EGFR lung cancer mutants get specialized

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In 2004, three groups independently identified mutations in the epidermal growth factor receptor (EGFR, ErbB1) that sensitized non-small cell lung cancers (NSCLCs) to the small-molecule EGFR inhibitors erlotinib and gefitinib (1–3). The most commonly occurring mutation, L834R (or L858R in numbering that includes the signal peptide), also leads to increased EGFR signaling. Despite impressive initial responses to the treatment of tumors containing the L834R substitution, patients inevitably developed resistance as a result of secondary mutations in the kinase domain, most frequently represented by modification of the gatekeeper residue (T766M) (4). After nearly a decade of research into the molecular basis by which activating mutations deregulate EGFR signaling, our understanding of these effects is greatly improved but not complete. In PNAS, Red Brewer et al. (5) use recent developments in our knowledge of EGFR activation to investigate how the L834R and L834R/T766M mutants drive aberrant signaling. Their work identifies a clever strategy in which the cancer mutants cooperate with wild-type EGFR and its close homolog, ErbB2, to produce enhanced activity. These results put a fresh spin on targeting EGFR in cancer.

The molecular mechanism which underlies EGFR activation involves formation of a unique asymmetric dimer between two kinase domains. In this arrangement, the C-terminal lobe of one kinase (the donor/activator) interacts with the N-terminal lobe of its partner (the acceptor/receiver), resulting in allosteric activation of the acceptor kinase (Fig. 1*A*) (6). Conformational changes in the acceptor kinase involve movement of the catalytically important αC helix toward the active site, and extension of the activation loop. These changes open a hydrophobic interface in the N-lobe, promoting association with the donor. Formation of the asymmetric dimer is absolutely essential for activation of EGFR, and the receptor fails to signal as a monomer (6).

Leucine 834 is located within a set of hydrophobic interactions that stabilize the inactive state of the activation loop. Its mutation to an arginine (L834R) was proposed to destabilize the inactive state, causing the kinase to increasingly populate the active conformation. In support of this notion came studies showing that an isolated L834R kinase domain is ∼20-fold more active than wild-type (6, 7). The first crystal structures of the L834R kinase also demonstrated that the mutant adopts an active conformation (7). Because erlotinib and gefitinib were shown to target the active conformation, this provided a justifiable explanation for their increased potency toward the L834R mutant (7, 8).

Because of stabilization of the active state, the L834R mutant was also predicted to bypass the requirement for asymmetric dimerization, and consequently acquire independence from the activating ligand EGF. However, results from two key cell-based studies suggested that the L834R receptor cannot signal as a monomer. First, the L834R mutant still requires EGF to become fully phosphorylated (9). Second, mutations that disrupt the asymmetric dimer significantly decrease phosphorylation of the L834R mutant (10). Thus, the main mechanism by which cancer mutations activate EGFR is not by freeing it from activation through the asymmetric dimer. Indeed, although Red Brewer et al. show that the T766M mutation further increases the activity of the L834R mutant, L834R/T766M receptors still retain dependence on asymmetric dimerization for full activation (5).

If the lung cancer mutations stabilize the active state of the EGFR kinase, why are mutant receptors still dependent on dimerization? A caveat of many EGFR kinase domain structures is that the packing of molecules results in a daisy chain of asymmetric dimers. Thus, although the first crystal structures of the L834R kinase were in an active conformation, it was not clear whether this conformation was stabilized by the mutation or by dimerization. When a dimerization-deficient L834R kinase was subsequently crystallized, the kinase did not adopt an active conformation but the hydrophobic acceptor interface in the N-lobe was partially opened (11). This finding is in contrast

