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REVIEW

Endocytic Regulation of EGFR Signaling

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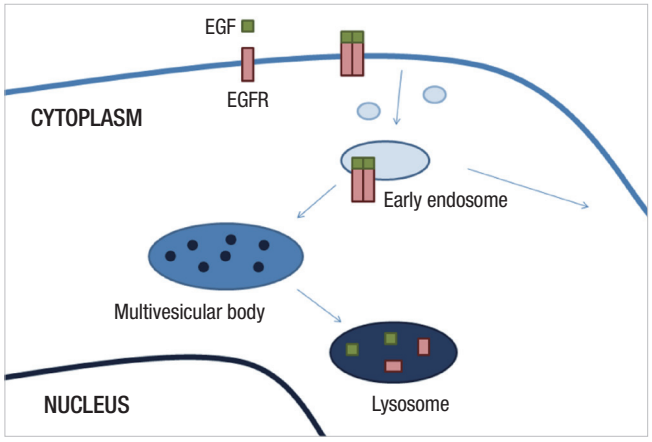
SYNOPSIS

Epidermal growth factor receptor (EGFR) is a member of the ErbB family (ErbB1-4) of receptor tyrosine kinases (RTKs). EGFR controls numerous physiological functions, including cell proliferation, migration, differentiation and survival. Importantly, aberrant signaling by EGFR has been linked to human cancers in which EGFR and its various ligands are frequently overexpressed or mutated. EGFR coordinates activation of multiple downstream factors and is subject of various regulatory processes as it mediates biology of the cell it resides in. Therefore, many studies have been devoted to understanding EGFR biology and targeting the protein for the goal of controlling tumor in clinical settings. Endocytic regulation of EGFR offers a promising area for targeting EGFR activity. Upon ligand binding, the activated receptor undergoes endocytosis and becomes degraded in lysosome, thereby terminating the signal. En route to lysosome, the receptor becomes engaged in activating various signaling pathways including PI-3K, MAPK and Src, and endocytosis may offer both spatial and temporal regulation of downstream target activation. Therefore, endocytosis is an important regulator of EGFR signaling, and increasing emphasis is being placed on endocytosis in terms of cancer treatment and understanding of the disease. In this review, EGFR signaling pathway and its intricate regulation by endocytosis will be discussed.



Key Words: EGFR; endocytosis; NSCLC EGFR mutants; tyrosine kinase; ubiquitin ligase

OVERVIEW

Epidermal growth factor receptor (EGFR), a member of the ErbB family (ErbB1-4) of receptor tyrosine kinases (RTKs), controls numerous physiological functions, including cell proliferation, migration, differentiation and survival. Importantly, aberrant signaling by EGFR has been linked to human cancers in which EGFR and its various ligands are frequently overexpressed. EGFR coordinates activation of multiple factors and is subject of various regulatory processes as it mediates biology of the cell it resides in. Therefore, many studies have been devoted to understanding EGFR biology and targeting the protein for the goal of controlling tumor in clinical settings. In this review, EGFR signaling pathway and its intricate regulation by endocytosis will be discussed.

EGFR AND CANCER

Composed of an extracellular growth factor binding site and a cytoplasmic kinase domain, RTKs confer a wide range of cellular functions including proliferation, apoptosis, migration and differentiation during development and tissue homeostasis. There are at least 58 receptors among the 20 subfamilies of RTKs that carry out such functional diversity[1]. Most importantly, RTKs have been intensely studied since aberrant expression and/or activation of RTKs is a major mechanism of oncogenic transformation.

EGFR is an ErbB family member of RTKs. In addition to EGFR (also known as ErbB-1/HER1), the ErbB family comprises of ErbB-2 (neu, HER2), ErbB-3 (HER3) and ErbB-4 (HER4). Since its initial discovery by Stanley Cohen in 1962, EGFR has been studied as a model RTK to provide much understanding of cellular and molecular mechanisms of RTK function and regulation[1]. As a transmembrane glycoprotein, EGFR binds to at least six extracellular ligands including epidermal growth factor (EGF), transforming growth factor α (TGFα), amphiregulin, heparin-binding EGF-like growth factor (HB-EGF), betacellulin, and epiregulin to regulate various functions within the cell[2]. In particular, EGFR is known to play crucial roles in cellular proliferation, survival, migration, and differentiation. Indeed, impaired epithelial development in several organs followed by embryonic lethality among EGFR knockout animal models illustrates the essential nature of EGFR in the cell[3,4]. Furthermore, oncogenic viruses exploit EGFR signaling network in many different ways, altering both receptor tyrosine kinase activity and gene expression[5].

