Targeting ADAMS and ERBBs in lung cancer

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Aberrant ERBB receptor activity contributes to the development of many human cancers. Receptor overexpression, kinase domain (KD) mutations, and autocrine ligand production contribute to ERBB activation in human tumors. ERBB-targeted tyrosine kinase inhibitors (TKIs) and monoclonal antibodies are used in cancer treatment; however, clinical hurdles, including patient selection and TKI resistance, need to be overcome in order to optimize therapy. This minireview will discuss recent findings on possible mechanisms leading to ERBB-targeted therapy resistance and potential means to overcome them.

The ERBB receptor-ligand network comprises four receptors, EGFR, ERBB2, ERBB3, and ERBB4; and multiple ligands, the EGF-related peptides (Yarden and Sliwkowski, 2001). ERBB receptors are activated in response to peptide binding, leading to ERBB receptor homo- and heterodimerization (Holbro and Hynes, 2004). There are three ligand groups: EGF, TGF-α, amphiregulin, and epigen, which bind EGFR; BTC, HB-EGF, and epiregulin, which bind EGFR or ERBB4; and neuregulins (NRGs, also known as heregulins [HRG]), which bind ERB3 and ERBB4. Despite the fact that none of the EGF-related peptides binds ERBB2, this receptor is the preferred dimerization partner for the other ligand-activated ERBBs (Graus-Porta et al., 1997). Aberrant EGFR and ERBB2 activity contributes to human cancer. Numerous clinical studies have implicated these receptors in the pathology of specific tumor types (Nicholson et al., 2001; Ross et al., 2003). The three major mechanisms leading to ERBB activation in cancer are gene amplification, altered ligand expression, and mutations in the receptor kinase or extracellular domain (Hynes and Lane, 2005). Intense efforts have gone into developing ERBB-targeted inhibitors, focusing either on the extracellular domain with antibody-based approaches or on blockade of the intracellular kinase domain (KD) with tyrosine kinase inhibitors (TKIs) (Baselga and Arteaga, 2005; Blackhall et al., 2006; Hynes and Lane, 2005). A mechanistic understanding of how these inhibitors impact on tumor cells will be essential to further enhance the current clinical successes (see, for example, Piccart-Gebhart et al., 2005). Furthermore, in order to improve patient selection optimization of factors that predict response or resistance to a specific therapeutic must continue. Two papers in this issue of *Cancer Cell* shed more light on these problems (Wang et al., 2006; Zhou et al., 2006). We will briefly present some background before discussing these papers.

The first large-scale clinical trials using EGFR TKIs were carried out in non-small cell lung cancer (NSCLC) patients. The overall response rate to the inhibitors was low (reviewed in Blackhall et al., 2006). However, the first somatic mutations in the KD of *EGFR* were discovered in tumors from those patients who were sensitive to the EGFR-selective TKIs gefitinib and erlotinib; >80% (n = 31) of the responders had KD mutations (Lynch et al., 2004; Paez et al., 2004; Pao et al., 2004). Although to a lower extent, KD mutations in *ERBB2* have also been detected in NSCLC (Figure 1 and Table 1) (Stephens et al., 2004; Shigematsu et al., 2005; Takano et al., 2005; Lee et al., 2006a; Sasaki et al., 2006). Needless to say, the pattern of response to ERBB-targeted TKIs and *EGFR* mutation status have been closely monitored during the past few years. Newer studies reported response rates for NSCLC patients with *EGFR* KD mutations ranging from 16% (Tsao et al., 2005) to 65% (Johnson and Janne, 2005). These wide differences might reflect diverse patient populations. The expression level of EGFR has also been associated with gefitinib and erlotinib response (Cappuzzo et al., 2005a; Takano et al., 2005; Tsao et al., 2005; Shepherd et al., 2005). However, objective responses to TKIs have also been observed in the absence of *EGFR* KD mutations or overexpression (Pao et al., 2004; Tsao et al., 2005; Takano et al., 2005), suggesting that other factors also contribute to TKI sensitivity. These clinical studies have led to intense efforts, first to clarify if ERBB KD mutations and/or additional factors predict TKI response, and second, to characterize the role of mutant ERBBs in cancer biology.

