Extracellular Signal-regulated Kinase (ERK) Regulates Cortactin Ubiquitination and Degradation in Lung Epithelial Cells*

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Background: Cortactin is essential for barrier integrity.
Results: ERK inhibition attenuates cortactin phosphorylation, ubiquitination, and degradation via the β-Trcp-mediated ubiquitin-proteasome system.
Conclusion: Cortactin stability is coordinately regulated by stress kinases and the ubiquitin-proteasomal network.
Significance: Understanding cortactin stability provides new targets to regulate epithelial barrier integrity.

Cortactin, an actin-binding protein, is essential for cell growth and motility. We have shown that cortactin is regulated by reversible phosphorylation, but little is known regarding cortactin protein stability. Here, we show that lipopolysaccharide (LPS)-induced cortactin degradation is mediated by extracellular regulated signal kinase (ERK). LPS induces cortactin serine phosphorylation, ubiquitination, and degradation in mouse lung epithelia, an effect abrogated by ERK inhibition. Serine phosphorylation sites mutant, cortactin S405A/S418A, enhances its protein stability. Cortactin is polyubiquitinated and degraded within the proteasome, whereas a cortactin K79R mutant exhibited proteolytic stability during cyclohexamide (CHX) or LPS treatment. The E3 ligase subunit β-Trcp interacts with cortactin, and its overexpression reduced cortactin protein levels, an effect attenuated by ERK inhibition. Overexpression of β-Trcp was sufficient to reduce the protective effects of exogenous cortactin on epithelial cell barrier integrity, an effect not observed after expression of a cortactin K79R mutant. These results provide evidence that LPS modulation of cortactin stability is coordinately regulated by stress kinases and the ubiquitin-proteasomal network.

Cortactin was originally identified as a substrate of Src kinase, which plays a critical role in actin polymerization via interaction with the Arp2/3 complex (1, 2). Cortactin regulates cell migration (3–5) and cell-cell junctions (6–8). It accumulates in lamellipodia (5, 9), an area that represents the leading edge in migratory cells. Down-regulation of cortactin decreases cell migration and invasion (5, 10, 11), whereas overexpression or activation of cortactin has the opposite effect (10, 11). Cortactin also associates with cadherin (7, 12) and β-catenin (13), which plays a critical role in maintaining cell-cell adherens junction. Cortactin depletion increases pulmonary endothelial permeability in vitro (14, 15) and in vivo (16). Cortactin expression promotes barrier function via interacting with myosin light chain kinase in pulmonary endothelial cells (17, 18). However, the role of cortactin in epithelial barrier function is still unclear. Another major function of cortactin is to regulate receptor-mediated endocytosis. Zhu et al. showed that suppression of cortactin expression by siRNA reduced transferrin uptake (19). Cortactin regulates clathrin-coated vesicle formation via association with dynamin-2 (19). Recent studies suggest that cortactin regulates NADPH oxidase activation and reactive oxygen species formation by association with p47phox (20). Thus, cortactin exerts multifunctional roles in cellular behavior underscoring the importance in defining mechanisms for its regulation. Both tyrosine and serine phosphorylation of cortactin affect actin polymerization and cell migration (5, 21–24). Src kinase catalyzes Tyr421, Tyr466, and Tyr482 phosphorylation of cortactin; these modifications reduce F-actin cross-linking activity of cortactin (25). However, several studies have suggested that tyrosine phosphorylation of cortactin by Src kinase enhances actin assembly (26–28). Head et al. showed that tyrosine phosphorylated cortactin is localized with F-actin in lamellipodia and podosomes (28). In vascular smooth muscle cells, tyrosine phosphorylation of cortactin is involved in the stability and turnover of podosomes (29). Tyrosine phosphorylation of cortactin significantly increases its association with myosin light chain kinase in pulmonary endothelial cells (8, 18). Serine phosphorylation of cortactin is mediated by extracellular signal-regulated kinases (ERKs) (23, 24, 30) and other serine/threonine kinases such as Pak1 (31). Cortactin serine phosphorylation (at Ser405 and Ser418) by ERK promotes actin polymerization and tumor cell movement (24, 32). In addition, serine phosphorylation of cortactin binds focal adhesion kinase, leading to its activation to control the level of cell scattering (22). As phosphorylation of proteins regulates their sta-
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bility, these studies raise the possibility that stress kinases could modulate cortactin concentrations in cells.

