Cortactin Overexpression Inhibits Ligand-Induced Down-regulation of the Epidermal Growth Factor Receptor

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Abstract

Ligand-induced receptor down-regulation by endocytosis is a critical process regulating the intensity and duration of receptor tyrosine kinase signaling. Ubiquitylation of specific receptor tyrosine kinases, for example, the epidermal growth factor receptor (EGFR) by the E3 ubiquitin ligase c-Cbl, provides a sorting signal for lysosomal degradation and leads to termination of receptor signaling. Cortactin, which couples the endocytic machinery to dynamic actin networks, is encoded by EMS1, a gene commonly amplified in breast and head and neck cancers. One mechanism whereby cortactin overexpression contributes to tumor progression is by enhancing tumor cell invasion and metastasis. However, in this study, we show that overexpression of cortactin in HeLa cells markedly inhibits ligand-induced down-regulation of the EGFR. This is independent of alterations in receptor auto-phosphorylation and correlates with impaired c-Cbl phosphorylation and association with the EGFR, reduced EGFR ubiquitylation, and sustained EGFR-induced extracellular signal-regulated kinase activation. Furthermore, analysis of a panel of head and neck squamous cell carcinoma (HNSCC) cell lines revealed that cortactin overexpression is associated with attenuated ligand-induced EGFR down-regulation. Importantly, RNAi-mediated reduction of cortactin expression in an 11q13-amplified HNSCC cell line accelerates EGFR degradation. This represents the first demonstration of modulation of growth factor receptor signaling by cortactin. Moreover, enhanced EGFR signaling due to cortactin overexpression may provide an alternative explanation for EMS1 gene amplification in human cancers. (Cancer Res 2005; 65(8): 3273–80)

Introduction

Control of growth factor receptor signaling is fundamental to a wide variety of cellular processes including cell proliferation, differentiation, motility, and survival. Therefore, growth factor receptors are subject to tight regulation in order to induce signaling of an appropriate magnitude for the task required. Inappropriate activation and aberrant signaling by particular growth factor receptors has been linked with a number of human diseases including cancer (1–3). Various oncogenic versions of the EGFR including the v-erbB protein of the avian erythroblastosis virus and the EGFR mutant EGFRv, harbor deletions spanning the c-Cbl docking site (1). Furthermore, a point mutation in the direct c-Cbl binding site of the EGFR impairs down-regulation of the receptor and elicits enhanced mitogenic signaling (12). Also, the most oncogenic member of the EGFR family, ErbB2/HER2, is poorly coupled to c-Cbl-mediated degradation (17). The EGFR can also escape c-Cbl-mediated down-regulation by its heterodimerization with ErbB2, which impairs its normal recruitment of c-Cbl and results in both receptor recycling to the cell surface and enhanced, sustained signaling (18–20).

Cortactin is a multidomain protein consisting of an NH₂-terminal acidic region, which binds the actin related protein (Arp)2/3 complex, a repeat region, which associates with filamentous actin, and a COOH-terminal src homology (SH)3 domain, which recruits a variety of cellular proteins (21). Cortactin can stimulate the actin-nucleation activity of the Arp2/3 complex alone or in combination with N-WASP (22, 23). In addition, cortactin also inhibits debranching and disassembly of dendritic actin networks (24). One cellular role for cortactin is...
to bridge the endocytic machinery with components and regulators of the actin cytoskeleton. For example, cortactin binds the mecha
chemo
, 2 which regulates the fission of endocytic vesicles. This interaction has recently been shown to participate in receptor-mediated endocytosis (25–27). Furthermore, a close relative of CIN85, CD2AP, binds to cortactin in an endocytic complex containing c-Cbl, the EGFR, and endophilin (16). Finally, cortactin and the Arp2/3 complex have been shown to colocalize and associate with endosomal vesicles, suggesting a role for the actin nucleation activity of cortactin in vesicle trafficking (28).

