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CELL BIOLOGY

EGFR-induced cytoskeletal changes drive complex cell behaviors: The tip of the iceberg

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Cytoskeletal networks are dramatically reorganized upon EGF stimulation to enable complex cell behaviors such as cell-cell communication, migration and invasion, and cell division. In this issue of *Science Signaling*, Roth *et al.* and Pike *et al.* use proteomic methods to identify several effectors of EGF responses. The findings show the interdependent nature of growth factor signaling and the cytoskeleton and identify potential new therapeutic targets for treating cancer and other growth factor–driven diseases.

Growth factor signaling through receptor tyrosine kinases (RTKs) is crucial to many aspects of development and morphogenesis (Fig. 1) (*1*). The importance of these signaling networks is underscored by the deregulation of RTKs in almost all types of human cancer. As such, decades of research have focused on elucidating and pharmacologically inhibiting the now canonical downstream kinase cascades that are induced by RTK activation and required for cell proliferation and survival (*2*, *3*). In contrast, how growth factor–initiated biochemical responses are translated into complex cell behaviors, such as cell-cell communication, migration and invasion, or even the act of cell division, is not well understood (*4*). As our understanding of the importance of these behaviors in disease progression increases, so does our appreciation of their potential as points of therapeutic intervention.

Recent studies have begun to highlight and unravel an interdependent relationship between RTKs and cellular cytoskeletal networks that is fundamental to the regulation of these complex cell behaviors and can be hijacked by tumor cells to drive tumor invasion and proliferation (*5*–*8*). For example, tight coordination between the activity of the epidermal growth factor receptor (EGFR) and the cortical actomyosin cytoskeleton enables the localized modulation of cell-cell junctions and other actin-based structures to be translated into tissue scale changes in cell shape and organization (*1*). In turn, cell junctions can regulate EGFR activity by controlling receptor distribution or access to ligand, and the associated actomyosin network can transmit mechanical forces that affect EGFR signaling and trafficking at the plasma membrane (*9*). The actin cytoskeletal network is spatially and functionally coordinated with that of microtubules, which direct the polarized movement of vesicles that deliver, remove, or recycle plasma membrane receptors (*10*). Even less well understood are mechanisms by which signaling from receptors like EGFR can functionally influence the microtubule networks that control their spatial distribution.

In this issue of *Science Signaling*, Roth *et al.* (*11*) and Pike *et al.* (*12*) from the Yarden and Parsons groups, respectively, used different proteomic approaches to uncover new insight into the complex question of how EGF-induced signaling modulates cytoskeletal remodeling during cell migration, adhesion, and division. Roth *et al.* used stable isotope labeling by amino acids in cell culture (SILAC)–based phosphoproteomic analysis to identify previously unknown proteins involved in growth factor–induced migration in mammary epithelial cells (*11*). Taking advantage of the fact that EGF, but not serum, is sufficient to stimulate migration of nontransformed mammary epithelial cells, they analyzed the differential patterns of phosphorylation distal to either stimulus. They found that the panel of substrates that was phosphorylated in response to either stimulus was remarkably similar, but many were phosphorylated at multiple sites and with modestly different kinetics. This work highlights the complexity of growth factor–mediated control of cell architecture and suggests that the ability of EGF to induce migration is the result of a collection of subtle changes in phosphorylation kinetics, rather than the modification of a unique set of substrates. One example of these subtle differences is ladinin-1 (LAD-1), a largely uncharacterized protein that emerged as a candidate in the screen due to the fact that of seven residues that are phosphorylated in response to EGF or serum stimulation, one is EGF-specific. LAD-1 is also transcriptionally induced by EGF, and the phosphorylated form specifically associates with actin filaments. The authors identified two proteins, filamin A (FLNA) and 14-3-3 sigma (SFN), as binding partners of LAD-1 and suggest a model wherein LAD-1 modulates actin remodeling via the phosphorylation-dependent recruitment of SFN to filamin-bundled actin filaments to promote F-actin depolymerization and treadmilling (Fig. 1, inset #1). The importance of further defining this mechanism is underscored by the authors' discovery of an important role for LAD-1 in mediating mammary cell migration and proliferation and association of LAD-1 with poor prognosis in breast cancer patients.