As a model RTK, EGFR has been under special attention over the years due to its implication in oncogenesis. For instance, deregulating EGFR signaling has become an important issue in cancer treatment, since the overexpression of EGFR in various epithelial tumors was first described in the 1980s[1]. Indeed, types of cancer where overexpression of EGFR is found include breast cancer, head-and-cancer, non small cell lung cancer (NSCLC), renal cancer, ovarian cancer, and colon cancer[6]. Oncogenic potency of EGFR is also well documented. High-level expression of EGFR and EGF ligand can transform mouse fibroblast NIH 3T3 cells[7]. In addition, EGFR activation initiates cytoprotective signaling, enabling tumor cells to become resistant to radiation and chemotherapy[8]. Thus, high expression of the receptor is associated with poorer survival, and EGFR serves as a strong prognostic indicator in certain cancer types[9].

Due to its implication in development of cancer, much effort has been contributed towards targeting EGFR for cancer therapy. Specifically, anti-EGFR monoclonal antibodies and tyrosine kinase inhibitors (TKIs) are currently in clinical usage[10]. Anti-EGFR monoclonal antibodies primarily function to inhibit ligand binding by binding to the extracellular domain of EGFR. Currently, cetuximab and panitumumab are approved for clinical usage while matuzumab, zalutumumab, MDX-447, hR3, and 806 are being investigated[11]. TKIs are usually ATP analog and act by inhibiting ATP binding to the kinase domain. TKIs against EGFR include gefitinib, erlotinib, lapatinib, AE788, PKI166, EKB-569, canertinib, HKI-272, HKI-357, CL-387.785 and BIBW 2992[11].

EGFR SIGNALING PATHWAY

Upon binding to its ligand, EGFR undergoes dimerization which promotes auto-phosphorylation on tyrosine residues of its cytoplasmic tail. These phosphorylated tyrosines, in turn, become docking sites for distinct downstream effectors harboring SH2 (Src homology 2) or PTB (phosphotyrosine binding) domains[5]. These EGFR interacting proteins possess intrinsic enzymatic activities or serve as adaptor proteins to mediate interactions that link different proteins involved in signal transduction. Thus, various signaling pathways are activated, ultimately mediating diverse cellular responses. In addition to EGFR homodimerization, EGFR may also heterodimerize with its ErbB family members. ErbB family members show high homology in the kinase domain (59-81% identity), whereas the C-terminal domains are more divergent (11-25% identity), thus contributing to their differences and diversification in the signaling output[12].

EGFR stimulates MAPK signaling pathway primarily through Grb2 which complexes with Ras guanine exchange factor Sos[13]. Another adapter Shc has also been shown to mediate MAPK pathway activation in certain cellular systems[5]. In addition to the MAPK pathway, Ras is also known to activate Cdc42 and PI3-K as well. Furthermore, Grb2 interacting adapter Gab1 also mediates PI3-K activation upon EGF stimulation[14]. In addition, EGFR may directly activate PI3-K by interacting with p85 regulatory subunit to release an autoinhibitory constraint that stim-

ulates the catalytic subunit (reviewed in [Schlessinger, 2000][13]). Of note, the generation of phosphatidylinositol (3,4,5) trisphosphate (PIP_3) by PI3-K recruits Akt/PKB to the plasma membrane and allow subsequent phosphorylation by the phosphoinositide-dependent kinase-1 (PDK1)[13].

Another signaling molecule, Phospholipase (PLC) γ1, also interacts directly with EGFR and catalyzes the hydrolysis of phosphatidylinositol (4,5)-bisphosphate (PIP_2), generating the second messengers diacylglycerol and inositol triphosphate[15]. Both diacylglycerol and Ca^{2+}, stimulated by inositol triphosphate, then activate members of protein kinase-C (PKC), which phosphorylates Thr654 of EGFR and inhibit the tyrosine kinase activity, thus providing a negative feedback mechanism to control EGFR activity[13]. Phosphatases can also be modulating the signaling output of EGFR. For example, MAPK responses are inhibited by protein phosphatases that dephosphorylate and inactivate this enzyme[13]. Furthermore, two phosphoinositide phosphatases PTEN and SHIP dephosphorylate the PIP_3, leading to the inhibition of cellular response mediated by PI3-Ks[16]. The signal diversification may also be achieved through heterodimerization with other ErbB family member receptors, thus creating multilayers of signaling complexity. For example, heterodimerization with ErbB3 is thought to enhance PI3-K pathway activation. Also, the heterodimerization between EGFR with ErbB2 typically confers signaling potency[16].