Importantly, cortactin is implicated in the progression of certain human cancers. The gene encoding cortactin, EMSI, localizes to chromosomal locus 11q13, a region commonly amplified in breast cancers and head and neck squamous cell carcinoma (HNSCC; refs. 29, 30). Since cortactin overexpression increases the motility of fibroblasts and endothelial cells (31, 32) and enhances bone metastasis of MDAMB-231 breast cancer cells in a nude mouse model (33), the explanation to date for EMSI amplification in human cancers has been that cortactin overexpression promotes tumor cell invasion or metastasis.

The overexpression of cortactin in specific cancers, coupled with its involvement in endocytic events and vesicle trafficking, led us to investigate the effects of enhanced cortactin expression on EGFR endocytosis. Unexpectedly, cortactin overexpression inhibited ligand-induced down-regulation of the EGFR, correlating with a decrease in c-Cbl phosphorylation and EGFR ubiquitylation and sustained downstream signaling. These findings may provide a novel explanation for EMSI amplification, and hence cortactin overexpression, in malignancies in which the EGFR plays an important role, such as breast and head and neck cancers.

Materials and Methods

Tissue culture and transient transfections. HeLa cells were maintained in DMEM (Invitrogen Corp., Carlsbad, CA) supplemented with 10% FCS (ThermoTrace Ltd., Melbourne, Victoria, Australia) and 2 mmol/L L-glutamine (Invitrogen). The human squamous cell carcinoma cell lines FaDu, Detroit 562, SCC9, SCC15, and SCC25 were maintained as previously described (34). Cells were transfected using Polyfect (Qiagen, Pty. Ltd., Clifton Hill, Victoria, Australia) following the manufacturers protocol.

Plasmids. The pliCMV-cortactin construct and hemagglutinin (HA)-tagged ubiquitin-encoding plasmid were as described previously (35, 36).

Suppression of cortactin expression by RNAi. Nineteen-nucleotide RNAs were chemically synthesized (Ambion, Inc., Austin, TX) based on the sequence 5′-GACGUGUUUGGACCACUUUU-3′ (27) or 5′-AGCGU
AGGGAGAGUGUUCCU-3′ and 5′-AGACUGAGAGCAUGCCCUTCC-3′ (37). Small interfering RNA (siRNA) against lamin A/C was used as a control. For annealing of siRNA, 20 μL of each single-stranded RNA (50 μmol/L) was incubated with 10 μL 5× annealing buffer [100 mmol/L potassium acetate, 2 mmol/L magnesium acetate, 30 mmol/L HEPES (pH 7.4)] for 1 minute at 90°C and then 1 hour at 37°C. The RNA duplexes were then stored at −20°C until use.

One day before transfection, Detroit 562 cells were plated at 1.4 × 10^5 cells per well in six-well plates. Each well contained 2 mL of medium. For transfection of the cells in one well, 3 μL of siRNA duplex (20 μmol/L) was dialyzed into 200 μL OptiMem (Invitrogen) in tube 1. In tube 2, 12 μL OligoFAMEM (Invitrogen) was added to 48 μL OptiMem. Tubes 1 and 2 were incubated for 7 to 10 minutes at room temperature before combining the solutions. Following incubation for 20 to 25 minutes at room temperature, 152 μL of OptiMem was added to the mixture. This was then added to the cells, which were incubated with the siRNA for 2 to 4 days prior to starvation and EGF stimulation.

Results

Cortactin overexpression inhibits ligand-induced down-regulation of the epidermal growth factor receptor. Inhibition of cortactin function via expression of the cortactin SH3 domain or microinjection of blocking antibodies reduces receptor-mediated endocytosis (26). However, the effect of cortactin overexpression, which occurs in certain human cancers, on endocytic receptor down-regulation has not been investigated. In order to address this, HeLa cells were transiently transfected with a cortactin expression construct. The cells were subsequently serum-starved and then stimulated with EGF over a 120-minute time course. EGFR down-regulation was determined by Western blotting total cell lysates and quantitative analysis by densitometry provided a profile of ligand-induced receptor down-regulation over time (Fig. 1A and B, respectively). In control (vector-transfected) cells, a decrease in total EGFR levels was detectable after 30 minutes of EGF treatment.
Receptor levels continued to decrease between 30 and 90 minutes, and by 120 minutes, the EGFR was almost undetectable. However, in cortactin-overexpressing cells, the EGF-induced down-regulation of the receptor was both delayed and impaired. Minimal down-regulation occurred in the first 60 minutes of the time course, and although receptor levels decreased between 60 and 90 minutes, significant EGFR expression persisted at 120 minutes.

