The EGF Receptor Hokey-Cokey

Dan Niculescu-Duvaz,[1] Steven Whittaker,[2] Caroline Springer,[1] and Richard Marais[2,3,*]
[1]The Institute of Cancer Research, Gene and Oncogene Targeting Team, Cancer Research UK Centre for Cancer Therapeutics, Sutton, Surrey SM2 5NG, United Kingdom
[2]The Institute of Cancer Research, Signal Transduction Team, Cancer Research UK Centre for Cell and Molecular Biology, London SW3 6JB, United Kingdom
*Correspondence: richard.marais@icr.ac.uk
DOI 10.1016/j.ccr.2007.02.021

In cancer, the epidermal growth factor (EGF) receptor (EGFR) can be activated by mutations that disrupt the inactive conformation and allow the active conformation to predominate. Structural studies have elucidated the molecular events that lead to EGFR activation and shown that small-molecule anti-EGFR drugs can bind to either the inactive or the active conformation of the kinase domain. In this issue of *Cancer Cell*, Yun et al. present 12 crystal structures of the wild-type or mutant forms of the EGFR kinase domain bound to four different ligands. This study will prove invaluable to those developing novel anti-EGFR drugs.

The epidermal growth factor (EGF) receptor (EGFR) is a membrane-spanning receptor tyrosine kinase (RTK) that regulates cell proliferation, survival, and migration (Hynes and Lane, 2005). EGFR is implicated in non-small-cell lung cancer (NSCLC), glioblastoma, and breast cancer, where its oncogenic potential is stimulated by protein overexpression or by somatic gain-of-function mutations (Hynes and Lane, 2005). Small deletions/insertions that affect the critical "C helix," or point mutations that affect the "glycine-rich loop" or the "activation segment" occur in 10%–20% of NSCLC cases (Lynch et al., 2004; Paez et al., 2004). The most common mutation (~40% of NSCLC cases) is an arginine for leucine substitution at position 858 (L858R), but surprisingly, it is the presence of a mutation such as this that determines patient responses to drugs such as gefitinib and erlotinib. This is unexpected because these small-molecule drugs were developed to target overexpressed wild-type EGFR ($EGFR^{WT}$) and not the mutated proteins (Lynch et al., 2004; Paez et al., 2004).

EGFR regulation has been intensely studied, particularly by structural biologists. EGF binds to the extracellular domain, inducing receptor dimerization, stimulating the kinase activity, and resulting in autophosphorylation of the cytosolic domain, although activation segment phosphorylation is not required for kinase activity (Stamos et al., 2002). Rather, cytosolic domain phosphorylation produces binding sites for other proteins, forming a signaling complex that regulates cell functions.

You put the C helix in, the C helix out,
In out, in out, you move it all about,
You do the Hokey-Cokey and you turn around
And that's what it's all about . . .

Adapted from www.bbc.co.uk/cbeebies/tweenies/songtime/songs/h/hokeycokey.shtml

Structural studies have revealed two conformations of the EGFR kinase domain: active and inactive (Stamos et al., 2002; Wood et al., 2004; Zhang et al., 2006). In each, the kinase domain adopts a typical kinase fold, with two lobes separated by the catalytic cleft (Figure 1), but in the inactive conformation the kinase domain is thought to be intrinsically autoinhibited. When EGF binding induces receptor dimerization, the local concentration of the kinase domain at the plasma membrane is effectively increased (Zhang et al., 2006). This stimulates the formation of asymmetric kinase domain dimers, in which the C lobe of one of the monomer (monomer B) binds to the N lobe of the other (monomer A). In this dimer, monomer B acts as an allosteric activator of monomer A by inducing several structural changes, one of which is to "push" the C helix of monomer A into the correct position for catalysis (Zhang et al., 2006). This elegant model explains how EGFR is activated.

