EGF receptor inhibition: Attacks on multiple fronts

The epidermal growth factor receptor (EGFR) drives tumor growth in a subset of human epithelial carcinomas. A crystallographic study by Li et al. in this issue of *Cancer Cell* provides the molecular basis for inhibition of EGFR by cetuximab (Erbitux), a monoclonal antibody that has been approved by the Food and Drug Administration as a therapeutic for advanced-stage colorectal cancers. Cetuximab targets one of the ligand binding domains of EGFR, thus preventing ligand activation of the receptor.

The epidermal growth factor receptor (EGFR), along with ErbB2 (HER2/Neu), ErbB3 (HER3), and ErbB4 (HER4), is a member of a subclass of cell surface receptors with intrinsic tyrosine kinase activity, known as receptor tyrosine kinases (RTKs) (reviewed in Schlessinger, 2000). Receptors of the EGFR subclass play critical roles in normal embryonic development, and as mediators of cell proliferation, they can also drive the growth of tumors through ligand or receptor overexpression or a receptor gain-of-function mutation (reviewed in Yarden and Sliwkowski, 2001). Excessive proliferative signaling from EGFR and ErbB2 has been implicated in a broad spectrum of epithelial carcinomas, including breast, ovarian, head and neck, lung, pancreatic, and colorectal.

Two therapeutic strategies have met with success for suppressing aberrant EGFR and ErbB2 signaling: antibody targeting of the extracellular region of the receptors and small-molecule inhibition of the cytoplasmic tyrosine kinase domains (reviewed in Mendelsohn and Baselga, 2003). The antibody approach provides high target specificity, but because the therapeutic agent is a protein, there are greater challenges in drug development. Small-molecule inhibitors are relatively less difficult to convert into a drug, but because of their small size, a suitably deep pocket/cleft in the target protein is usually necessary to achieve high affinity binding and specificity. For EGFR and ErbB2 (and other protein kinases), the ATP binding cleft of the tyrosine kinase domain satisfies this requirement.

Trastuzumab (Herceptin), a monoclonal antibody that targets ErbB2, was approved by the Food and Drug Administration (FDA) in 1998 for the treatment of metastatic breast cancers overexpressing ErbB2. The monoclonal antibody cetuximab (Erbitux), which targets EGFR, was approved last year by the FDA for advanced-stage, EGFR-driven colorectal cancers. Two small-molecule inhibitors of EGFR/ErbB2 have been approved by the FDA, gefitinib (Iressa) and erlotinib (Tarceva). Gefitinib and erlotinib, both of which target the ATP binding cleft of EGFR, have been approved for advanced non-small cell lung cancer. Importantly, recent studies of non-small cell lung cancer patients have shown that gefitinib is only effective (and then, highly effective) for a subpopulation of these patients, those who harbor particular mutations in the tyrosine kinase domain of EGFR (reviewed in Minna et al., 2004). Several other drugs targeting EGFR and ErbB2 are at various stages of clinical trials.

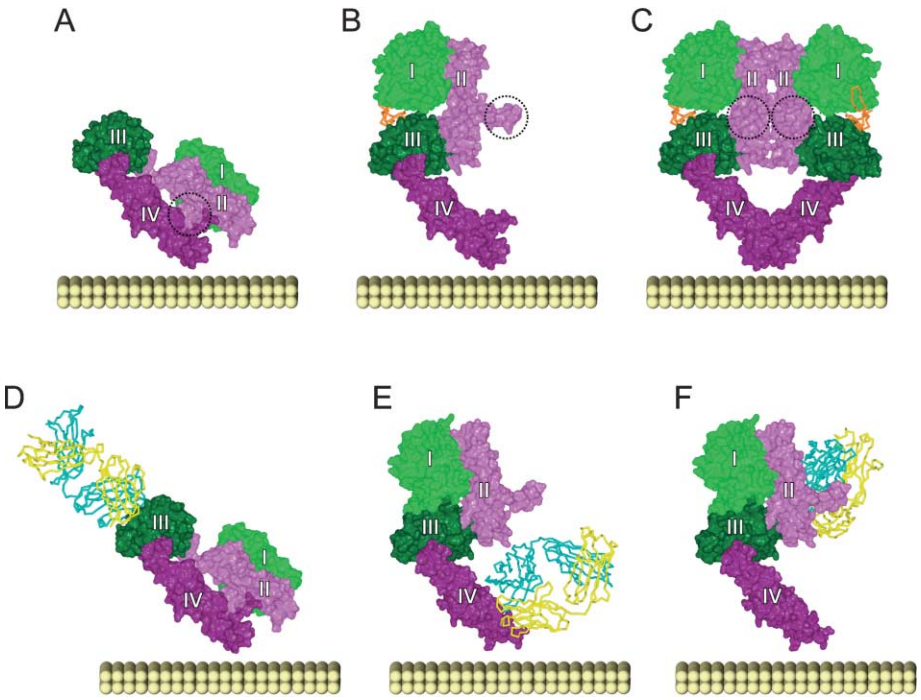

Figure 1. Ligand activation of EGFR and antibody targeting of EGFR and ErbB2

The ectodomains of EGFR and ErbB2 are shown in surface representation, with domain I (L1) colored light green, domain II (CR1) colored light purple, domain III (L2) colored dark green, and domain IV (CR2) colored dark purple. The dimerization arm in domain II is indicated by a dashed circle in **A**–**C**. For each antibody Fab (**D**–**F**), the Cα trace of the light chain is colored yellow and the Cα trace of the heavy chain is colored cyan. The receptor ectodomains have been oriented such that the position of domain IV is common. The membrane bilayer is represented (not to scale) by the yellow spheres.

A: Structure of the unbound EGFR ectodomain (autoinhibited, tethered state).

