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Researchers Learn How Epidermal Growth Factor Receptor Is Activated

In a discovery that may help scientists design new cancer drugs, Howard Hughes Medical Institute researchers have provided scientists with the first definitive look at how the catalytic center of the epidermal growth factor receptor -- a protein often implicated in cancer development -- turns itself on to promote cell growth.

The epidermal growth factor receptor (EGFR) is overactive in many breast, lung, colon, and pancreatic cancers. Because of its key role in driving the proliferation of cells, EGFR is a target of several cancer drugs currently in development, as well as several approved therapies. The researchers said their findings offer fresh insight into how these drugs work and clues for the design of the next generation of EGFR inhibitors.

The researchers, led by Howard Hughes Medical Institute (HHMI) investigator John Kuriyan, published their findings in the June 15, 2006, issue of the journal *Cell*. Xuewu Zhang, who is in Kuriyan's laboratory at the University of California, Berkeley, was the first author of the article. Other co-authors were from the Johns Hopkins University School of Medicine.

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EGFR is nestled into the cell membrane on the surface of cells. When EGFR is activated by molecules called ligands, members of the EGFR receptor family pair up (dimerize), which then activates a region of the receptor inside

the cell called a tyrosine kinase domain, which is the catalytic center of the protein. Kinases are protein switches that activate other proteins by adding a phosphate group to them via a process called phosphorylation.

In healthy cells, EGFR triggers growth in response to an activating signal that comes from outside the cell. When the signal is encountered, EGFR receptors on the surface of the cell form pairs. HHMI researchers have now learned that this pairing causes a physical change in the shape of the kinase domain. This change of shape converts the normally inactive kinase into an active one, which then sends signals inside the cell that trigger cell growth.

“It had long been known that the EGFR ligand dimerizes the receptor and that this dimerization converts into an activation of the kinase domain; but it wasn't understood how that happens,” said Kuriyan. “This paper provides for the first time a very specific and detailed molecular model for how the EGF receptor switches on at the level of the kinase domain.”

In the first set of experiments, the researchers demonstrated that the kinase domain of EGFR is normally maintained in the off state. They found that a particular mutation that activates EGFR in a large percentage of patients with lung cancer caused a 20-fold increase in the kinase domain's activity. And when Zhang forced kinase domains into close proximity to one another -- as happens when the receptors dimerize -- he found that the kinase switched on. This result suggests that the activation involves some kind of inter-molecular interaction, that is, the kinases activate each other when they are brought together.

The next step was to pinpoint how one EGFR kinase domain would switch on another. A clue came from earlier work by other researchers who were using x-ray crystallography to determine the structure of the kinase domain alone. In x-ray crystallography, protein crystals are bombarded with x-ray beams. As the x-rays pass through and bounce off of atoms in the crystal, they leave a diffraction pattern, which can then be analyzed to determine the three-dimensional shape of the protein.

In their structural studies, those scientists had found only the active conformation of EGFR in their protein crystals. “Those earlier findings made us realize that the crystals must hold the answer to how EGFR switches on,” said Kuriyan.

Thus, Kuriyan and his colleagues performed detailed analyses of the active conformation of this crystal structure and their own new structures. These analyses revealed two types of dimers -- a symmetric form, in which both units had the same relative position to each other, and an asymmetric form, in which one unit took a different position relative to the other. Their subsequent experiments determined that the asymmetric conformation was important for activation.

Their studies also determined in structural detail how this activation takes place. They found that the asymmetric activation of one EGFR kinase domain by another is analogous to the activation of cyclin-dependent kinase (CDK) by cyclin, which are involved in regulating cell growth.

“Our model now is that EGFR normally sits in an inactive conformation, which we call Src/CDK-like. But when it is brought into high local concentration -- that is, when it's dimerized -- that overcomes the barrier for activation through an intermolecular interaction, and one EGFR molecule then pushes the active site of the other into the active state, and that switches it on,” said Kuriyan.

Kuriyan said that the activation mechanism they discovered is more complex than might be expected, but for good evolutionary reason. “When the ligand arrives outside the cell, the receptor has to transmit that information to the inside of the cell. I suspect the way this worked when the first transmembrane receptors tyrosine kinase evolved was simply to make activation contingent on phosphorylation of one receptor by the other. My suspicion is that as the EGF receptor evolved, it moved away from that primordial mechanism.”

Evolution of the more specialized mechanism has ultimately enabled more specific and responsive control of the family of EGFRs. For example, this evolutionary fine-tuning has enabled different combinations of EGFR family members (including ErbB2/HER2, ErbB3/HER3 and ErbB4/HER4) to switch on one another and result in a wide spectrum of specific signals. This kind of specificity is critical to the cell, given the powerful role EGFRs play in cell proliferation, differentiation and migration, Kuriyan noted.