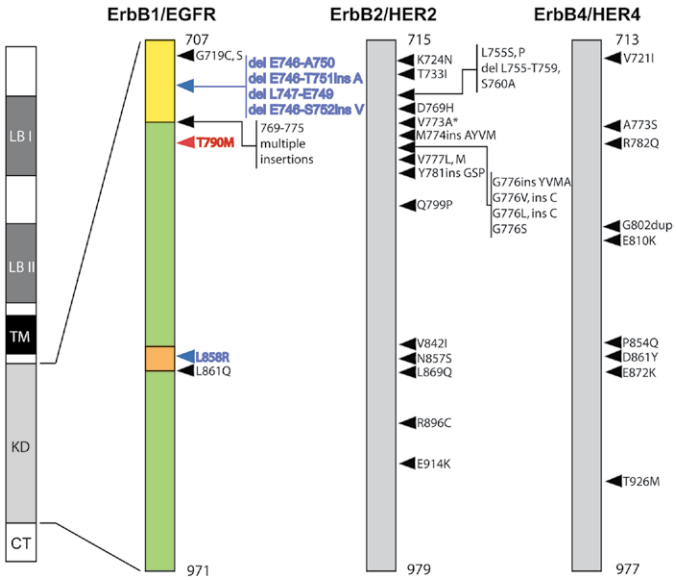

Figure 1. Kinase domain mutations in ERBB receptors

Structure of a full-length ERBB with the indicated domains: ligand binding (LB I and II), transmembrane (TM), kinase (KD), and cytoplasmic tail (CT). The KDs of EGFR, ERBB2, and ERBB4 are shown enlarged, with the elucidated structure of the EGFR KD consisting of the N-terminal lobe with the ATP binding site (yellow) and the C-terminal lobe (green) containing the activation loop (orange). Alterations in EGFR labeled in blue are associated with increased transforming activity and increased sensitivity to the TKIs gefitinib and erlotinib; T790M (red) confers resistance to both. Mutations, deletions, and insertions in ERBB2 are also clustered in the N lobe of the receptor. The G776^{YVMA} and VC insertions enhance ERBB2 activity (Wang et al., 2006). One mutation (*) was reported in a gefitinib-responsive SCCHN patient in the absence of EGFR mutation (Cohen et al., 2005). ERBB4 mutations are found in a variety of cancers (Soung et al., 2006). del, deletion; ins, insertion; dup, duplication.

Table 1. Frequency of ERBB2 kinase domain mutations in various cancers

Cancer	Frequency	Remarks	Reference
NSCLC	5/120 (4.2%), 5/51 (9.8%)	adenocarcinoma, insertions in exon 20 (4/5), L755P in exon 19	Stephens et al., 2004
	11/671 (1.6%), 11/394 (2.8%)	adenocarcinoma, in-frame duplications/insertions in exon 20	Shigematsu et al., 2005
	1/80 (1.3%)	tumor cell lines, (+) from adenocarcinoma, in-frame duplications/insertions in exon 20	
	0/66	gefitinib-treated patients	Takano et al., 2005
	1/114 (0.8%)	nonadenocarcinoma, G776ins YVMA exon 20	Lee et al., 2006a
	1/122 (0.8%)	adenocarcinoma, G776ins YVMA	Sasaki et al., 2006
Total	18/1093 (1.6%)	17 adenocarcinomas	
SCLC	0/36		Shigematsu et al., 2005
Gastric	9/180 (5%)	various mutations	Lee et al., 2006b
CRC	0/28		Shigematsu et al., 2005
	3/104 (2.9%)	all carry K-ras mutations, V777L, M; V842I	Lee et al., 2006b
Total	3/132 (2.3%)		
Breast	0/28		Shigematsu et al., 2005
	4/94 (4.3%)	none with ErbB2 amplification, del L755-T759, L755S, P; R896C	Lee et al., 2006b
Total	4/122 (3.3%)		
SCCHN	1/4 (25%)	Gefitinib-responsive patient, V773A	Cohen et al., 2005
Bladder	0/15		Shigematsu et al., 2005
Prostate	0/14		Shigematsu et al., 2005
Ovary	1/188 (0.5%)	G776ins YVMA exon 20	Lassus et al., 2006