Cortactin increases recycling of ligand-activated epidermal growth factor receptors. Cortactin overexpression may perturb EGFR down-regulation by decreasing the internalization rate of ligand-occupied receptors, or by impairing post-internalization sorting to the lysosome, resulting in increased recycling back to the cell surface. Measurement of EGFR internalization rates did not reveal a significant difference between control cells and those overexpressing cortactin (Fig. 2A). However, following an initial phase of EGFR internalization, the reappearance of EGFRs at the cell surface was observed between 60 and 120 minutes in cortactin-overexpressing cells, presumably due to the recycling of endocytosed receptors (Fig. 2B). Therefore, in cortactin-overexpressing cells, a pool of internalized receptors persists which is ultimately recycled rather than degraded, an effect likely to contribute to the impaired receptor degradation at 120 minutes evident in Fig. 1B.

Overexpression of cortactin does not alter ligand-induced tyrosine phosphorylation of the epidermal growth factor receptor. In order to investigate how cortactin overexpression affects EGFR signaling, we first analyzed receptor autophosphorylation, which is required for the recruitment of signaling proteins including those regulating receptor down-regulation (12, 13). Cell lysates were subjected to immunoprecipitation with an anti-EGFR antibody and the degree of receptor tyrosine phosphorylation assessed by immunoblotting with an anti-phosphotyrosine antibody. No significant difference in total EGFR tyrosine phosphorylation was detected between control and cortactin-overexpressing HeLa cells (Fig. 3A). Since the specific EGFR tyrosine phosphorylation sites involved in c-Cbl recruitment are tyrosine (Y) 1045, the binding site for the c-Cbl SH2 domain (13), and Y1068, which recruits c-Cbl indirectly via Grb2 (12), we investigated the status of these sites using specific phosphorylation state–specific antibodies. Ligand-induced phosphorylation of both Y1045 and Y1068 was equivalent in control and cortactin-overexpressing cells (data not shown). Also, there was no alteration in c-Cbl/Grb2 association in cortactin-overexpressing cells versus control cells (data not shown). These data indicate that the mechanism responsible for the impaired EGFR down-regulation lies downstream from receptor tyrosine phosphorylation.

Overexpression of cortactin impairs coupling of the epidermal growth factor receptor to c-Cbl and epidermal growth factor receptor ubiquitylation. The known role of c-Cbl in both EGFR internalization and down-regulation (3, 4), led us to examine whether this regulatory pathway was altered in cortactin-overexpressing cells. Western blotting of EGFR immunoprecipitates

![Figure 1. Overexpression of cortactin inhibits ligand-induced EGFR down-regulation. A, HeLa cells were transiently transfected with a cortactin expression vector (C) or with vector alone (V) and then stimulated with EGF for the indicated time periods. Cell lysates were prepared and Western blotted for the EGFR and cortactin as indicated. A CD2AP blot was also done as a loading control; B, a profile of EGFR levels over time was obtained by densitometric analysis and normalization for loading. Results are expressed as a percentage of EGFR levels in cortactin-overexpressing cells at 0 minutes; points, mean; bars, SE.](image1)

![Figure 2. The effect of cortactin overexpression on EGFR internalization and recycling. HeLa cells were transiently transfected with a cortactin expression vector or with vector alone. Following transfection, cells were subjected to internalization (A) or recycling (B) assays as described in Materials and Methods. A, the internalization rates are plotted to show the ratio of internal to cell surface receptors over time. B, the data are presented as (cpm bound at T_x / cpm bound at T_0) × 100; points, mean; bars, SD.](image2)
revealed that cortactin overexpression markedly reduced EGF-induced association of c-Cbl with the EGFR (Fig. 3B). Furthermore, Western blotting of c-Cbl immunoprecipitates with an anti-phosphotyrosine antibody revealed that EGF-induced c-Cbl tyrosine phosphorylation was also attenuated in cortactin-overexpressing cells (Fig. 3C), an effect which persisted until 120 minutes (data not shown).

