The Role of the ErbB Family Members in Non–Small Cell Lung Cancers Sensitive to Epidermal Growth Factor Receptor Kinase Inhibitors

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Abstract Inhibitors targeting the epidermal growth factor receptor (EGFR) are effective in a subset of non–small cell lung cancers. Such cancers often harbor EGFR mutations and/or amplification. These cancers require EGFR activity for the maintenance of critical intracellular survival and growth signaling pathways. Evidence is now accruing that EGFR works in concert with other ErbB family members, particularly HER2 and ErbB3, to activate these signaling pathways in a subset of lung cancers. These findings have important implications regarding the biology of these cancers and may lead to improved methods for identifying tumors that are responsive to EGFR kinase inhibitors and alternative therapies to treat cancers driven by ErbB signaling.

The epidermal growth factor receptor (EGFR) is a member of a family of closely related growth factor receptor tyrosine kinases that includes EGFR (ErbB1), HER2/*neu* (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). Upon ligand binding, these receptors homodimerize or heterodimerize leading to autophosphorylation and subsequent activation of intracellular signaling cascades such as the phosphoinositide 3-kinase (PI3K)/Akt, Raf/MEK/Erk, and Jak/Stat signaling pathways. Because EGFR is expressed in a majority of non–small cell lung carcinomas (NSCLC), it has been an attractive target for the development of therapeutic agents (1–3). The small-molecule EGFR tyrosine kinase inhibitors (TKI), including gefitinib and erlotinib, have been evaluated in clinical trials for patients with NSCLC. Both agents produce partial responses in 10% to 20% of all NSCLC patients (4–7).

As highlighted in other articles in this issue of Clinical Cancer Research, lung cancers with *EGFR* mutation and/or amplification are the most likely to shrink in response to EGFR inhibitors (8–14). In such cancers, EGFR is the major activator of critical growth and survival signaling pathways, and thus these cancers are addicted to EGFR activity. When exposed to EGFR inhibitors, these key growth and survival signaling pathways are aborted, resulting in apoptosis and/or cell cycle arrest. Over the past 2 years, it has become increasingly clear that EGFR works in concert with other ErbB family members, particularly HER2 and ErbB3, to function as an oncogene in these cancers. Thus, the ErbB family of receptor tyrosine kinases works collectively to drive these EGFR TKI–sensitive lung cancers. This review will focus primarily on the data demonstrating that ErbB3 and HER2 have critical roles in this subset of NSCLCs. The role of the other ErbB family member, ErbB4, remains much less defined and will not be discussed.

ErbB3 Is Used to Activate PI3K/Akt Signaling

ErbB3 is unique among the ErbB family members in that it is has weak or no tyrosine kinase activity (15). However, upon heterodimerization with other ErbB family members, it is phosphorylated on tyrosine residues. Tyrosine-phosphorylated ErbB3 then serves as a scaffold to propagate intracellular signaling events. In particular, tyrosine-phosphorylated ErbB3 directly binds to and activates PI3K. The PI3K/Akt pathway provides a critical oncogenic stimulus whose increased activity is implicated in a wide range of cancers (for review, see refs. 16, 17). ErbB3 possesses six tyrosine phosphorylation sites with YXXM motifs that serve as excellent binding sites for PI3K (18–20). Studies have shown that PI3K/Akt signaling is tightly regulated by EGFR in TKI-sensitive NSCLCs, and EGFR TKIs down-regulate the PI3K/Akt pathway exclusively in those NSCLC cell lines in which they also inhibit growth (21–24). Furthermore, expression of oncogenic mutants of the PI3K/Akt pathway into *EGFR* mutant, gefitinib-sensitive NSCLC cell lines leads to constitutive activation of PI3K/Akt signaling and abrogates gefitinib-induced apoptosis.[4]

EGFR is known to activate the PI3K/Akt pathway via two major mechanisms: ErbB3 and Gab1. Gab1-mediated activation of PI3K/Akt signaling seems to be the major mechanism in