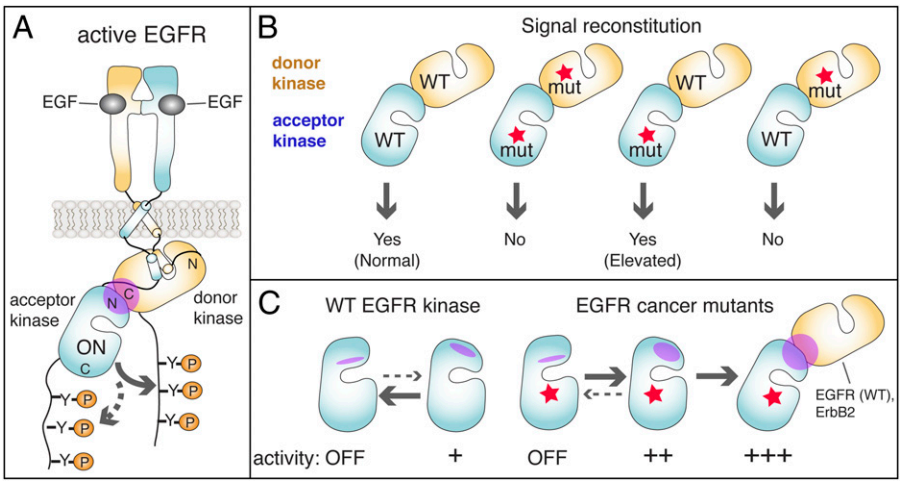

Fig. 1. (*A*) Activation of EGFR receptors is dependent on asymmetric dimerization between the kinase domains. The donor kinase stabilizes the active conformation of the acceptor kinase through a set of hydrophobic interactions (magenta circle) between the N-lobe (N) of the acceptor kinase and the C-lobe (C) of the donor kinase. (*B*) When positioned as an acceptor kinase, NSCLC mutants L834R and L834R/T766M ("mut," red star), result in more efficient signaling than wild-type (WT) EGFR. However, NSCLC mutants fail to serve as donor kinases. (*C*) Monomeric EGFR kinase is primarily inactive because of the occlusion of the activator interface (magenta) within the N-lobe. Activating mutations in the acceptor kinase promote dimerization through partial destabilization of the inactive conformation.

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to the dimerization-deficient wild-type EGFR kinase, which shows a fully sequestered acceptor interface (6). Hence, although the monomeric L834R mutant cannot adopt an active conformation, it attains a partially acceptor-competent state. Long time-scale molecular dynamics simulations confirmed that the L834R kinase faces a lower energy barrier to adopt an acceptor-competent conformation compared with wild-type (12). An important consequence of this behavior is an increased propensity of the L834R mutant to dimerize because of exposure of the hydrophobic acceptor interface (12). Thus, enhanced signaling by EGFR lung cancer mutants seems to be primarily driven by increasing the affinity of the asymmetric dimer.

The functional asymmetry within the active EGFR dimer raises an intriguing possibility that ErbB receptors might specialize as acceptors or donors. Although the catalytically inactive member of the EGFR family, ErbB3, is already known to function only as a donor because of substitutions in its acceptor interface, the acceptor/donor specialization of active ErbB receptors is unclear (6). Most recently, EGFR/ErbB2 heterodimeric signaling has been shown to proceed specifically through catalytic activation of the EGFR kinase, suggesting that EGFR is the preferred acceptor in this pair (13). Red Brewer et al. (5) investigate whether this preference can be modulated by cancer mutations. The authors use a complementation assay in which the asymmetric dimer is reconstituted using designated donor and acceptor constructs. Although donors and acceptors are individually inactive, coexpression reconstitutes an active dimer (Fig. 1*B*). Pairing of an L834R or L834R/T766M mutant acceptor with a wild-type donor results in increased receptor phosphorylation compared with the pairing of a wild-type acceptor and a wild-type donor. This outcome is in agreement with the hypothesis that activating mutations in the EGFR kinase lower the energetic barrier to attain an acceptor-competent conformation (Fig. 1*C*). Another observation made during this analysis is more puzzling. The L834R and L834R/T766M mutants fail to efficiently complement an acceptor kinase, regardless of the mutational status of the acceptor (Fig. 1*B*). Thus, the cancer mutations appear to improve the activity of an acceptor kinase but concomitantly weaken donor function.

A consequence of the specialization of cancer mutants as acceptor kinases is their acquired preference for wild-type dimerization partners. Red Brewer et al. (5) demonstrate that wild-type EGFR and ErbB2 are efficiently phosphorylated by the mutant receptors when the latter take the place of the acceptor kinase. Moreover, it seems that the most prolific signaling by cancer mutants occurs in the presence of wild-type heterodimerization partners, because more mutant receptors can now serve in the catalytically active acceptor position. As noted by the authors, this cooperation provides a probable reason for the preservation of wild-type EGFR alleles—and frequent ErbB2 coexpression—in tumor cells. The cross-talk also likely results in the diversification of signaling by oncogenic mutants, because ErbB receptors exhibit distinct signaling characteristics depending on their dimerization partners (14).

The results of Red Brewer et al. encourage investigation into alternative routes by which drug-resistant NSCLC could be treated.

Aside from important implications for EGFR signaling in cancer, the present study raises an interesting question about the molecular determinants required for efficient acceptor/donor function. Past studies provide a compelling explanation for improved acceptor function of cancer mutants, but the mechanism by which L834R/T766M mutations could diminish donor function is not immediately clear. The mutations are too removed from the donor interface (~30 Å) to have a direct effect. However, the present work opens up the possibility that the donor interface might be allosterically regulated by the activation state of the kinase. This aspect would provide a unique point of regulation in EGFR receptor signaling.

Most importantly, the results of Red Brewer et al. (5) encourage investigation into alternative routes by which drug-resistant NSCLC could be treated. Based on the apparent negative effect of cancer mutations on the donor function of EGFR, it is plausible that small molecules that alter the conformation of the wild-type donor kinase to produce a donor-incompetent state might be effective in inhibiting EGFR signaling. Another strategy should take advantage of the retained dependence of NSCLC mutants on asymmetric dimerization. Inhibitors of EGFR dimerization would likely be very effective in curtailing aberrant signaling mediated by mutant receptors. Next-generation EGFR therapeutics will likely have to be used in combination with traditional kinase inhibitors, but the need for their development is becoming increasingly clear.

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