Notably, most of the published structures of the EGFR kinase domain are in the active conformation. This is partly because some drugs only bind to the active conformation, but also because the high concentration of protein required for crystallization studies induces the formation of the asymmetric units normally induced by receptor dimerization, thereby forcing monomer A into the active conformation. The inactive conformation is only observed in crystals formed by kinase domains that cannot form dimers (due to the introduction of carefully selected mutations), or when the domain binds to inhibitors such as lapatinib, which only binds to the inactive conformation (Wood et al., 2004; Zhang et al., 2006). However, this conformation is highly instructive, because in it the activation segment forms a short helix that inserts

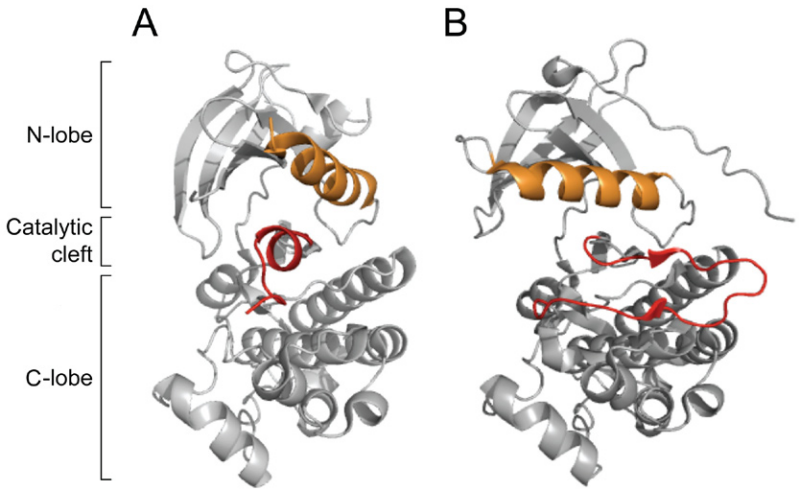

Figure 1. Comparison of the Inactive and Active Conformations of the EGFR Kinase Domain

(A) Inactive EGFR kinase domain from the structure bound to lapatinib (removed for clarity) is shown (coordinates published in Wood et al. [2004]).

(B) Active EGFR kinase domain from the structure bound to erlotinib (removed for clarity) is shown (coordinates published in Stamos et al. [2002]). In each case the position of the N lobe, the C lobe, and the catalytic cleft are indicated. The C helix regions are highlighted in orange, and the activation segments are in red. The kinases are aligned with each other such that their C lobes are in the same relative orientation. This reveals a subtle twist in the relative orientations of the N lobes and the insertion of the activation segment behind the C helix in the inactive (A) structure.

into a hydrophobic pocket behind the C helix (Figure 1). This disrupts the alignment of the C helix and is a key feature of the inactive kinase. L858 inserts into the hydrophobic pocket, and structural studies suggest that the larger, charged side chain of arginine could not be accommodated. The arginine is therefore predicted to disrupt the inactive conformation of EGFR, leading to the conclusion that EGFR mutations do not so much activate the kinase as disrupt the inactive conformation. This allows the C helix to occupy the correct position for catalytic activity, thereby favoring the active conformation (Zhang et al., 2006). This is similar to the protein kinase BRAF, which is also activated by mutations that destabilize the inactive conformation (Wan et al., 2004), and suggests that inactive conformation destabilization could be a common mechanism by which cancer mutations to activate kinases.

In this issue of *Cancer Cell*, Yun and coworkers add to this fast-moving story by presenting the structures of the kinase domain of $EGFR^{WT}$ and two gefitinib-sensitive mutants, L858R and G719S (a glycine-rich loop mutant) bound to the ATP analog AMP-PNP and three small-molecule inhibitors, gefitinib, AEE788 (a pyrrolopyrimidine), and AFN941 (a staurosporine analog) (Yun et al., 2007). All 12 structures adopt the active conformation, confirming that L858R and G719S cause minimal disruption to the protein backbone in the active state and suggesting that glycine-rich loop mutations also activate EGFR by destabilizing the inactive conformation. Yun et al. demonstrate that $EGFR^{G719S}$ and $EGFR^{L858R}$ are activated 10- and 50-fold, respectively, which is consistent with the theoretical contribution that these residues make to the stability of the inactive conformation. Also, the higher-activity $EGFR^{L858R}$ is more sensitive to gefitinib and AEE788 than the lower-activity $EGFR^{G719S}$. It is suggested that this is a reflection of the fact that these inhibitors only bind to the active conformation. However, this is a circular argument. If the inhibitors only bind to the active conformation, why then do they not bind to activated $EGFR^{WT}$ in cells and inhibit it similarly to the mutants? This may simply reflect the length of time that the wild-type protein spends in the active conformation, and because it can exchange between active and inactive conformations, it can displace the inhibitors, allowing ATP to rebind and thus retain some catalytic activity.