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fibroblasts, as mouse embryonic fibroblasts deficient in Gab1 lose EGF-induced activation of PI3K signaling (25). A recent study suggests that as lung cells become transformed, they switch from using Gab1 to ErbB3 as the means for activating PI3K (26). Additionally, we observed that EGFR links to PI3K signaling via ErbB3 in NSCLC cell lines that are sensitive to EGFR TKIs ("sensitive" defined as an IC_{50} <1 μmol/L) whether the sensitive cell line possesses wild-type or mutant *EGFR* (21). This mechanism for activating the PI3K/Akt pathway has been observed in all EGFR TKI–sensitive cell lines examined to date, including HCC827 (*EGFR* exon 19 deletion; amplified), H3255 (*EGFR* L858R; amplified), PC-9 (*EGFR* exon 19 deletion; amplified), DFCI-LU011 (*EGFR* exon 19 deletion; amplified), Calu-3 (*EGFR* wild type; *Her2* amplified), H358 (*EGFR* wild type), and A431 (*EGFR* wild type; amplified). Furthermore, down-regulation of ErbB3 expression via RNA interference is sufficient to decrease the amount of active Akt (21). The connection between ErbB3 to PI3K is observed almost exclusively in gefitinib-sensitive NSCLC cell lines. NSCLC cell lines with *de novo* resistance to EGFR TKIs tend to use non-ErbB3-mediated mechanisms of activating PI3K (21). In fact, most NSCLC cell lines with *de novo* resistance to EGFR TKIs do not express significant levels of ErbB3 (21). These observations have lead to the following proposed model: For a cell to be inhibited by anti-EGFR therapy, EGFR must be a critical driving force for its growth and survival, and thus EGFR controls PI3K activity. When EGFR regulates PI3K in lung cancers, it uses ErbB3 to directly link to PI3K. Therefore, those cancers that respond to EGFR TKIs likely express ErbB3.

There are now several studies that have observed a correlation between gefitinib sensitivity and ErbB3 expression in NSCLC cell lines (21, 27, 28). Thus, assessment of ErbB3 expression may help predict patients who are likely to benefit from EGFR TKI therapy. In fact, a recent small study that examined ErbB3 expression by immunohistochemistry observed that tumor ErbB3 expression correlates with patient responses to EGFR TKI (29). Further studies on larger sample sizes will help determine if ErbB3 expression will be a clinically useful tool to further define who will benefit from EGFR TKIs.

Why do these cancers use ErbB3 to activate the PI3K/Akt pathway? Perhaps ErbB3 provides a more efficient activation of the PI3K pathway than Gab1, either by increased amplitude of signaling or longer duration of kinetics. In fact, we did observe that cotransfection of *ErbB3* with *EGFR* (mutant or wild type) prolongs EGF-induced activation of Akt (21). Alternatively, expression of ErbB3 in these cancers may promote survival of cancer for other reasons. For example, expression of ErbB3 endows a cancer cell with the ability to respond to neuregulins, a group of ligands that activate ErbB3 (and ErbB4) but not EGFR. Additionally, ErbB3 may affect the functioning, internalization, or localization of other ErbB receptors in manners not yet appreciated.

Her2 Amplification Is Associated with Response to EGFR TKIs

HER2 has recently been identified as a potentially important component of the ErbB signaling network in the EGFR TKI–sensitive lung cancers. Remarkably, *Her2* amplification, as determined by fluorescence *in situ* hybridization analysis, was identified as a positive predictor of response to EGFR TKIs (30). In fact, the most powerful predictor of response was the presence of both *Her2* amplification and an *EGFR* mutation. The presence of both alterations was associated with an 88% (seven of eight) response rate, whereas either alone was a very poor predictor of response rate [*Her2* amplification alone = 0% (0 of 11), *EGFR* mutation alone = 14% (1 of 7); ref. 30]. Thus, high expression of HER2 likely has dramatic effects on EGFR signaling and oncogenicity. This is supported by the finding that RNA interference targeting either *Her2* or *EGFR* is equally effective at inhibiting growth in the H1650 cell line (*EGFR* deletion mutation; ref. 31).