EGFR DEGRADATION THROUGH ENDOCYTOSIS

In terms of tumorigenic potency conferred by EGFR, regulation of signaling is just as critical as the EGFR signaling itself. The key component of EGFR regulation involves ligand-induced receptor endocytosis which leads to the degradation of the receptor and termination of signal intensity, or recycling for continued signaling. Because of two radically different outcomes, understanding mechanisms of receptor internalization and its endosomal sorting confers a promising potential for the regulation of EGFR activity. The balance between the various stimulatory and inhibitory responses will ultimately determine the strength and duration of the signals that are transmitted through the networks of signaling cascades following their initiation at the cell surface in response to receptor stimulation.

Once ligand bound, EGFR, located mostly at caveolae and noncaveolae rafts, becomes internalized into the endosomal compartments before reaching lysosomes, resulting in the signal termination and receptor degradation[17] (Figure 1). In this context, endocytosed EGFR migrates down a system of heterogeneous compartments that have generally been characterized as 'early' or 'late' endosomes depending on the kinetics with which the compartments are endocytically loaded. These early and late endosomes can be distinguished on the basis of their morphological appearances. While early endosomes are primarily located towards the cell periphery, late endosomes are more spherical and are often positioned closer to the nucleus[18]. Furthermore, late endosomes frequently have multivesicular appearance and are, therefore, referred to as multivesicular bodies (MVBs). It is in MVBs, that EGFR sorting into the internal vesicles is facilitated, and the receptor becomes degraded upon subsequent fusion with the lysosome. Alternatively, internalized receptors may instead be recycled back onto the cell surface for more signaling[19].

While seemingly simple, the endocytic trafficking of EGFR is a complex process involving multiple factors. First, the type of ligand bound to EGFR may dictate the fate of the ligand-receptor complex. In particular, EGF facilitates the lysosomal degradation while TGFα seems to cause recycling of the receptor, thereby causing much more potent signaling response[17]. The dimerization partner may also affect the regulation of EGFR, as an overexpression of HER2 has been demonstrated to inhibit down-regulation of the EGFR and of itself, as well as increasing the recycling rate of EGFR[20].

The endosomal sorting process is thought to involve various mechanisms including sorting motifs, such as sorting nexin 1 (SNX1) associating tyrosine-leucine motif ^{954}YLVI on the cytoplasmic domains[20]. Importantly, a number of studies have identified ubiquitin as a signal sufficient for both receptor internalization and degradation. In fact, ubiquitination of not just the cargo itself, but also the endocytic machinery is becoming evident as factors such as Eps15 and Hepatocyte growth factor-

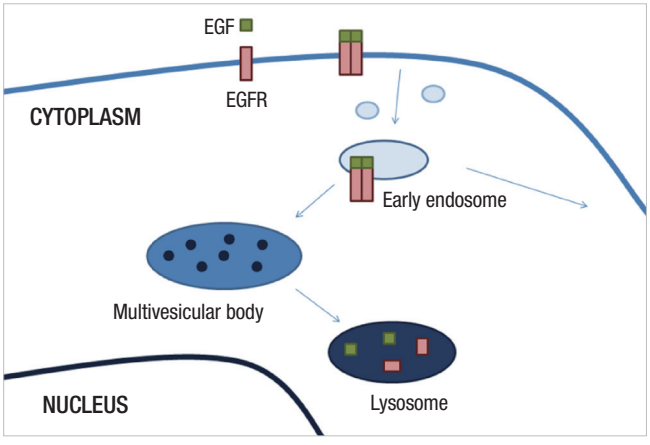


Figure 1. Ligand-induced degradation of EGFR. Ligand-free, mature EGFR is primarily localized at the cell surface. Upon ligand binding, activated EGFR becomes internalized, and becomes localized to endosomes. Depending on type of ligand bound, dimerization partner, mutational statuses and/or key factors, EGFR may recycle to the cell surface or be sorted to lysosomes. EGFR bound to EGF is mostly targeted for lysosomes, where it becomes degraded. En route, activated EGFR serves as docking site for many adapter proteins, leading to activation of various downstream targets. EGFR signaling affects various cellular processes such as proliferation, differentiation, survival, growth, and migration.

regulated tyrosine kinase substrate (Hrs) were shown to be ubiquitinated by Nedd4 family of ubiquitin ligase, which is phosphorylated upon EGF treatment[3].

