EGF receptor activation by heterologous mechanisms

A myriad of growth factor-dependent and -independent mechanisms for activating the EGF receptor and the related ErbB receptors underscore the importance of receptor inhibitors in the treatment of some solid tumors.

The aberrant activation of ErbB family receptor tyrosine kinases (RTKs), including the EGF receptor, ErbB2 (also called HER2/neu), ErbB3, and ErbB4, has been implicated in tumor growth and progression. One mechanism by which these receptors may be activated in tumor cells is through overexpression, which can either promote constitutive receptor dimerization or overwhelm negative regulatory mechanisms that keep receptor activity in check. EGF receptor and ErbB2 are overexpressed in a variety of solid tumors, and overexpression often correlates with reduced time to relapse and poor prognosis. These observations point to ErbB receptors as therapeutic targets, and the clinical success and administrative approval of the ErbB2-directed Herceptin for breast cancer patients whose tumors overexpress this receptor provide proof of this principle (Shawver et al., 2002).

Certainly ErbB receptor overexpression can lead to ligand-independent activation, constitutive signaling, and the promotion of tumor growth and progression. However, while receptor overexpression is a readily recognizable clinical marker for aggressive tumors, mechanistically overexpression may underrepresent the role of ErbB receptors in tumor growth. The autocrine production of ErbB binding epidermal growth factor (EGF)-like ligands by tumor cells or the tumor-induced production of ligands by surrounding stromal cells has also been implicated in ErbB-mediated tumor growth. Moreover, progress in the field in recent years has uncovered a plethora of mechanisms leading to ErbB receptor activation by heterologous cell surface proteins. This point is highlighted by the findings of Liu et al. (2002) in this issue of *Cancer Cell,* which suggest that the overexpression of the urokinase plasminogen activator receptor (uPAR) can mediate the activation of normal levels of EGF receptor.

Urokinase plasminogen activator (uPA), an extracellular serine protease, functions in conjunction with its GPI-linked cell surface receptor uPAR in the creation of the active plasmin protease. Several decades of study have pointed to a causal role for the plasminogen activation system in tumor cell invasion and metastasis, and uPA and uPAR overexpression in many tumor types predicts a poor patient prognosis (Andreasen et al., 1997). Interestingly, at least some of the activities ascribed to the plasminogen activation system appear to be independent of protease function. uPAR is capable of mediating the activation of intracellular signaling cascades such as the Erk1/2 MAP kinases despite its lack of an intracellular domain. The interaction of uPAR with integrins accounts for its signaling activity and suggests a role for this receptor in the coordination of cellular adhesion, migration, and growth (Ossowski and Aguirre-Ghiso, 2000; Preissner et al., 2000).

Liu et al. report that the EGF receptor plays a central role in mediating cellular growth signals initiated by uPAR and integrins. When overexpressed in a head and neck carcinoma cell line that expresses modest levels of EGF receptor, uPAR associated with and activated the $\alpha_5\beta_1$ integrin complex. Plating these cells on fibronectin (FN) promoted the recruitment of the EGF receptor to integrins, stimulated the tyrosine phosphorylation of the EGF receptor, and enhanced Erk activity (Figure 1). FN-stimulated EGF receptor activity appeared to be independent of RTK overexpression or the expression or release of EGF-like growth factors, and was only dependent on the overexpression of uPAR and the functional integrity of the uPA/uPAR/FN/integrin complex. Inhibitor studies suggested that EGF receptor activity was responsible for FN-stimulated Erk activation and for tumor cell growth in a chick chorioallantoic membrane (CAM) assay. One implication of their results is that uPAR overexpression could facilitate both tumor growth and invasion through its multifunctional nature.

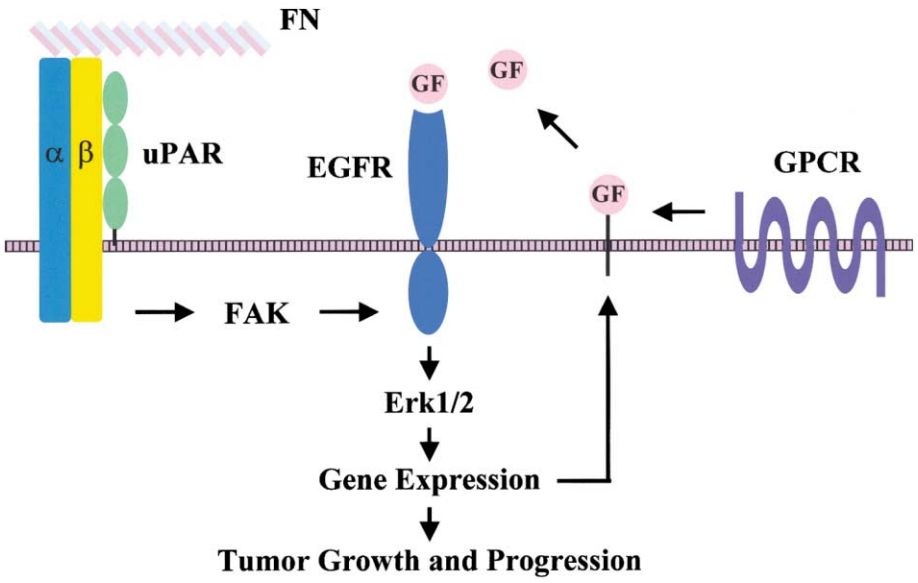

Figure 1. Heterologous ligand-dependent and -independent EGF receptor activation mechanisms