Interestingly, experiments on cell lines have shown that breast cancer and NSCLC cell lines with *Her2* amplification have increased sensitivity to EGFR TKIs (21, 32, 33). However, the IC_{50} for several of these cell lines is >1 μmol/L, and it is quite possible that off-target effects of EGFR TKIs on HER2 are the reason for their effectiveness in these cell lines. The data from Cappuzzo et al. (30) suggests that EGFR TKIs, at the concentrations currently achieved in patients, are unlikely to be effective in *Her2*-amplified NSCLCs that lack *EGFR* mutations [0% response rate (0/11)]. As discussed by Swanton et al. in this issue of Clinical Cancer Research, it remains to be determined if trastuzumab or other anti-HER2 agents will be effective in this subset of NSCLCs.

The observations highlighted above suggest that HER2 likely augments EGFR-mediated oncogenicity and addiction to EGFR signaling. One possible reason is the effect that HER2 has on EGFR cycling. EGFR/HER2 heterodimers are preferentially recycled back to the plasma membrane compared with EGFR homodimers that are more often targeted for degradation (for review, see ref. 34). Additionally, HER2, which has no known endogenous ligand, seems to exist primarily in an active conformation ready to heterodimerize with other ErbB family members (35). Thus, in settings of limiting ligand, there may be more efficient activation of HER2-containing heterodimers. Furthermore, HER2 is seems to be the "preferred dimerization partner" for the other ErbB family members (36).Thus, EGFR may preferentially bind to HER2 rather than homodimerize or heterodimerize with ErbB3.

Lateral Signaling between EGFR, Her2, and ErbB3

From the data discussed above, the presence of mutant/amplified *EGFR*, amplified *Her2*, and ErbB3 expression all correlate with responsiveness to EGFR TKIs. These findings suggest that these ErbB family members likely work together to promote tumorigenesis. Thus, we propose the following simplistic model to depict this hypothesis (Fig. 1). As mentioned above, HER2 seems to be the preferred heterodimer partner for the other ErbB family members, and thus may serve to transduce EGFR activation to ErbB3 phosphorylation. HER2 likely heterodimerizes with EGFR, leading to the activation of EGFR and HER2. Once HER2 is phosphorylated and activated, it may undergo "lateral signaling" by heterodimerizing with ErbB3, thereby leading to its phosphorylation (36). This may be essential if EGFR/ErbB3 heterodimers do not occur efficiently. ErbB3, upon tyrosine phosphorylation by HER2, binds directly to PI3K, thereby activating the critical PI3K/Akt pathway. This model predicts

