Insider Influence on ErbB Activity

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The receptor tyrosine kinase ErbB is activated by ligand-induced dimerization, leading to trans-phosphorylation of the cytoplasmic kinase domains. Bill et al. (2010) now demonstrate that trans-phosphorylation can be modulated from within the cell by the cytoplasmic protein cytohesin, providing new insights into ErbB-dependent processes during normal development and cancer.

Receptor tyrosine kinases (RTKs) are a large family of single-pass transmembrane receptors that convert extracellular information, conveyed by ligands, to the activation of intracellular signaling cascades. Ligand binding induces receptor dimerization and leads to trans-phosphorylation of tyrosines in the cytoplasmic kinase domains. The phosphorylation creates docking sites for SH2 domains, thus recruiting to the activated receptor complex proteins that trigger intracellular signal transduction cascades. A variety of positive and negative regulatory interactions affect the signaling outcome. The mechanisms include ubiquitination and phoshorylation of the receptor, formation of nonproductive receptor dimers, trapping of the extracellular ligand, and modulation of the intracellular cascade. However, until now, no cytoplasmic components were known to directly affect the process of ligand-induced receptor phosphorylation. Bill et al. (2010) now show that cytohesins, guanine nucleotide exchange factors (GEFs), bind to the cytoplasmic domain of ErbB receptor dimers and facilitate conformational changes that promote transphosphorylation and signaling activity.

These findings add to an increasingly complex and nuanced understanding of the mechanisms of receptor tyrosine kinase activation. It was originally proposed that communication between the extracellular and intracellular domains of RTKs is sequential, that is, dimerization of the extracellular domains, triggered by ligand binding, leads to dimerization of the intracellular domains and subsequent kinase activation (Yarden and Schlessinger, 1987). However, further work has since revealed several surprising new features of RTK dimerization, a process that has been examined in the greatest detail for the epidermal growth factor (EGF) receptor/ErbB family. In the case of the Drosophila EGF receptor dimer, binding of the first ligand molecule induces a conformational change that reduces the affinity for binding of the second ligand molecule (Alvarado et al., 2010). The cytoplasmic juxtamembrane region also plays a role in activation of the kinase (Red Brewer et al., 2009; Thiel and Carpenter, 2007). Ligand binding relieves an inhibitory association between the juxtamembrane region and the kinase domain, facilitating dimerization between the two juxtamembrane domains that stabilizes the kinase domain dimer (Jura et al., 2009). Finally, recent work shows that kinase domains exist in an autoinhibited state. Activation of the kinase requires generation of an asymmetric dimer, where the C-terminal lobe of one kinase molecule activates the second kinase domain (Zhang et al., 2006). An extreme case of dimer asymmetry occurs in the formation of active heterodimeric ErbB complexes that comprise one receptor with an active kinase domain and one with a catalytically dead kinase domain (ErbB3).

One question, therefore, is whether and how cells regulate these additional steps in the activation of RTKs. A recent study on Dok-7, an SH2-containing adaptor protein for the MuSK RTK, suggested that Dok-7 facilitates MuSK activity by promoting the juxtaposition of the two kinase domains, forming a positive feedback loop. This loop enhances receptor activation in distinct domains along the muscle plasma membrane at neuromuscular junctions (Bergamin et al., 2010; Inoue et al., 2009).

The paper by Bill et al. (2010) identifies a new scenario in which cytoplasmic components impinge on the process of RTK activation. The work demonstrates that cytohesins play a critical role of facilitating ErbB receptor family activation. Cytohesin proteins were previously characterized as guanine nucleotide exchange factors for ADP ribosylation factors (ARFs). Interestingly, GEF activity is dispensable for their role in facilitating ErbB activation. Bill et al. (2010) show that the level of cytohesins directly affects the signaling outcome of ErbB receptors (Figure 1). Although overexpression of cytohesins does not affect EGF receptor clustering or endocytosis, it leads to an increase in the phosphorylation of EGF receptor dimers. Conversely, inhibition of cytohesin with the specific inhibitor SecinH3 reduces the phosphorylation of dimerized receptors. Furthermore, fluorescence resonance energy transfer (FRET) studies of EGF receptor dimers tagged with a fluorescent protein suggest that the addition of cytohesin leads to conformational changes in the cytoplasmic domains. These changes affect kinase activation, presumably by facilitating structural changes required for formation of an asymmetric kinase dimer.