Many of the regulation concepts described by Yun et al. have been discussed elsewhere, but this paper is important in the information it provides to those developing EGFR inhibitors. Inhibitors that bind to the active receptor have more rapid binding and dissociation kinetics than those that bind to the inactive conformation. Presumably this is because active conformation binders simply bind to the pre-existing cavity and therefore must only compete with ATP. In contrast, inhibitors that bind to the inactive kinase do so through an induced fit. This requires opening of the kinase domain, displacement of the resident ATP, competition with ATP, and then refolding of the domain around the inhibitor, all of which takes longer and requires significantly more association and dissociation energy. The perceived advantage of targeting the inactive conformation is that the inhibitors should be more selective, because whereas all active kinases adopt more or less the same shape, inactive kinases exist in a variety of structures, providing more opportunity to develop selective compounds.

An important conclusion from these studies is that drugs that bind to the active conformation will favor mutated EGFR, whereas those that bind to the inactive kinase will only inhibit wild-type EGFR or those mutants that can be forced into the inactive conformation. Accordingly, it would be interesting to use structural approaches to see if lapatinib can force a substituted arginine at position 858 into the hydrophobic pocket behind the C helix in the inactive conformation. Furthermore, the observation that gefitinib binds to $EGFR^{L858R}$, but not $EGFR^{WT}$ or $EGFR^{G719S}$, through two distinct modes and that AFN941 binds to $EGFR^{G719S}$ through a distinct mechanism compared to $EGFR^{WT}$ and $EGFR^{L858R}$ is also interesting, as it suggests the possibility of making muta-

tion-specific inhibitors. Patient stratification and matching the drug to the mutation will be an important strategy to ensure successful treatment of malignancies such as NSCLC. Furthermore, most patients on prolonged gefitinib and erlotinib treatment develop secondary mutations in the EGFR kinase domain that block drug binding, leading to clinical resistance and therapy failure (Kobayashi et al., 2005; Kwak et al., 2005; Pao et al., 2005). The ability to synthesize drugs that inhibit through different binding modes will be crucial if we are to tackle this increasingly important clinical problem. Finally, the information learned for the EGFR will have important ramifications for other kinases, such as BCR-ABL, which also binds to drugs in either the active and inactive conformations (Liu and Gray, 2006; Schindler et al., 2000).

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A Case of Mistaken Identity? Nonductal Origins of Pancreatic "Ductal" Cancers

L. Charles Murtaugh[1,*] and Steven D. Leach[2,*]
[1]Department of Human Genetics, University of Utah, Salt Lake City, UT 84112, USA
[2]Departments of Surgery and Cell Biology, Johns Hopkins University, Baltimore MD 21218, USA
*Correspondence: murtaugh@genetics.utah.edu, stleach@jhmi.edu
DOI 10.1016/j.ccr.2007.02.020

In this issue of *Cancer Cell*, Guerra and colleagues provide important new insights regarding the ability of specific pancreatic cell types to generate invasive pancreatic cancer. First, they demonstrate that classical pancreatic "ductal" neoplasia can be induced by activation of oncogenic *Kras* in nonductal exocrine cells. Second, they show that, while *Kras* activation in immature acinar and centroacinar cells is readily able to induce ductal neoplasia, *Kras*-mediated tumorigenesis in mature exocrine pancreas requires the induction of chronic epithelial injury. The results shed new light on the "cell of origin" of pancreatic ductal cancer and demonstrate that chronic pancreatitis provides a permissive environment for *Kras*-induced pancreatic neoplasia.

Among the many problems of cancer research, "cell-of-origin" questions may occasionally be viewed as trivial or semantic. Yet tumors are not born equal: for example, pancreatic ductal adenocarcinomas (PDACs) nearly always arise from precursors that sustain activating *KRAS* mutations, while such mutations are almost never seen in less common pancreatic cancers such as islet cell carcinomas. Some of these differences may reflect the internal wiring of the initiating cell types, such that *KRAS* activation favors transformation in one cell but not another. Deciphering these interactions between epigenetic determinants of cell identity and genetic changes leading to tumor formation might identify new targets for cancer treatment and prevention. In this issue of *Cancer Cell*, Guerra et al. (2007) make an important and surprising advance in clarifying the adult cell of origin for PDAC.