Ubiquitination regulates protein stability and involves the sequential modification of the targeted proteins by the action of an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme, and E3 ubiquitin-protein ligase (33). Phosphorylation is a molecular signature that often leads to recruitment of the ubiquitination E3 ligase complex to a target protein (34–36). Several studies have shown that calpain 2 regulates cortactin degradation (37, 38); however, cortactin degradation through the ubiquitin proteolytic system has not been studied. Here, we show for the first time that β-Trcp, an E3 ligase component, is sufficient to mediate elimination of cortactin by the ubiquitin-proteasome system. Further, ERK-dependent serine phosphorylation of cortactin is essential for cortactin ubiquitination and degradation in response to lipopolysaccharide (LPS). Hence, these results provide evidence that cortactin protein stability is regulated by the combinatorial activities of ERK and β-Trcp as key bioeffectors controlling epithelial barrier function.

EXPERIMENTAL PROCEDURES

Cells and Reagents—Murine lung epithelial (MLE12) cells (from ATCC) were cultured with HITES medium containing 10% fetal bovine serum (FBS) and antibiotics at 37 °C in 5% CO2. V5 antibody, mammalian expression plasmid pcDNA3.1/His-V5-topo, and Escherichia coli TTop10-competent cells were from Invitrogen. β-Trcp and ubiquitin antibodies were from Cell Signaling (Danvers, MA). CHX, leupeptin, PD98059, shcortactin, shβ-Trcp, and β-actin antibody were from Sigma. MG-132 was from EMD Chemicals (Philadelphia, PA). ERK and p-ERK antibodies, immunobilized protein A/G beads, and control IgG were from Santa Cruz Biotechnology (Santa Cruz, CA). All materials in highest grades used in the experiments are commercially available.

Construction of Cortactin and β-Trcp Plasmids—The primers for human cortactin cDNA with a C-terminal myc tag were: forward primer, 5′-CACCATGTTGAGAGCTTCCAGG-CGCC-3′ and reverse primer with myc tag, 5′-CTCAGATCT-TCTTCTGAAATAAGTTTTGTTCTCTGGCCAGCTC-CACATA-3′. The primers for human β-Trcp with C-terminal V5 tag were: forward primer, 5′-CACCATGACCCTGGCAGG-GGCGT-3′ and reverse primer, 5′-TCTGAGATGTAG-GTGATG-3′. The PCR conditions were as follows: 98 °C for 1 min and 35 cycles of 98 °C for 15 s, 58 °C for 15 s, and 72 °C for 30 s. The resulting PCR products were purified from agarose gel, followed by one-step cloning into a pcDNA3.1D/V5-His vector.

Site-directed Mutagenesis—Primers for lysine 314 to arginine mutant of human cortactin were: forward primer, 5′-GGAAAGTTATGGGTGCAGAGGATCGATGGA-TAA-3′ and reverse primer, 5′-TTATCCATCGATCCCT-CTGACCCCCATACTTC-3′. Primers for lysine 79 to arginine mutant of human cortactin were: forward primer, 5′-AAGGAACCTTGAAACAGGACAGCTTC-3′ and reverse primer, 5′-GGCATGGAGCTTTG-TCTGAGTCCAGTCTTTT-3′. Template was human cortactin cDNA with C-terminal myc tag in pcDNA3.1D/V5-His vector. The PCR conditions were as follows: 95 °C for 1 min and 18 cycles of 95 °C for 50 s, 68 °C for 8 min. PCR products were digested with Dpn1 enzyme prior to transformation. Mutations were confirmed by nucleotide sequencing.

Immunoblotting and Immunoprecipitation—Cell lysates were subjected to SDS-PAGE, electrotransferred to membranes, and immunoblotted. For immunoprecipitation, equal amounts of cell lysates (1,000 μg) were incubated with 2 μg/ml specific primary antibodies overnight at 4 °C followed by the addition of 40 μl of protein A/G-agarose for 2 h at 4 °C. The immunoprecipitated complex was washed three times with 1% Triton X-100 in PBS and analyzed by Western blotting with an enhanced ECL system.

Immunostaining—MLE12 cells were plated on 35-mm MetTek glass-bottom culture dishes. After treatments as indicated, cells were fixed in 3.7% formaldehyde for 20 min, followed by permeabilization with 0.1% Triton X-100 for 2 min. Cells were incubated with a 1:200 dilution of antibodies, followed by a 1:200 dilution of fluorescence-conjugated secondary antibody sequentially for immunostaining. Immunofluorescent cell imaging was performed on a Nikon confocal microscope.