The biochemical and biological consequences of EGFR mutations have been studied by various means. Most of the missense mutations and in-frame deletions that arise in exons 18–21 of the KD affect residues in the ATP binding pocket (Figure 1). Recent mutational and crystallographic studies revealed that the kinase activity of $EGFR^{WT}$ is autoinhibited and that this conformation is destabilized by a common mutation, L834R, conferring on the mutant a dramatic increase in basal and ligand-induced kinase activity (Zhang et al., 2006). In vitro data from cellular models suggest that KD mutations enhance coupling of the mutant receptor to prosurvival pathways (Greulich et al., 2005; Sordella et al., 2004). This hypothesis is supported by the clinical observation that increased *EGFR* copy number was observed more frequently in patients bearing *EGFR* KD mutations than in patients with $EGFR^{WT}$ (Cappuzzo et al., 2005a), suggesting that mutant *EGFR* alleles are selectively amplified and required for NSCLC survival. Results from transgenic models provide convincing support for the importance of *EGFR* KD mutations in lung cancer (Ji et al., 2006; Politi et al., 2006). Inducible expression of two common EGFR mutations in transgenic animals rapidly induced lung adenocarcinomas with features similar to those seen in human tumors with *EGFR* KD mutations. Withdrawal of the inducer led to tumor regression in the mice, demonstrating the importance of mutant EGFR in cancer cell survival. Importantly, the mice responded to EGFR-selective TKIs, rapidly demonstrating a significant tumor reduction (Ji et al., 2006). These mice provide excellent models for further studies on tumor biology and response.

ERBB2 KD mutations were initially detected in NSCLC (Stephens et al., 2004), although at a lower frequency than *EGFR* mutations. However, neither the biology of mutant ERBB2 nor its impact on clinical response to TKIs has been reported. This is where the new study of Arteaga and colleagues comes into the picture (Wang et al., 2006). The group examined the biological effects of two different *ERBB2* KD mutants, the $G776^{YVMA}$ and $G776^{VC}$ insertions. Following introduction into normal human breast and lung cells, mutant *ERBB2* was constitutively active and conferred oncogenic properties on the cells. Moreover, EGFR displayed elevated activity, and multiple downstream signaling pathways were stimulated. Mutant *ERBB2*-expressing cells were tested for their sensitivity to various ERBB inhibitors. Although these cells were resistant to the EGFR-selective TKIs gefitinib and erlotinib, they were sensitive to the dual EGFR/ERBB2 TKIs lapatinib and CI-1033, showing the dominance of mutant ERBB2 in this model. In the clinic it is not yet known if *ERBB2* KD mutations confer resistance to gefitinib or erlotinib in NSCLC patients. In one small study that included four patients with *ERBB2* KD mutation, none responded to gefitinib (Han et al., 2006), supporting the in vitro results presented by Arteaga and colleagues. In addition to their sensitivity to the dual ERBB TKIs, the transformed cells were also sensitive to the ERBB2-targeted monoclonal antibody trastuzumab (Wang et al., 2006). It is well documented that breast cancers with *ERBB2* amplification respond to trastuzumab (Hynes and Lane, 2005), and data are emerging that elevated *ERBB2* copy number might impact on response of NSCLC patients to ERBB-targeted therapies (Cappuzzo et al., 2005c). *ERBB2* amplification is found in 11% of NSCLC specimens (discussed in Rosell, 2004), and results from clinical trials suggest that the subgroup of patients with amplification achieve clinical benefit when trastuzumab is added to different chemotherapy regimes (Langer et al., 2004). Intriguingly, it was recently reported that trastuzumab in combination with paclitaxel led to a partial response of a NSCLC patient with increased *ERBB2* copy number and an *ERBB2* KD mutation (G776L) (Cappuzzo et al., 2006). Although currently based on one example, it is possible that screening for *ERBB2* amplification in combination with mutation will be the ultimate test for choosing NSCLC patients who might be considered for trastuzumab therapy. Based upon the results reported by Wang et al. (2006), these patients might also respond to a dual EGFR/ERBB2 inhibitor.