Stimulation of some GPCRs has been demonstrated to induce the release of membrane bound EGF-like growth factor (GF) ligands, which are then free to activate the EGF receptor in an autocrine manner. Overexpression of uPAR primes $\alpha_5\beta_1$ integrins to activate EGF receptor upon fibronectin (FN) binding, possibly through a FAK-dependent mechanism. Receptor activation leads to the activation of downstream kinases such as Erk1, Erk2, Erk5, and Akt, and the accompanying regulation of gene expression patterns that can lead to the growth and invasive properties of tumor cells. Genes encoding EGF-like growth factors are often targets receptor activation, leading to a positive feedback or amplification loop.

These results are consistent with numerous reports indicating that ErbB receptors are capable of mediating signaling from a variety of heterologous stimuli (Carpenter, 1999). Heterologous ErbB receptor activation can involve either growth factor ligand-dependent or -independent mechanisms, but result in MAP kinase activation via processes that are most often dependent on receptor tyrosine kinase activity. For example, transactivation of EGF receptors by G protein-coupled receptors (GPCRs) such as the lysophosphatidic acid (LPA) receptor has been demonstrated to induce the shedding of membrane-tethered EGF-like ligand precursors by metalloproteases (Gschwind et al., 2001). Cytokine receptors such as those for growth hormone and IL6 can interact with the EGF receptor and ErbB2, respectively, to mediate MAPK activation in response to their ligands. Finally, membrane depolarization and cellular stresses such as exposure to UV irradiation or oxidants can also lead to MAP kinase activation through the EGF receptor (Weiss et al., 1997).

The observations of Liu et al. are also consistent with the emerging theme that overexpression of some cell surface proteins could unmask or augment their ErbB activation activities. For example, the mucins MUC1 and MUC4 play roles in the normal protection and lubrication of epithelial surfaces, but are commonly found overexpressed in a variety of malignant tumors. Because their size and highly negative charge disrupt cell-cell and even cell-protein interactions, mucins are thought to contribute to tumor cell evasion of immune surveillance. However, both MUC1 and MUC4 have been shown to interact with ErbB receptor family members and to potentiate signaling (Schroeder et al., 2001) and cellular growth properties (Komatsu et al., 2001). While the physiological significance of this functional duality in normal tissue development or maintenance remains to be determined, aberrant ErbB activation by overexpressed mucins could actively contribute to the growth or progression of tumor cells (Carraway et al., 2001). Like uPAR, the mucins appear to exert their effects on ErbB receptors in the absence of RTK overexpression.

The results described above encompass dozens of individual reports and emphasize that there is remarkable plasticity in the activation of the ErbB RTKs. ErbB activation mechanisms appear to be more prevalent in tumor cells that overexpress some heterologous cell surface proteins, but ErbB receptor overexpression is not necessary. Hence, it is quite likely that aberrant ErbB receptor activation plays a more far-reaching role in tumor growth and progression than is represented by overexpression. Moreover, most of the described mechanisms involve the activation of receptor tyrosine kinase activity itself, as opposed to crossphosphorylation of receptors by other kinases to serve as a scaffold for the initiation of signaling events. These observations suggest that ErbB-directed tyrosine kinase inhibitors such as the small molecule EGF receptor inhibitor Iressa, which in preclinical studies exhibits some growth inhibitory effects toward EGF receptor-overexpressing non-small cell lung cancers (Raben et al., 2002), could possibly impact a wider subset of tumors than those that overexpress receptors. For the future it will be important to determine the extent to which ErbB receptors are aberrantly activated in tumors where overexpression is not observed. The development of highly sensitive phospho-specific antibodies directed toward active receptors could help alleviate some of the technical barriers in this regard.

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Selected reading

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Myc—Is this the oncogene from Hell?

A new paper implicates the Myc oncoprotein in the direct induction of DNA damage and consequent genome instability in cultured cells. However, it is less clear whether Myc induces the same genetic pandemonium in vivo.

The corrupted genomes of most human epithelial cancers are, like the Yucatan crater formed by the meteor that wiped out the dinosaurs, unambiguous relics of some catastrophic calamity within the tumor cell, a salient reminder of the genomic abyss that opens when the mechanisms that maintain chromosomal integrity fail or are overridden. Indeed, so