Lysosomal degradation of EGFR is in large part regulated by the Cbl family of ubiquitin ligases. Upon ligand activation and phosphorylation of EGFR, Cbl becomes associated with the active receptor and monoubiquitinates EGFR[21]. From there, its association with the receptor throughout endosomal compartments is required for the lysosomal sorting process of activated EGFR[21,22]. Cbl also functions as an adaptor protein interacting with factors such as Cbl-interacting protein of 85 kDa (CIN85), which also functions in Cbl-mediated endocytosis of EGFR[23]. Also, Grb2 has been shown to facilitate internalization as its knockdown using siRNA approach significantly inhibits the receptor internalization[24]. In addition, there are other factors that mediate EGFR endocytosis and downregulation through Cbl. For example, Sprouty, Sts-1/Sts-2 and Cortactin seem to inhibit efficient EGFR trafficking to the lysosome and block receptor downregulation[25-27]. It is also important to note that Cbl may play a critical role in migratory mechanism through cytoskeletal rearrangement. Cbl has been shown to promote degradation and regulate another regulator of cell morphology βPix/Cool-1, which is involved in cytoskeletal rearrangements. Formation of complex with Cbl results in regulation of Cbl-mediated EGFR degradation and thus, Cool-1 has been shown to play a key role in regulating EGFR degradation[28]. EGF-mediated downregulation of tension-3 and upregulation of cten has also been implicated in metastasis of breast cancer[29].

Similarly, numerous studies have established the importance of Hrs, a mammalian homologue of yeast vacuolar protein sorting (Vps) protein, and factors that exert their effects through Hrs. Hr regulates the MVB sorting of EGFR, receptor ubiquitination and degradation[3]. Hr interacting factors include SNX1, stimulatory G protein subunit (G s), signal-transducing adaptor molecule (STAM), and tumor susceptibility gene product 101 (TSG101), all of which seems to facilitate the lysosomal sorting of the receptor with Hrs[30,31].

Because of such complexity involving multiple factors, the understanding of the endocytic events sometimes takes a dramatic turn as well. For years, it had been widely believed that the activated EGFR, once ubiquitinated becomes endocytosed primarily via clathrin coated vesicles whose fission is mediated by dynamin[17]. For example, Eps15, a substrate phosphorylated by EGFR, localizes at clathrin coated pits where it interacts with the clathrin assembly complex AP-2 and AP-2 binding Epsin[23]. The presence of ubiquitin interacting motifs found in Epsin and Eps15 provided further support for these factors as components of the ligand-induced endocytic machinery[27].

However, subsequent studies demonstrated that the ubiquitinated cargo may be endocytosed via clathrin independent pathway. For example, ubiquitination-impaired EGFR mutant was internalized through the clathrin pathway, whereas an ubiquitin moiety was internalized exclusively by the non-clathrin pathway[32]. In addition, an extensive colocalization of epsin with the ubiquitin-GFP on endocytic structures could be observed in cells where clathrin levels were drastically reduced by RNA interference[33]. Such results reveal the mutually exclusive colocalization of epsin with membrane-bound ubiquitin or clathrin playing a role in controlling the endocytic route taken by ubiquitinated cargo[33]. Further supporting this idea, an immuno-EM study in HeLa cells showed double-labeling for EGF and epsin resulted in only a minor subset of coated pits labeled for both[27]. It has recently been shown that EGFR may utilize clathrin or non-clathrin pathway depending on the concentration of ligand available[34].

The requirements for ubiquitin and ubiquitin ligase have also been subjects of debate regarding EGFR internalization. The involvement of factors such as CIN85, Epsin and Eps15 as mentioned above and studies using ubiquitin moiety as the protein have implicated ubiquitin to be critical to EGFR internalization[17,23,35]. However, emerging literature evidence hints for dispensable roles of ubiquitin and ubiquitin ligase Cbl in EGFR internalization[22,36,37]. Nevertheless, activated EGFR endocytosis involves multiple factors forming layers of regulation, and the exact mechanism still remains unclear.