Table 2. ADAMs, ERBB ligand shedding, and potential roles in different cancer types

ADAM	Shedding of ERBB ligands	Expression in cancer	Reference
ADAM8	?	RCC, NSCLC, brain	Rocks et al., 2006
ADAM9	HB-EGF	myeloma, breast, gastric, prostate, NSCLC, melanoma, HCC	Sahin et al., 2004
ADAM10	HB-EGF, EGF, BTC	breast, CRC, uterus, ovary, prostate, hematological malignancies, gastric	Sahin et al., 2004
ADAM12	HB-EGF	breast, gastric, glioblastoma, hematological malignancies, liver, colon	Asakura et al., 2002
ADAM15	HB-EGF, AR, TGFα	breast, hematological malignancies, gastric, prostate, lung carcinoma	Hart et al., 2005
ADAM17	HB-EGF, AR, TGFα, EPR, NRGα2c, -β1, -β2	breast, prostate, gastric, CRC, HCC, ovary, RCC	Horiuchi et al., 2005
ADAM19	NRGβ1, -4	brain, RCC	Wildeboer et al., 2006
ADAM28	?	NSCLC	Ohtsuka et al., 2006

There is emerging evidence that ERBB3 and ERBB4 also have roles in common human cancers. *ERBB4* KD mutations have been detected in 2.0% of tumor tissues (n = 594), including 2.3% of NSCLCs (n = 217) (Soung et al., 2006). Neither their impact on response to ERBB TKIs nor their biology has been studied. Genomic gain of *ERBB3* does not appear to be a marker for response to TKI therapy in NSCLC (Cappuzzo et al., 2005b), which is not surprising since this kinase-impaired receptor acquires signaling activity only as a heterodimer with another ERBB (Hynes and Lane, 2005). However, there are data suggesting that lung cancers that depend upon EGFR for survival activate ERBB3 via upregulation of EGF-related peptides (Fujimoto et al., 2005). Although ERBB3 is kinase impaired, its ability to strongly couple to PI3K confers a special role on this receptor. Cancer cells driven by ERBB2 (Holbro et al., 2003) or EGFR (Engelman et al., 2005) coopt ERBB3 to activate the PI3K/AKT pathway. In summary, an assessment of ERBB3 levels combined with *ERBB2* copy number and/or mutation status in NSCLC may also prove useful for predicting response to ERBB-targeted TKIs.

Resistance to ERBB inhibitors, both TKIs and antibodies, has emerged as a significant clinical problem. Resistance may be de novo, for example, in those NSCLC patients with *EGFR* KD mutations who never responded to TKIs. Or it is acquired, as seen in some cases where tumors arising during gefitinib or erlotinib therapy contained a secondary mutation, T790M (Kobayashi et al., 2005; Pao et al., 2005). Based upon the model of erlotinib bound to the KD of EGFR, the change at residue 790 is predicted to keep the receptor active but prevent the TKI from binding (Kobayashi et al., 2005). Fortunately, this class of TKI-resistant mutants is sensitive to other EGFR inhibitors, such as CL-387,785 (Greulich et al., 2005).

Acquired resistance also results from alterations in EGFR trafficking (Kwak et al., 2005), a process that is subject to multiple control mechanisms (Lenferink et al., 1998) and plays an important role in receptor signaling activity (Wiley, 2003). In a NSCLC cell line model of acquired gefitinib resistance, EGFR internalization was more rapid in the resistant than in the parental cells (Kwak et al., 2005). Importantly, the gefitinib-resistant cells remained sensitive to irreversible ERBB-selective TKIs (Kwak et al., 2005). These results suggest that intracellular dissociation of reversible ERBB inhibitors may play a role in resistance and that an irreversible TKI might not be subject to this effect. In fact, differences in EGFR trafficking in cancer cells might have a general role in TKI response, and it will be interesting to develop additional in vitro models for testing this.