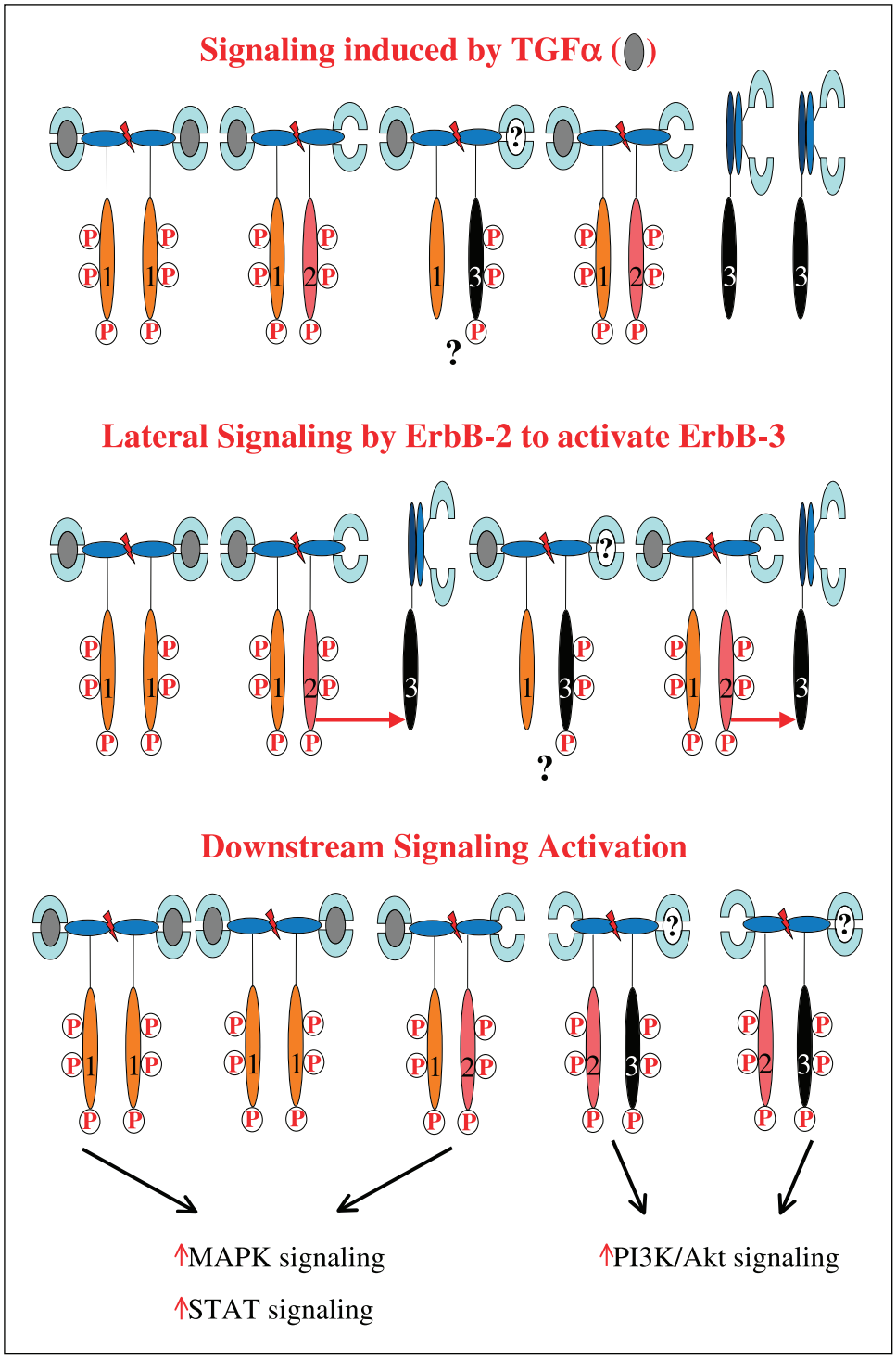

Fig. 1. Lateral signaling among ErbB family members. A model depicting the mechanisms by which EGFR, Her2, and ErbB3 may work in concert to activate downstream signaling pathways. In this model, Her2 is activated by EGFR. Activated Her2 is then capable of activating ErbB3. Top, EGFR/HER2 heterodimers are activated by EGFR ligands. It is unknown whether EGFR/ErbB3 heterodimers exist (*?*), but if they do EGFR would not be phosphorylated because ErbB3 is kinase dead. Middle, activated HER2 dissociates from EGFR and associates with ErbB3 and phosphorylates it [i.e., lateral signaling (*arrow*)]. Bottom, phosphorylated EGFR, HER2, and ErbB3 lead to activation of critical prosurvival and growth signaling cascades. It is unknown if EGFR/ErbB3 heterodimers are present in the cell (?).

that inhibition of EGFR should decrease both ErbB2 and ErbB3 phosphorylation as well as downstream signaling. Furthermore, ErbB2 inhibitors may also be effective in these TKI-sensitive NSCLCs, as they would be predicted to down-regulate ErbB3/PI3K/Akt signaling. In particular, inhibition of ErbB2 could prove effective in NSCLCs that become resistant to EGFR TKIs via mechanisms that still require active ErbB signaling (e.g., the acquisition of a T790M mutation in EGFR; refs. 37, 38).

Future Directions

As the understanding of how the ErbB family members work together to promote lung cancer matures, this information may be used to guide treatment strategies and therapeutic designs. Future studies should inform us if assessment of *Her2* amplification and/or ErbB3 expression has an effect on our ability to make wiser clinical decisions regarding the use of EGFR TKIs. Determining if PI3K couples to ErbB3 may inform

us if tumors are ErbB driven (whether via EGFR and/or HER2). Furthermore, therapies either targeting HER2 (kinase inhibitors and/or antibodies) or blocking ErbB3 activation may be effective for the treatment of some lung cancers that acquire resistance to EGFR. Such therapies, when added to EGFR TKIs, may also improve initial responses to EGFR kinase inhibitors by simultaneously attacking partners of EGFR in cancer.

