2). Enhanced MET signaling caused by amplification of *MET* or overexpression of its ligand, hepatocyte growth factor (HGF), has been found in EGFR TKI–resistant tumors. Enhanced MET signaling is thought to promote resistance by preventing effective suppression of the PI3K pathway. Consistent with the importance of PI3K in some resistance mechanisms, *PTEN* loss and *PIK3CA* mutations have been detected in TKI-resistant NSCLC cell lines and patients, albeit with low frequency. Conversely, hyperactivation of the RAS–MEK–ERK pathway has also been implicated in EGFR TKI resistance, although the molecular mechanisms have not been fully elucidated. Lung cancers with acquired resistance to EGFR TKIs occasionally harbor activating *BRAF* mutations, although *KRAS* and *NRAS* mutations have not been observed. More recently, hyperactivation of the MEK pathway has been shown to broadly mediate resistance to mutant-specific inhibitors in T790M-expressing human and mouse cancers via *MAPK1* amplification and suppression of feedback mechanisms (3). Additional mechanisms of resistance include AXL upregulation, *MED12* loss, IGF-IR upregulation, and HER2 activation (1, 2). Nevertheless, the mechanism of acquired resistance is still unknown for about one third of resistant NSCLCs. Moreover, it is likely that heterogeneous tumors use multiple mechanisms.

To discover new mechanisms of resistance, de Bruin and colleagues (4) performed a genome-wide RNA interference screen to identify genes that when inactivated confer resistance to erlotinib. In this study, published in this issue of *Cancer Discovery*, the authors identified the *NF1* tumor suppressor as one such gene, and then validated their findings in functional cell-based assays, mouse models, and human tumor samples. Importantly, these studies not only reveal a new resistance gene, but also provide a mechanistic rationale for developing potential combination therapies.

NF1 encodes a RAS GTPase-activating protein (RAS GAP) known as neurofibromin. Accordingly, *NF1* mutations or suppression trigger the activation of RAS and downstream effector pathways. Loss-of-function mutations in *NF1* underlie the familial cancer syndrome neurofibromatosis type I; however, *NF1* plays a broader role in sporadic cancers and has been shown to be mutated and/or inactivated in glioblastoma, melanoma, and NSCLC, among others (5–9). Cellular and/or animal models have established a functional role for *NF1* inactivation in glioblastoma and melanoma (7, 8, 10); however, this is the first study to investigate the biologic consequences of *NF1* inactivation in lung cancer.

After identifying *NF1* in the erlotinib screen, de Bruin and colleagues (4) confirmed that *NF1* ablation promoted resistance to erlotinib in both cell culture and xenograft models.

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doi: 10.1158/2159-8290.CD-14-0286

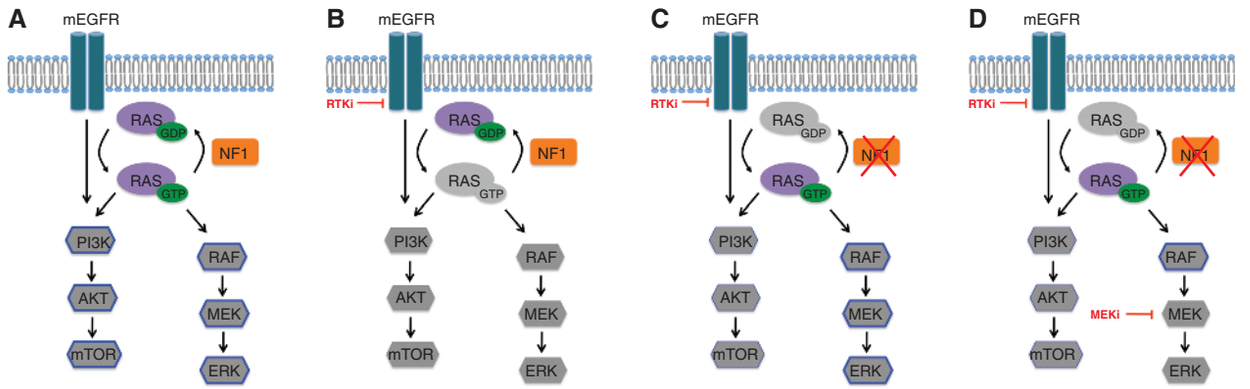

Figure 1. *NF1* loss confers resistance to EGFR TKIs in NSCLC. **A,** in the presence of activating *EGFR* mutations (mEGFR) the PI3K and ERK pathways are hyperactivated. **B** and **C,** EGFR TKIs suppress these pathways; however, when *NF1* is lost the MEK–ERK pathway cannot be effectively inhibited. **D,** cotargeting MEK resensitizes these tumors to EGFR inhibitors. Notably, *NF1* loss may also enhance resistance driven by secondary *EGFR* mutations as well as other RTKs. A thicker border around the hexagons indicates hyperactivation. RTKi, RTK inhibitor; MEKi, MEK inhibitor.