The paper by Zhou and colleagues presents evidence for another resistance mechanism in NSCLC, one that involves autocrine ligand production (Zhou et al., 2006). The EGF-related peptides are synthesized as transmembrane precursors that are cleaved by cell surface proteases (Harris et al., 2003), leading to the release of soluble ligands. This cleavage is an important step in the control of ligand availability and receptor activation (Borrell-Pages et al., 2003). The ADAMs (*a d*isintegrin *a*nd *m*etalloproteases), zinc-dependent membrane-associated proteases, control the cleavage of most EGF-related ligands (Blobel, 2005). The ADAMs family has 29 mammalian members that share a common molecular structure. Catalytically active ADAMs cleave a variety of substrates, including growth factors and extracellular matrix proteins (Blobel, 2005; Seals and Courtneidge, 2003; Zhou et al., 2005). Various lines of evidence suggest that ADAM10 and ADAM17 are the principle proteases for the ERBB ligands (Sahin et al., 2004).

Zhou and colleagues provide evidence suggesting that HRG-mediated ERBB3 activation might be an important gefitinib resistance mechanism. An examination of primary NSCLC tissues revealed that most expressed HRG and ERBB3; active ERBB3 was found in a subset. Since NSCLCs also express EGFR and ERBB2, they hypothesized that autocrine HRG activation of ERBB3-containing heterodimers might prevent gefitinib response, since these heterodimers are unlikely to respond to the EGFR-selective TKI. This was tested using NSCLC cell lines, where they showed that gefitinib insensitivity significantly correlated with HRG expression. Based upon ADAM17 overexpression in tumors with ERBB3 activity, and the role of ADAM10 and ADAM17 in ERBB ligand cleavage, they identified INCB3619, a small molecule that strongly inhibits both ADAMs. Importantly, this dual ADAM inhibitor blocked HRG release in a gefitinib-resistant NSCLC cell line, sensitizing the tumor cells to the TKI.

These are exciting results, since ADAM10 has also been implicated in autocrine EGFR activity in breast cancer (Borrell-Pages et al., 2003; see Table 2 for other ADAMs implicated in cancer). Furthermore, in ERBB2-positive breast cancer, autocrine ligand production might also interfere with trastuzumab response, since trastuzumab-sensitive breast tumor cell lines become resistant to the antibody in the presence of EGF-related ligands (Motoyama et al., 2002). Trastuzumab binds domain IV of ERBB2, a region not involved in receptor dimerization (Cho et al., 2003), which explains why ERBB ligands can induce activation of ERBB2-containing dimers in the presence of the antibody. At this point one might ask whether a dual EGFR/ERBB2 inhibitor that should also block HRG-induced ERBB2/ERBB3 heterodimers might be sufficient to overcome HRG-mediated resistance, making combined inhibition of ERBBs and ADAMs superfluous. Since Zhou and colleagues only examined sensitivity of NSCLC cell

lines to gefitinib, these data are not available. However, it should be kept in mind that ADAMs have many substrates whose processing might circumvent ERBB inhibition by other mechanisms. In fact, ADAMs cleave the ectodomain of ERBB2, leaving behind an active truncated receptor (Molina et al., 2001). Furthermore, ADAM-mediated proteolysis of IGFBPs (discussed in Zhou et al., 2005) releases IGF-1, a peptide known to play a role in circumventing ERBB inhibitors (Lu et al., 2001). In summary, information from the new papers in this issue of *Cancer Cell* suggest that dual EGFR/ERBB2 inhibitors either alone or combined with a selective ADAM inhibitor might be effective in cancers with autocrine ligand activation of ERBB receptors and ERBB KD mutations.

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