An important function of c-Cbl in receptor down-regulation is to ubiquitylate the EGFR (40). Tyrosine phosphorylation is required for the ubiquitin ligase activity of c-Cbl (13), resulting in the continuous ubiquitylation of the receptor and its subsequent targeting to lysosomal degradation (5, 6, 14, 15). Since both c-Cbl tyrosine phosphorylation and the association of c-Cbl with the EGFR was reduced in cortactin-overexpressing cells, we investigated the effect of these alterations on EGF-induced EGFR ubiquitylation. A plasmid encoding HA-tagged ubiquitin was transfected into HeLa cells in combination with either vector alone or a cortactin-encoding plasmid. Following EGF simulation, the EGFR was immunoprecipitated and analyzed for the extent of ubiquitylation by immunoblotting for the HA tag. Receptor ubiquitylation was undetectable in unstimulated cells (Fig. 4). Upon EGF treatment, EGFR ubiquitylation in control cells could be readily detected and peaked at 5 minutes of stimulation. However, overexpression of cortactin caused a substantial decrease in the level of EGFR ubiquitylation, such that this modification was undetectable at the 15-minute time point. Since sustained c-Cbl mediated ubiquitylation of the EGFR is necessary for efficient endosomal sorting and lysosomal degradation (5, 15), this provides an explanation for the impaired EGF down-regulation in cortactin overexpressing cells (Fig. 1).

Impaired epidermal growth factor receptor down-regulation upon overexpression of cortactin correlates with sustained Erk signaling. The Ras/Erk cascade is a key intracellular signaling pathway linking activation of growth factor receptors to cytoplasmic and nuclear effectors. Therefore, we investigated whether the inhibition of EGF down-regulation by cortactin overexpression altered this downstream signaling pathway. Erk activation over a 30-minute time course was assayed by Western blotting with a phosphospecific antibody against active Erk1/2 (Fig. 5A and B). In cortactin-overexpressing and control cells, a
similar increase in Erk activation relative to unstimulated cells was evident after 2 minutes of stimulation. However, in cortactin-overexpressing cells, Erk activity then decayed more slowly. Reflecting this, there was a trend for Erk phosphorylation in these cells to be higher at 5 and 15 minutes, although the difference in Erk activation did not reach statistical significance until 30 minutes post-stimulation. To examine this further, HeLa cells were subjected to a more prolonged time course of EGF stimulation (Fig. 5C and D). This confirmed that cortactin overexpression leads to sustained Erk signaling, since significantly enhanced Erk activation was detected in cortactin-overexpressing cells between 30 and 120 minutes after EGF stimulation.

Together, these data show that increased expression of cortactin inhibits the coupling of c-Cbl to the EGFR and thereby EGFR ubiquitylation, leading to a decrease in ligand-induced down-regulation of the receptor and sustained EGF-induced Erk activation. Similar results were obtained in COS cells (data not shown).