B: Structure of the 1:1 EGF:EGFR ectodomain complex (dimerization-competent state). The Cα trace of EGF is colored orange.

C: Structure of the 2:2 EGF:EGFR ectodomain complex (dimerized, activated state). The 2-fold axis of the dimer is vertical, in the plane of the page.

D: Structure of cetuximab bound to domain III of the EGFR ectodomain.

E: Structure of trastuzumab bound to domain IV of the ErbB2 ectodomain.

F: Structure of pertuzumab bound to domain II of the ErbB2 ectodomain.

The extracellular region (ectodomain) of EGFR and its family members is composed of two types of domains, a so-called L domain and a cysteine-rich (CR) domain. These domains are arranged (N- to C-terminal) as two repeats of an L-CR pair: L1-CR1-L2-CR2. The ligand binding site comprises domains I (L1) and III (L2), the discontinuous (in a linear domain sense) nature of which hints at a complicated ligand-mediated activation process. The natural ligands for EGFR include EGF, transforming growth factor-α (TFGα), amphiregulin, and betacellulin (among several others), while neuregulins are the ligands for ErbB3 and ErbB4.

Several differences among the four EGFR family members are notable. One receptor, ErbB3, is devoid of catalytic activity, even though it possesses a tyrosine kinase domain (critical catalytic residues have been substituted), and one receptor, ErbB2, is ligandless. These peculiarities figure prominently in an important mechanistic feature of this subclass of RTKs: heterodimerization (reviewed in Schlessinger, 2000; Yarden and Sliwkowski, 2001). Through heterodimerization, ErbB3, without an active kinase domain, can still be activated (phosphorylated) by ligandless ErbB2, which will recruit an ErbB3-specific set of downstream signaling proteins to the receptor heterodimer.

Over the past several years, a burgeoning of structural data has illuminated the molecular mechanisms governing ligand-mediated activation of EGFR and related family members (reviewed in Burgess et al., 2003). Crystal structures of the ectodomains of EGFR, ErbB2, and ErbB3, in various ligand-bound states, have revealed that binding of monomeric EGF or TGFα to the EGFR ectodomain stabilizes a conformation of the ectodomain that is competent for homodimerization or for heterodimerization with other EGFR family members. More precisely, a dimerization arm in domain II (CR1) of the receptor, which is sequestered by domain IV (CR2) of the unliganded receptor (tethered state; Figure 1A), becomes exposed upon EGF binding to domains I (L1) and III (L2) (Figure 1B). Rather than a 2:2 ligand:receptor complex in which the ligands bridge the two receptors, a common mode of RTK activation, the receptors themselves are the bridging elements in the 2:2 EGF:EGFR complex (Figure 1C). The stabilized receptor dimer then undergoes *trans*-phosphorylation on specific tyrosine residues in the cytoplasmic domain.

In the paper by Li et al. (2005), the crystal structure of the EGFR ectodomain in complex with the antigen binding fragment (Fab) of cetuximab is presented, which reveals that the antibody binds exclusively to domain III (L2) of the receptor (Figure 1D). A comparison with a previous EGFR structure with bound EGF indicates that the cetuximab binding footprint on domain III overlaps that of EGF on domain III. Through in vitro binding studies, Li et al. (2005) show that the cetuximab Fab binds ~50 times tighter to the (soluble) EGFR ectodomain than does EGF. Thus, the principal mechanism by which cetuximab inhibits EGFR signaling and elicits an antitumor effect is likely through direct ligand competition.

A second inhibitory mechanism may also be operative as a consequence of cetuximab binding to domain III of EGFR. To adopt a dimerization-competent conformation, in which the dimerization arm of domain II is exposed, domains I and III must be juxtaposed. For EGFR, ErbB3, and ErbB4, this is accomplished through ligand binding (Figure 1B), whereas ErbB2 adopts this conformation constitutively, without ligand (Figures 1E and 1F). Cetuximab binding to domain III of EGFR will, through steric hindrance, prevent the spontaneous (ligand-independent) juxtaposition of domains I and III (compare Figures 1B and 1D), thereby thwarting any basal EGFR dimerization and signaling. The relative contribution of this mechanism to the overall antitumor effect of cetuximab is difficult to assess.

The crystal structure of cetuximab bound to the EGFR ectodomain is the third structure of a therapeutic antibody bound to the ectodomain of an EGFR family member. The other two antibodies are trastuzumab and pertuzumab (Omnitarg), both of which (Fab fragments) were cocrystallized with the ErbB2 ectodomain (Cho et al., 2003; Franklin et al., 2004). The mode of antibody binding is distinct in each case (Figures 1D–1F). As discussed above, cetuximab binds to domain III (L2) of EGFR, whereas trastuzumab and pertuzumab bind to domains IV (CR2) and II (CR1), respectively, of ErbB2. Moreover, cetuximab binds to the tethered state of EGFR, whereas trastuzumab and pertuzumab bind to the untethered, dimerization-competent state of ErbB2. The antitumor activity of trastuzumab appears to derive from several mechanisms, including stimulation of ErbB2 endocytosis and antibody-mediated cytotoxicity, whereas pertuzumab directly interferes with the ability of ErbB2 to heterodimerize.

This is an exciting and cautiously optimistic time in the quest to develop anticancer therapeutics designed to target specific protein tyrosine kinases, best exemplified by the success of imatinib (Gleevec) in inhibiting Bcr-Abl in chronic myeloid leukemia (Druker et al., 2001). Through a better understanding of the cellular signaling processes underlying tumorigenesis and metastasis, and of the precise molecular interactions between a drug—antibody or small molecule—and its kinase target, our arsenal of rationally designed therapeutics should continue to expand.

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Selected reading

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