EGFR SIGNLING AND ENDOCYTOSIS

In addition to its negative regulatory role on RTK signaling through lysosomal sorting, more emphasis has been placed on endocytosis as signaling pathway may require active endocytic machinery for proper signaling, and different signaling pathways may originate from different subcellular compartments[38]. Specifically, endocytic trafficking may be effecting the receptor and its signaling by two methods. It could control the magnitude of the signaling (quantitative) and/ or it could regulate the specificity of the signaling (qualitative). For instance, an altered endocytic trafficking leading to the decreased lysosomal targeting of receptors would simply increase a pool of receptors available to interact with their substrates. It's also known that endosomal receptors are capable of triggering different signaling cascades as surface-localized receptors. In other words, EGFR preferentially interacts with specific molecules depending on its cellular localization. For example, in NR6 fibroblasts transfected with EGFR, internalized EGFR were deficient in stimulating PLCγ1 function as measured by level of PIP_2 hydrolysis[39]. Studies using dynamin mutant to inhibit internalization of EGFR have shown that endocytic trafficking is required for full phosphorylation of EGFR, PI3K and Erk[40]. Also, proliferative responses to EGF were severely impaired in calcium-modulating cyclo-

philin ligand (CAML) deficient cells, where recycling of internalized receptors to the plasma membrane was defective[41].

EGFR kinase domain mutants identified in NSCLC induce anchorage-independent cell growth and constitutive phosphorylation of EGFR as well as of major downstream targets STAT3, Akt and Erk[42,43]. It has been demonstrate that interaction and cooperativity between mutant EGFRs and Src play a critical role in constitutive engagement of the downstream signaling pathways that allow NSCLC-associated mutant EGFRs to mediate oncogenesis[42], and proposed that the altered endocytic trafficking of mutant EGFRs via recycling pathway provides a way in which mutant EGFR may engage in prolonged signaling and preferential interaction with Src[44]. However, it is not clear how mutant receptors become more engaged with Src or the reasons mutant receptors undertake the recycling pathway.

Endocytic regulation of EGFR signaling becomes especially important in terms of cell migration. In border cells of drosophila, the subcellular localization of EGFR signaling is actively maintained. In the absence of proteins involved in EGFR endocytosis (Cbl and Rab5 GEF), localization fails and migration is disturbed[45]. Thus, events of receptor endocytosis are necessary for localized RTK signaling, preserving spatial information inherent in ligand gradients and thereby allowing RTKs to be used for guidance[45]. Also, factors such as a Src substrate p120-catenin regulates actin dynamics and cadherin abundance and activity, indicating that it may play a critical role in motility of cells[46]. In this context, enhanced adhesion to the extracellular matrix due to the EMT transition may allow engagement of integrin-induced activation of EGFR through Src. Thus, regulating cellular localizations of receptors may contribute to the specificity of the response, and there are a growing number of evidences associating receptor signals with endocytic trafficking.

Signaling molecules downstream of EGFR may also be involved in the regulation of the receptor trafficking. For instance, Ras seems to directly regulate the Rab5 nucleotide exchange activity of Rin1, thereby possibly activating endocytosis[47]. Thus, Ras activation by EGFR may also provide a mechanism by which EGFR may be initiating its own internalization, although a study using dominant negative Ras showed no inhibition of EGF endocytosis in HeLa cells[27]. Likewise, RhoB activation through the Vav2 exchange factor by EGFR slows the trafficking of internalized receptor to the lysosome[48]. Furthermore, EGFR phosphorylation site on Cbl (Y731) becomes a docking site for p85 subunit of PI3-K, providing a mechanism for the endocytic machinery to activate signaling[49]. In fact, PI3-K can itself control membrane trafficking and endosome recycling via modulating local levels of phosphoinositidies[49].

Likewise, other signaling molecules that mediate the activity of EGFR are also found to modulate the trafficking pattern. PKC diverts internalized EGFR molecules from the degradative fate to a recycling pathway. In addition, Src phosphorylates clathrin in addition to EGFR, and the overexpression of Src accelerates clathrin mediated internalization of EGFR[50]. Similarly, Src-mediated tyrosine phosphorylation is required for the function of dynamin in ligand-induced EGFR internalization[51]. Most importantly, Src is found to antagonize the function of Cbl by mediating phosphorylation and degradation of Cbl[52].