Figure 5. Cortactin overexpression results in sustained Erk activation in response to EGF stimulation. A, HeLa cells were transiently transfected with a cortactin expression vector (C) or with vector alone (V) and stimulated with EGF as previously described. Cell lysates were Western blotted for the activated forms of Erks 1 and 2 using a phosphorylation state-specific antibody (top). Levels of Erk and cortactin in the same lysates were determined using anti-Erk and anti-cortactin antibodies (middle and bottom, respectively); B, phospho-Erk1/2 signals were quantified by densitometry, normalized for protein loading, and represented as a percentage of maximal Erk activity (with Erk activation in cortactin-overexpressing cells at 2 minutes of stimulation treated as 100%). Points, mean; bars, SE; C and D, analysis of Erk activation over a prolonged time course was analyzed as for (A) and (B); D, Erk activation in cortactin-overexpressing cells at 30 minutes of stimulation is treated as 100%. Columns, mean; bars, SE; B and D, *, P < 0.02; **, P < 0.005 by unpaired Student’s t test, indicating significant differences between cortactin-overexpressing and control cells.

Figure 6. Determination of the rates of EGFR down-regulation in a panel of HNSCC cell lines. A, EGFR and cortactin expression in HNSCC cell lines. Equivalent amounts of cell lysate were Western blotted as indicated, with β-actin providing a loading control; B, EGF-induced down-regulation of the EGFR and Erk activation in a high (FaDu) and low (SCC25) cortactin-expressing cell line. Cells were serum-starved and then stimulated with EGF for the indicated times. Cell lysates were prepared and Western blotted as indicated; C, quantitative analysis of EGFR down-regulation in HNSCC cell lines. Analyses were done as in (B), and a profile of EGFR levels over time was obtained by densitometric analysis and normalization for loading. Results are expressed as a percentage of EGFR levels at 0 minutes; points, mean; bars, SE. To simplify the graph, only the upper half of error bars are shown, i.e., above the data points.
Figure 7. Cortactin overexpression contributes to attenuation of EGFR down-regulation in HNSCC cells. A, SCC9 cells were transiently transfected with a cortactin expression vector or with vector alone, and then stimulated with EGF for the indicated time period. Cell lysates were prepared and Western blotted as indicated. The data is representative of triplicate experiments. B, Detroit 562 cells were transfected with a cortactin-selective siRNA or a lamin A/C-selective siRNA as a control, and then stimulated with EGF for the indicated time periods. Cell lysates were prepared and Western blotted as indicated. The data is representative of triplicate experiments.

Cortactin overexpression attenuates ligand-induced epidermal growth factor receptor down-regulation in head and neck squamous cell carcinoma cell lines. In the previous experiments, we utilized HeLa cells as a model system. In order to investigate whether comparable effects of cortactin could be detected in cancer cells exhibiting EMSI amplification and/or overexpression of endogenous cortactin, we utilized a panel of HNSCC cell lines. This cell type was chosen since HNSCCs commonly exhibit amplification of the 11q13 locus in which the EMSI gene resides (30). The panel comprised five cell lines; FaDu and Detroit 562, which feature 11q13 amplification, and SCC9, SCC15 and SCC25, in which the 11q13 copy number is relatively normal (34, 41). Determination of cortactin expression in this panel by Western blot analysis revealed that FaDu cells express the highest levels of cortactin, approximately 6-fold higher than the lowest expressing line, SCC9 (Fig. 6A). The next highest expressers were Detroit 562 cells and SCC15 cells. Since SCC15 cells expressed comparable levels of cortactin to Detroit 562, which are 11q13-amplified, we categorized FaDu, Detroit 562, and SCC15 as cortactin-overexpressing HNSCC cell lines. SCC9 and SCC25 served as low-expressing controls.

Interestingly, analysis of ligand-induced EGFR down-regulation rates revealed that the cell lines segregated into the same two groups as previously defined by their cortactin expression status. In SCC9 and SCC25 cells, which express low amounts of cortactin, receptor down-regulation was rapid, with only 10% to 20% of the receptor remaining after 90 minutes (Fig. 6B and C). In contrast, receptor down-regulation was much slower in the cortactin-overexpressing FaDu, Detroit 562, and SCC15 cell lines. In these lines, 60% to 80% of the EGFR persisted at the end of the time course. Furthermore, Erk activation was also more sustained in cortactin-overexpressing HNSCC cells (Fig. 6B and data not shown). These data cannot be explained by differences in EGFR expression, since EGFR levels are comparable in SCC9 cells, which exhibit rapid receptor degradation, and FaDu cells, in which EGFR down-regulation is attenuated (Fig. 6A).