This study is important because it identifies a new way for the signal-receiving cell to modify the RTK signal early in the signaling process, at the level of receptor phosphorylation. And as may be expected, cancer cells point the way to pathological abrogation of this circuit. Elevated EGF receptor/ErbB signaling is characteristic of many cancers. Bill et al. show that
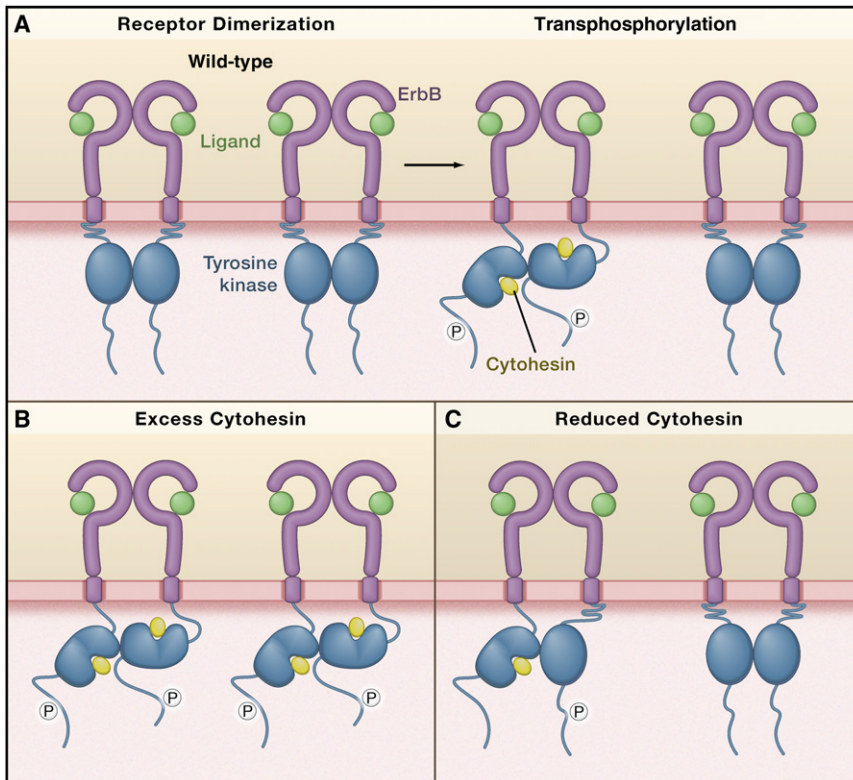
Figure 1. Cytohesin Levels Modulate ErbB Dimer Phosphorylation
(A) Upon ligand binding, ErbB receptor tyrosine kinases form dimers. In order to activate the kinase domain and trigger transphosphorylation, conformational changes that induce formation of asymmetric dimers must take place. Direct binding of cytohesin to the cytoplasmic domain facilitates these conformational changes.
(B) Overexpression of cytohesins, which occurs in some lung adenocarcinomas, elevates EGF receptor phosphorylation.
(C) Reduction in cytohesin levels, for example following treatment with the drug SecinH3 or cytohesin RNA interference, leads to a decrease in receptor phosphorylation.

there is an increase in ErbB transphosphorylation in human lung adenocarcinomas with elevated levels of cytohesins, without a corresponding increase in receptor protein levels. These observations raise the possibility of attenuating ErbB activity in tumors by antagonizing cytohesins. Indeed, Bill et al. show that addition of SecinH3 reduced the proliferation of an EGF receptor-dependent lung cancer cell line. When the same cells were injected into mice, treatment of the mice with the inhibitor also resulted in reduced proliferation of tumor cells.

Similar modulations of RTK activity from within the cell may also occur during normal organismal development to adjust the responsiveness of tissues to a given RTK signaling pathway. In *Drosophila*, for example, immunohistochemical staining for the activated form of MAP kinase has shown dramatic differences among tissues in the range of EGF receptor signaling activity around a ligand source (Gabay et al., 1997). In some tissues, the range of signaling is clearly modulated by the level of active ligand. However, it now seems feasible that the local level of cytohesins or other, yet to be identified cytoplasmic modulators also determines the sensitivity of individual tissues to EGF receptor activation.

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