CONCLUSION

Their association with cancer, mechanism of signaling pathways, and potential defects in regulatory processes are some of the essential questions one needs to address in order to treat patients with EGFR amplification and/or mutation. By better understanding which are the critical partners and processes in EGFR-mediated biology, more effective treatment methods can be designed and targeted.

REFERENCES

1. Gschwind, A., Fischer, O.M., and Ullrich, A. (2004). The discovery of receptor tyrosine kinases: targets for cancer therapy. *Nat Rev Cancer 4,* 361-370.
2. Wiley, H.S. (2003). Trafficking of the ErbB receptors and its influence on signaling. *Exp Cell Res 284,* 78-88.
3. Miettinen, P.J., Berger, J.E., Meneses, J., Phung, Y., Pedersen, R.A., Werb, Z., and Derynck, R. (1995). Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. *Nature 376,* 337-341.
4. Threadgill, D.W., Dlugosz, A.A., Hansen, L.A., Tennenbaum, T., Lichti, U., Yee, D., LaMantia, C., Mourton, T., Herrup, K., Harris, R.C., et al. (1995). Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. *Science 269,* 230-234.
5. Yarden, Y., and Sliwkowski, M.X. (2001). Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol 2,* 127-137.
6. Herbst, R.S. (2004). Review of epidermal growth factor receptor biology. *Int J Radiat Oncol Biol Phys 59,* 21-26.
7. Di Marco, E., Pierce, J.H., Fleming, T.P., Kraus, M.H., Molloy, C.J., Aaronson, S.A., and Di Fiore, P.P. (1989). Autocrine interaction between TGF alpha and the EGF-receptor: quantitative requirements for induction of the malignant phenotype. *Oncogene 4,* 831-838.
8. Sturla, L.M., Amorino, G., Alexander, M.S., Mikkelsen, R.B., Valerie, K., and Schmidt-Ullrichr, R.K. (2005). Requirement of Tyr-992 and Tyr-1173 in phosphorylation of the epidermal growth factor receptor by ionizing radiation and modulation by SHP2. *J Biol Chem 280,* 14597-14604.
9. Nicholson, R.I., Gee, J.M., and Harper, M.E. (2001). EGFR and cancer prognosis. *Eur J Cancer 37* Suppl 4, S9-15.
10. Hynes, N.E., and MacDonald, G. (2009). ErbB receptors and signaling pathways in cancer. *Curr Opin Cell Biol 21,* 177-184.
11. Sattler, M., Abidoye, O., and Salgia, R. (2008). EGFR-targeted therapeutics: focus on SCCHN and NSCLC. *Scientific World Journal 8,* 909-919.
12. Schulze, W.X., Deng, L., and Mann, M. (2005). Phosphotyrosine inter-