Open Discussion

Dr. Daniel Haber: Outside of lung cancer, there are a number of reports of dramatic responses to gefitinib and erlotinib in other tumor types, and there are really no good explanations of mechanisms in terms of expressional patterns of other HER2 family members. Do you have any thoughts on that?

Dr. Engelman: I would think that most of these cancers that respond to the normal doses do so probably by inhibiting ErbB1. The gliomas are different—they may use different adaptors—but the epithelial tumors that are using ErbB1 to drive cancer are probably networking with these proteins as well. In terms of predicting response, without genetic data, I am a bit skeptical about using immunohistochemistry for ErbB3 and ErbB2 at this time.

Dr. Jeffrey Settleman: With regard to the H358 cell line, which has an activating Ras mutation, we also find that it is quite sensitive to EGFR inhibition. Any thoughts as to the data that are out there about Ras and primary resistance?

Dr. Engelman: I don't think K-Ras confers resistance per se, meaning, if you take an activated K-Ras and put it into a sensitive cell line, you will not confer resistance. I have data to support that. As we know, the K-Ras is often associated with smoking and the EGFR TKI – sensitive lung cancers are associated with nonsmoking. I think these are two different processes tumorigenesis, and K-Ras and EGFR are markers for these two different tumor types. I don't believe the concept that K-Ras can activate the downstream signal and thereby confer resistance. I don't think it confers resistance.

Dr. Settleman: So, the implication of that is that these drugs can work in multiple types of tumor.

Dr. Engelman: They could; they probably don't work in most of the K-Ras tumors because these tumors are driven by different processes and are not addicted to ErbB signaling. We have data that the PI3K pathway is actually addicted to other processes in those tumor types.

Dr. Lewis Cantley: In addition to the transcriptional regulation, I am curious about the possibility there may also be a gene silencing mechanism here because ErbB3, as opposed EGFR, is not normally epithelial tissue, it is more in neuronal tissue, so one might expect there is a mechanism of silencing this gene in epithelial tissues. Something might be going on there.

Dr. Paul Bunn: So it would be a negative transcriptional regulation, not a positive one.

Dr. Bruce Johnson: When you did the P85 immunoprecipitation studies, you got multiple bands in the sensitive cell lines that you don't see in the resistant ones. Do you want to comment on what you think: (*a*) are those real, and (*b*) what do you think they might be?

Dr. Engelman: I'll comment on the one cell line that I have data for. In the 3255 cell line, the other protein that PI3K is coupling to is IRS1, which is a known, very potent coupler to PI3K. If you look at the blots, P85 comes off of ErbB3 and goes more onto IRS1, so one might ponder, if you have so much going to IRS1, why you don't continue to have PI3K activity? We are investigating why that is so.

Dr. John Heymach: Any thoughts about whether HER2 might be an obligate link between EGFR and HER3? If it is obligate in certain situations, why don't we see more activity for HER2-directed therapy in lung cancer?

Dr. Engelman: That is a great question. There is paucity of data about the causal role of ErbB2. However, I think it may be possible to intercept the network at that level. The fact that trastuzumab failed in real clinical trials may have to do with the effectiveness of an antibody-mediated therapy versus a kinase inhibitor. It will be interesting to see how other ErbB2 inhibitors do in the clinical environment.

Dr. Glenwood Goss: It is my understanding that with the Glaxo compound lapatinib, the dual kinase inhibitor of ErbB1 and ErbB2, virtually no responses have been seen in NSCLC. How does that fit into your model?

Dr. Engelman: It doesn't fit well. As I said, the model is easily testable, but in reality, I don't know about the pharmacodynamics. I'd like to test it some preclinical models and see where it fails in the preclinical models to draw a real conclusion.

Dr. Bunn: Lapatinib has a 3% response rate—who knows whether 3% is different from 8%? Also, it was done with a single dose. It may not be the right dose. That is another drug that may not have the right dose. So I would say we don't know.

Dr. Thomas Lynch: There are several other dual kinase inhibitors coming down the pike that hit both ErbB1 and ErbB2, so lapatinib won't be the only proof of principle with this; hopefully, there will be other ones that we will look at.

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