Although there was an inverse correlation between the levels of cortactin expression in this panel of HNSCC cell lines and the rates of ligand-induced EGFR down-regulation, it was possible that other differences between the cell lines could underlie this observation. Although heterodimerization of the EGFR with erbB2 results in enhanced recycling of the former receptor (18), this does not explain the differences between the HNSCC cell lines, as SCC9 cells express more erbB2 than Detroit 562s, and comparable levels to the FaDu line (data not shown). Rather than investigating further alternative explanations for the differential rates of EGFR degradation, we decided to directly test the role of cortactin. First, we overexpressed cortactin in SCC9 cells. This reduced EGFr-induced receptor degradation at 60 and 90 minutes of stimulation, and led to more sustained Erk activation (Fig. 7A, representative of triplicate experiments). Second, in order to determine the contribution from cortactin in 11q13-amplified cells, we suppressed cortactin expression in Detroit 562 cells by transfection of a selective siRNA (27). Although we were unable to reduce cortactin levels to those of the low-expressing lines, we achieved a level of expression midway between SCC25 and SCC15 cells (Fig. 7B). Strikingly, this accelerated ligand-induced EGFR down-regulation and attenuated Erk activation, as indicated by a significant reduction in Erk phosphorylation at the 90-minute time point (Fig. 7B, representative of triplicate experiments). Similar results were obtained with different cortactin-selective siRNAs (ref. 37 and data not shown). Therefore, although other factors probably also contribute to the contrasting receptor down-regulation profiles among HNSCC cell lines, these data clearly show that cortactin overexpression in HNSCC cells acts to attenuate EGFR down-regulation and prolong Erk activation, and identify a novel functional role for cortactin in human cancers.

Discussion

Aberrant signaling by receptor tyrosine kinases has been associated with many human cancers, and escape from c-Cbl-mediated receptor down-regulation has recently been shown to be an important event in receptor deregulation (1). In this study, we show that cortactin overexpression impairs c-Cbl phosphorylation and association with the EGFR and attenuates ligand-induced EGFR down-regulation. This results in sustained EGFR signaling in cortactin-overexpressing cells and provides a novel explanation for EMSI gene amplification in human cancers.
Tyrosine phosphorylation of c-Cbl stimulates its ubiquitin ligase activity and CIN85 binding, which are both implicated in ligand-induced receptor down-regulation (4, 7, 8). In this study, inhibition of c-Cbl tyrosine phosphorylation by cortactin overexpression had no significant effect on receptor internalization. Presumably, either sufficient CIN85 is recruited, or other endocytic pathways compensate, to allow normal ligand-induced receptor internalization to occur when cortactin is overexpressed. However, receptor ubiquitylation was markedly impaired, resulting in an increase in receptor recycling at late time points. Consistent with these observations, analyses of c-Cbl knockout fibroblasts and Chinese hamster ovary cells conditionally defective in ubiquitylation show a requirement for c-Cbl-mediated receptor ubiquitylation in late endosomal sorting and lysosomal degradation of the EGFR, but not receptor internalization (5). Also, expression of ubiquitylation-defective mutants of Cbl results in impaired ubiquitylation of receptor tyrosine kinases, reduced lysosomal targeting and enhanced receptor recycling and signaling (4, 13, 40). Therefore, impaired receptor ubiquitylation due to reduced c-Cbl activation and coupling to the EGFR represents the most likely explanation for the observed effect of cortactin overexpression on EGFR down-regulation and recycling.