actome of the ErbB-receptor kinase family. *Mol Syst Biol 1,* 2005 0008.
13. Schlessinger, J. (2000). Cell signaling by receptor tyrosine kinases. *Cell 103,* 211-225.
14. Mattoon, D.R., Lamothe, B., Lax, I., and Schlessinger, J. (2004). The docking protein Gab1 is the primary mediator of EGF-stimulated activation of the PI-3K/Akt cell survival pathway. *BMC Biol 2,* 24.
15. Haugh, J.M., Huang, A.C., Wiley, H.S., Wells, A., and Lauffenburger, D.A. (1999). Internalized epidermal growth factor receptors participate in the activation of p21(ras) in fibroblasts. *J Biol Chem 274,* 34350-34360.
16. Marmor, M.D., and Yarden, Y. (2004). Role of protein ubiquitylation in regulating endocytosis of receptor tyrosine kinases. *Oncogene 23,* 2057-2070.
17. Conner, S.D., and Schmid, S.L. (2003). Regulated portals of entry into the cell. *Nature 422,* 37-44.
18. Katzmann, D.J., Odorizzi, G., and Emr, S.D. (2002). Receptor downregulation and multivesicular-body sorting. *Nat Rev Mol Cell Biol 3,* 893-905.
19. Maxfield, F.R., and McGraw, T.E. (2004). Endocytic recycling. *Nat Rev Mol Cell Biol 5,* 121-132.
20. Worby, C.A., and Dixon, J.E. (2002). Sorting out the cellular functions of sorting nexins. *Nat Rev Mol Cell Biol 3,* 919-931.
21. de Melker, A.A., van der Horst, G., Calafat, J., Jansen, H., and Borst, J. (2001). c-Cbl ubiquitinates the EGF receptor at the plasma membrane and remains receptor associated throughout the endocytic route. *J Cell Sci 114,* 2167-2178.
22. Duan, L., Miura, Y., Dimri, M., Majumder, B., Dodge, I.L., Reddi, A.L., Ghosh, A., Fernandes, N., Zhou, P., Mullane-Robinson, K., et al. (2003). Cbl-mediated ubiquitinylation is required for lysosomal sorting of epidermal growth factor receptor but is dispensable for endocytosis. *J Biol Chem 278,* 28950-28960.
23. Haglund, K., Sigismund, S., Polo, S., Szymkiewicz, I., Di Fiore, P.P., and Dikic, I. (2003). Multiple monoubiquitination of RTKs is sufficient for their endocytosis and degradation. *Nat Cell Biol 5,* 461-466.
24. Huang, F., Khvorova, A., Marshall, W., and Sorkin, A. (2004). Analysis of clathrin-mediated endocytosis of epidermal growth factor receptor by RNA interference. *J Biol Chem 279,* 16657-16661.
25. Kowanetz, K., Crosetto, N., Haglund, K., Schmidt, M.H., Heldin, C.H., and Dikic, I. (2004). Suppressors of T-cell receptor signaling Sts-1 and Sts-2 bind to Cbl and inhibit endocytosis of receptor tyrosine kinases. *J Biol Chem 279,* 32786-32795.
26. Lynch, D.K., Winata, S.C., Lyons, R.J., Hughes, W.E., Lehrbach, G.M., Wasinger, V., Corthals, G., Cordwell, S., and Daly, R.J. (2003). A Cortactin-CD2-associated protein (CD2AP) complex provides a novel link between epidermal growth factor receptor endocytosis and the actin cytoskeleton. *J Biol Chem 278,* 21805-21813.
27. Stang, E., Blystad, F.D., Kazazic, M., Bertelsen, V., Brodahl, T., Raiborg, C., Stenmark, H., and Madshus, I.H. (2004). Cbl-dependent ubiquitination is required for progression of EGF receptors into clathrin-coated pits. *Mol Biol Cell 15,* 3591-3604.
28. Feng, Q., Baird, D., Peng, X., Wang, J., Ly, T., Guan, J.L., and Cerione, R.A. (2006). Cool-1 functions as an essential regulatory node for EGF receptor- and Src-mediated cell growth. *Nat Cell Biol 8,* 945-956.
29. Katz, M., Amit, I., Citri, A., Shay, T., Carvalho, S., Lavi, S., Milanezi, F., Lyass, L., Amariglio, N., Jacob-Hirsch, J., et al. (2007). A reciprocal tensin-3-cten switch mediates EGF-driven mammary cell migration. *Nat Cell Biol 9,* 961-969.
30. Lu, Q., Hope, L.W., Brasch, M., Reinhard, C., and Cohen, S.N. (2003). TSG101 interaction with HRS mediates endosomal trafficking and receptor down-regulation. *Proc Natl Acad Sci U S A 100,* 7626-7631.
31. Mizuno, E., Kawahata, K., Kato, M., Kitamura, N., and Komada, M. (2003). STAM proteins bind ubiquitinated proteins on the early endosome via the VHS domain and ubiquitin-interacting motif. *Mol Biol Cell 14,* 3675-3689.
32. Sigismund, S., Woelk, T., Puri, C., Maspero, E., Tacchetti, C., Transidico, P., Di Fiore, P.P., and Polo, S. (2005). Clathrin-independent endocytosis of ubiquitinated cargos. *Proc Natl Acad Sci U S A 102,* 2760-2765.
33. Chen, H., and De Camilli, P. (2005). The association of epsin with ubiquitinated cargo along the endocytic pathway is negatively regulated by its interaction with clathrin. *Proc Natl Acad Sci U S A 102,* 2766-2771.
34. Sigismund, S., Argenzio, E., Tosoni, D., Cavallaro, E., Polo, S., and Di Fiore, P.P. (2008). Clathrin-mediated internalization is essential for sustained EGFR signaling but dispensable for degradation. *Dev Cell 15,* 209-219.
35. Soubeyran, P., Kowanetz, K., Szymkiewicz, I., Langdon, W.Y., and Dikic, I. (2002). Cbl-CIN85-endophilin complex mediates ligand-induced downregulation of EGF receptors. *Nature 416,* 183-187.
36. Huang, F., and Sorkin, A. (2005). Growth factor receptor binding protein 2-mediated recruitment of the RING domain of Cbl to the epidermal growth factor receptor is essential and sufficient to support receptor endocytosis. *Mol Biol Cell 16,* 1268-1281.
37. Pennock, S., and Wang, Z. (2008). A tale of two Cbls: interplay of c-Cbl and Cbl-b in epidermal growth factor receptor downregulation. *Mol Cell Biol 28,* 3020-3037.
38. Sadowski, L., Pilecka, I., and Miaczynska, M. (2009). Signaling from endosomes: location makes a difference. *Exp Cell Res 315,* 1601-1609.
39. Haugh, J.M., Schooler, K., Wells, A., Wiley, H.S., and Lauffenburger, D.A. (1999). Effect of epidermal growth factor receptor internalization on regulation of the phospholipase C-gamma1 signaling pathway. *J Biol Chem 274,* 8958-8965.
40. Vieira, A.V., Lamaze, C., and Schmid, S.L. (1996). Control of EGF receptor signaling by clathrin-mediated endocytosis. *Science 274,* 2086-2089.
41. Tran, D.D., Russell, H.R., Sutor, S.L., van Deursen, J., and Bram, R.J. (2003). CAML is required for efficient EGF receptor recycling. *Dev Cell 5,* 245-256.
42. Chung, B.M., Dimri, M., George, M., Reddi, A.L., Chen, G., Band, V., and Band, H. (2009). The role of cooperativity with Src in oncogenic transformation mediated by non-small cell lung cancer-associated EGF receptor mutants. *Oncogene 28,* 1821-1832.
43. Sordella, R., Bell, D.W., Haber, D.A., and Settleman, J. (2004). Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science 305,* 1163-1167.
44. Chung, B.M., Raja, S.M., Clubb, R.J., Tu, C., George, M., Band, V., and Band, H. (2009). Aberrant trafficking of NSCLC-associated EGFR mutants through the endocytic recycling pathway promotes interaction with Src. *BMC Cell Biol 10,* 84.
45. Jekely, G., Sung, H.H., Luque, C.M., and Rorth, P. (2005). Regulators of endocytosis maintain localized receptor tyrosine kinase signaling in guided migration. *Dev Cell 9,* 197-207.