The importance of the dynamic interrelationship of EGFR and the microtubule cytoskeleton in complex cell behaviors is highlighted by the studies of Pike *et al.* (*12*). After finding that the Coxsackie and adenovirus receptor (CAR), a junction adhesion molecule family member, is important for EGF-dependent proliferation in lung cancer cells and establishing that protein kinase C δ–dependent phosphorylation of CAR alters its distribution at cell-cell junctions, the authors used mass spectrometry to identify the chromokinesin KIF22 as a phospho-specific binding partner for CAR (Fig. 1, inset #2). In KIF22-depleted cells, EGFR is rapidly internalized from the plasma membrane, and the activity of its canonical effector extracellular signal–regulated kinase (ERK) is decreased. Their work suggests that this is due to a novel cytoplasmic role for KIF22 in stabilizing the peripheral microtubule network, which would normally stabilize EGFR at the plasma membrane and promote sustained downstream signaling through ERK. This study identifies a potentially novel interaction module that could dynamically regulate the spatial distribution of both signaling

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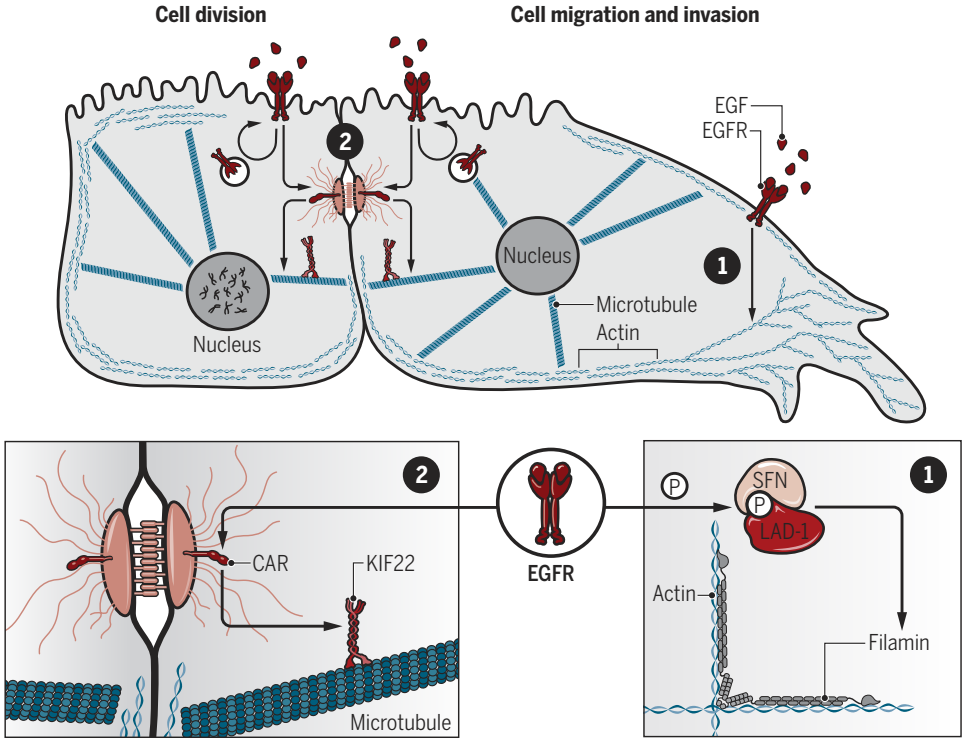

Fig. 1. Both the actomyosin and microtubule networks are markedly reorganized upon EGF stimulation to modulate cell division, migration, and invasion. Two new studies use proteomic methods to identify effectors of these important growth factor responses. Roth *et al.* (*11*) identified LAD-1 as a target of EGF-induced phosphorylation that is important for migration and proliferation of mammary epithelial cells through the modulation of actin dynamics (inset #1). They propose a model in which EGFR signaling activates the phosphorylation-dependent recruitment of SFN to FLNA-bundled actin filaments to promote F-actin depolymerization and treadmilling that facilitates invasive cell behavior. Pike *et al.* (*12*) identified the chromokinesin KIF22 as a phospho-specific binding partner of the CAR cell adhesion receptor and suggest that KIF22 regulates both EGFR signaling and CAR distribution by stabilizing the peripheral microtubule network (inset #2). This highlights important coordination between cell-cell junctions, microtubule dynamics, and EGFR activity.

and adhesion receptors. It is not yet understood how the dynamic reorganization of CAR at cell junctions contributes to cell proliferation, but it will be important in future studies to understand how actomyosin-dependent mechanical forces at the cell junctions contribute to changes in CAR localization. It is likely that coordinated interplay between the actin and microtubule cytoskeletal networks contributes to these processes.

While these studies begin to chip away at the complex issue of understanding how growth factor signaling coordinates with the cellular cytoskeletal networks to modulate complex cell behaviors, it is clear that sophisticated new approaches will be necessary to fully unravel the complexities of the problem. Future studies will need to bring together high-throughput proteomic approaches such as those described here with novel bioengineering tools designed to isolate specific aspects of cell behavior in physiologically relevant settings. New high-resolution imaging techniques now make it possible to monitor local and specific cytoskeletal changes that occur in response to growth factor stimulation in real time. These two studies provide a glimpse of what will likely be a flood of new information regarding the complete cellular response to growth factor stimulation—a positive outcome of melting this particular iceberg.

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