Using an inducible transgenic model of NSCLC, they also showed that *Nf1* (mRNA) was significantly downregulated in 10 of 18 tumors that had acquired resistance to erlotinib *in vivo*, supporting their functional cellular studies in an independent model. To evaluate the clinical relevance of these findings, the authors examined matched pre- and post-EGFR TKI-treated human tumor samples and found that *NF1* mRNA levels were also decreased by more than 2-fold in 4 of 10 tumors that had acquired resistance to TKIs, with little variation of *NF1* expression in other samples. Finally, they showed that low *NF1* mRNA levels in tumors from patients before EGFR TKI therapy were associated with decreased overall median survival (7.6 vs. 19.1 months), suggesting that the level of *NF1* expression may predict responsiveness to TKIs.

As noted previously, several mechanisms of resistance to EGFR inhibitors have been reported, many of which affect PI3K–AKT or ERK signaling. Given that both pathways are regulated by the *NF1* tumor suppressor, *NF1* (loss) is uniquely poised to mediate resistance to EGFR TKIs. On a mechanistic level, de Bruin and colleagues (4) demonstrated that *NF1* loss confers its effects by hyperactivating RAS and, conversely, that the catalytic RAS GAP domain is sufficient to restore sensitivity to erlotinib. The authors further suggest that it is the hyperactivation of MEK–ERK, or more precisely the inability of erlotinib to completely inhibit ERK, that promotes TKI resistance in NSCLC. Notably, combined erlotinib and selumetinib, a MEK inhibitor, exert more potent effects in *NF1*-deficient xenografts than erlotinib alone (Fig. 1). Synergistic effects were also observed in the transgenic NSCLC model, although the spectrum of resistance mechanisms in the latter model is likely a mix of both *Nf1*- and non-*Nf1*–related events. In both instances, however, it is difficult to rule out a potential contributory role for the PI3K pathway, especially under physiologic *in vivo* conditions, where the NF1–RAS–PI3K axis may also be important. Nevertheless, these studies clearly demonstrate that aberrant MEK–ERK pathway activation, which is triggered by loss of *NF1*, is an important mechanism that mediates resistance to erlotinib, suggesting that the combination of EGFR and MEK inhibitors may be more effective in *EGFR*-mutant NSCLC than monotherapy (Fig. 1). These drug combinations are currently being evaluated in the clinic, although increased toxicity has been observed in patients receiving both agents (4). The hope is that second-generation mutant-specific EGFR inhibitors might reduce this toxicity.

An additional interesting facet of this study is that *NF1* loss occurs in the context of both primary *EGFR* mutations and the secondary T790M mutation. In this respect, it is important to be precise about neurofibromin function. Loss of *NF1* alone is not sufficient to promote RAS activation in the absence of growth factors. Instead, neurofibromin normally attenuates RAS signaling downstream of specific growth factor receptors. As such, loss of *NF1* might be expected to enhance signaling downstream of $EGFR^{T790M}$. Similarly, it is conceivable that *NF1* loss may cooperate with other mechanisms of resistance, amplifying signaling downstream of other receptor tyrosine kinases (RTK) such as MET and HER2. Interestingly, NF1 seems to be playing a broader role in drug resistance. For example, *NF1* loss has been shown to confer resistance to BRAF inhibitors in melanoma through both the PI3K and MEK pathways in mouse models and in human cells, and the NF1 protein is absent in a subset of BRAF inhibitor–treated human tumors (7). Subsequent BRAF inhibitor resistance screens independently identified *NF1* as a resistance gene (11, 12). Although sequencing studies have now confirmed that *NF1* is mutated in a subset of BRAF inhibitor–treated melanomas (12, 13), to date homozygous mutations/deletions have not been observed. However, like PTEN, NF1 and other RAS GAPs are frequently inactivated by epigenetic and nongenetic mechanisms in cancer, and loss of NF1 protein expression seems to be more common than biallelic alterations in both melanoma and glioblastoma (6, 8, 9). A similar paradigm may be occurring in NSCLC. In this study, the authors found no evidence of *NF1* mutations but readily detected suppression of *NF1* mRNA levels in EGFR TKI–resistant human and mouse tumors. The unanswered question is what is the primary mechanism of *NF1* loss,

especially in the context of drug resistance? Moreover, how low must *NF1* levels be reduced to promote a phenotype? The current study reveals the *NF1* tumor suppressor gene as a new mediator of resistance to EGFR TKIs, although additional work is required to discover the specific non-mutational mechanisms that inactivate *NF1*. In the meantime, the challenge will be to develop a reliable means of quantifying NF1 suppression and to better understand the threshold and consequences of reduced NF1 levels, so that these insights may ultimately be exploited in the clinic.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Published online May 2, 2014.

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CANCER DISCOVERY

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Cancer Discovery 2014;4:519-521.