46. Reynolds, A.B., and Roczniak-Ferguson, A. (2004). Emerging roles for p120-catenin in cell adhesion and cancer. *Oncogene 23,* 7947-7956.
47. Clague, M.J., and Urbe, S. (2001). The interface of receptor trafficking and signalling. *J Cell Sci 114,* 3075-3081.
48. Wherlock, M., Gampel, A., Futter, C., and Mellor, H. (2004). Farnesyltransferase inhibitors disrupt EGF receptor traffic through modulation of the RhoB GTPase. *J Cell Sci 117,* 3221-3231.
49. Kassenbrock, C.K., Hunter, S., Garl, P., Johnson, G.L., and Anderson, S.M. (2002). Inhibition of Src family kinases blocks epidermal growth factor (EGF)-induced activation of Akt, phosphorylation of c-Cbl, and ubiquitination of the EGF receptor. *J Biol Chem 277,* 24967-24975.
50. Wilde, A., Beattie, E.C., Lem, L., Riethof, D.A., Liu, S.H., Mobley, W.C., Soriano, P., and Brodsky, F.M. (1999). EGF receptor signaling stimulates SRC kinase phosphorylation of clathrin, influencing clathrin redistribution and EGF uptake. *Cell 96,* 677-687.
51. Ahn, S., Kim, J., Lucaveche, C.L., Reedy, M.C., Luttrell, L.M., Lefkowitz, R.J., and Daaka, Y. (2002). Src-dependent tyrosine phosphorylation regulates dynamin self-assembly and ligand-induced endocytosis of the epidermal growth factor receptor. *J Biol Chem 277,* 26642-26651.
52. Yokouchi, M., Kondo, T., Sanjay, A., Houghton, A., Yoshimura, A., Komiya, S., Zhang, H., and Baron, R. (2001). Src-catalyzed phosphorylation of c-Cbl leads to the interdependent ubiquitination of both proteins. *J Biol Chem 276,* 35185-35193.