The mechanism for the observed effects of cortactin overexpression on c-Cbl function are unclear. The activity of c-Cbl towards the EGFR is regulated by both protein-protein interactions and tyrosine phosphorylation. For example, Sprouty2 attenuates EGFR ubiquitylation and down-regulation by binding to the SH2 domain of c-Cbl and inhibiting its association with the EGFR (42). However, we did not observe an increase in Sprouty2 expression or association with c-Cbl in cortactin-overexpressing cells (results not shown). Also, activated Cdc42 binds to c-Cbl via p85Cool-1 (for cloned-out-of-library)/β-Pix (for Pak-interactive exchange factor) and inhibits the coupling of c-Cbl to the EGFR and thus receptor ubiquitylation (43). Fgd1, a Cdc42 guanine nucleotide exchange factor, directly interacts with and is targeted within the cell by cortactin (44). Therefore, overexpression of cortactin may activate Cdc42 via Fgd1, leading to Cdc42-β-Pix-c-Cbl complex formation and reduction of the interaction of c-Cbl with the EGFR. Finally, the nonreceptor tyrosine kinase Src phosphorylates and activates the ubiquitin ligase activity of c-Cbl, leading to ubiquitylation of Src itself and c-Cbl (45, 46). Inhibition of Src kinases using the specific inhibitor PP1 impairs c-Cbl phosphorylation and association with the EGFR leading to reduced ubiquitylation of the receptor (47). Since cortactin is a known Src substrate and also binds to this tyrosine kinase (48), it is possible that overexpression of cortactin in cells may act to sequester Src from c-Cbl and thereby inhibit c-Cbl phosphorylation and function towards the EGFR. The roles of Cdc42 and Src in the observed effects of cortactin are currently under investigation.

Based upon the receptor down-regulation, internalization, and recycling data for cortactin-overexpressing cells presented in Figs. 1 and 2, internalized EGFRs accumulate until an apparent threshold is reached at 60 minutes. At this point, the receptors begin to be either degraded, or recycled back to the surface. The degradation observed could be triggered by low-level ubiquitylation of the receptor by c-Cbl or another ubiquitin ligase, and/or other sorting mechanisms. Interestingly, a similar delay in ligand-induced EGFR degradation was recently observed in c-Cbl-deficient MEFs (5). It is likely that a persistence of the EGFR in particular endosomal compartments underlies the sustained Erk activation in cortactin-overexpressing cells, since endosomal EGFRs play an important role in stimulation of the Shc/Ras/Erk pathway (49, 50). Since we did not detect significant EGFR degradation in HeLa cells until 30 minutes of EGF stimulation (Fig. 1 and data not shown), the modest enhancement of Erk activation at 5 and 15 minutes of EGF stimulation in cortactin-overexpressing cells (Fig. 5) may reflect altered trafficking of the EGFRs among specific endosomal compartments. This is currently under investigation.

Interestingly, the EGFR is implicated in the progression of head and neck cancers and estrogen receptor–negative breast cancers (51), both cancers where EMS1 amplification has prognostic significance (29, 30). This highlights the possibility that amplification of EMS1 in primary tumors may provide a proliferative advantage via enhanced EGFR mitogenic signaling, with effects on cellular invasion and metastasis functioning later in tumor progression (52). Studies using fibroblasts, hepatocellular carcinoma or MDA-MB-231 breast cancer cells have not detected effects of cortactin overexpression on proliferative end-points (31, 33, 53).

However, these studies did not specifically examine EGF-induced responses, and in the case of MDA-MB-231 breast cancer cells, the presence of mutant K-Ras and B-Raf proteins may mask any effect of cortactin on Erk activation (54). It is also possible that cortactin overexpression requires additional events during tumor progression to exert mitogenic effects. Finally, it should be noted that potentiation of Erk signaling in cortactin-overexpressing cells may also contribute to the known role of cortactin in cell migration, since phosphorylation of cortactin by Erk has recently been shown to increase the ability of cortactin to enhance N-WASP-mediated actin polymerization (55). Increased Erk activation may also promote cell migration through effects on focal adhesion dynamics and activation of myosin light chain kinase (56). Therefore, enhanced EGF-induced Erk signaling due to cortactin overexpression could contribute to the multifaceted process of tumor progression by affecting both the proliferation and motility of cancer cells.

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