Epithelial Permeability Assay with Measuring Dextran Leak—MLE12 cells were plated at 100% confluence on permeable inserts containing 0.4-μm pores. Further, overexpression of HA-tagged ubiquitin (1–4 kDa) on the upper chamber for 20 h, fluorescence levels in the upper medium and bottom membrane were measured by a fluorescence microplate reader with excitation at 488 nm and emission at 520 nm.

Measurement of Transepithelial Resistance (TER)—MLE12 cells were grown to confluence on Transwells with 0.4-μm pores. TER was measured by EVOM-Epithelial Voltmeter according to the manufacturer’s instruction (World Precision Instruments Inc, Aston, Stevenage, UK).

Statistics—All results were subjected to statistical analysis using one-way ANOVA and, wherever appropriate, analyzed by Student-Newman-Keuls test. Data are expressed as mean ± S.D. of triplicate samples from at least three independent experiments, and values that were p < 0.05 were considered statistically significant.

RESULTS

Cortactin Degradation Is Mediated by Ubiquitin-Proteasome System—To investigate the mechanisms of cortactin degradation, we first examined the stability of cortactin in MLE12 cells. Treatment with protein synthesis inhibitor, CHX, revealed that cortactin half-life (t1/2) is ~ 8.9 h (Fig. 1, A and B). To identify which pathway is involved in cortactin degradation, MLE12 cells were treated with proteasomal (MG-132) or lysosomal (leupeptin) inhibitors prior to CHX treatment. MG-132, not leupeptin, blocked cortactin degradation. In addition, inhibition of the proteasome system by MG-132 induced the accumulation of cortactin in a time-dependent manner (Fig. 1, C and D). Further, overexpression of HA-tagged ubiquitin (1–4 μg, 24 h) induced panubiquitination of proteins in whole cell
lysates and triggered cortactin degradation (Fig. 1, E and F). These results indicate that cortactin is degraded by the ubiquitin-proteasome machinery in lung epithelia.

**LPS Induces Cortactin Degradation in Lung Epithelial Cells and Lung Tissues**—Lung epithelium is the first site for pathogen contact. We have shown that LPS potently induces cytokine release (39) and epithelial barrier disruption (40, 41). LPS treatment also induced cortactin degradation in a time- (Fig. 2, A and B) and dose- (Fig. 2, C and D) dependent fashion. Cortactin is localized in the cytoplasm and within the leading edge of migratory cells. LPS treatment (20 μg/ml H9262 g/ml, 8 h) significantly reduced fluorescence immunostaining of cortactin in both the cytoplasm and leading edge of MLE12 cells (Fig. 2E). Further, cortactin expression in lung tissues from LPS (1 and 5 mg/kg, 20 h) challenged mice was examined. Intratracheal administration of LPS, compared with control, significantly induced cortactin degradation in mouse lung tissue (Fig. 2, F and G). These results demonstrate that LPS induces cortactin degradation.

**Lys79 is a Molecular Site Regulating Cortactin Stability**—We substituted several candidate lysine (K) residues of cortactin with Arg (R). Of several mutants tested, only cortactinK79R exhibited protein stability in response to CHX treatment (Fig. 3, A and B). Further, we examined whether the cortactinK79R mutant exhibits resistance to LPS treatment. LPS (20 μg/ml, 8 h) reduced levels of a cortactin wild type and a cortactinK314R mutant, whereas cortactinK79R mutant levels remained unchanged in response to LPS treatment (Fig. 3, C and D), suggesting that Lys79 is a critical residue for regulating cortactin stability.

**LPS Induces Cortactin Serine Phosphorylation via ERK Activity**—Protein phosphorylation serves as a key molecular signal for the ubiquitination of proteins targeted by Skp-Cul-Cullin1-F box (SCF)-based E3 ligases (34–36). To investigate whether LPS-induced cortactin degradation is regulated by its phosphorylation, first we examined serine phosphorylation of cortactin in response to LPS treatment. MLE12 cells were treated with LPS for 1–4 h, serine-phosphorylated proteins were immunoprecipitated with an antibody to panphosphoserine, followed by immunoblotting with cortactin antibody. Fig. 4A shows that LPS-induced serine phosphorylation in a time-dependent manner. LPS had no effect on tyrosine phosphorylation of cortactin (data not shown). Consistent with prior studies (42), LPS induced phosphorylation of ERK in a time-dependent manner (Fig. 4A). Further, we found that the LPS treatment promoted association of cortactin with ERK (Fig. 4B). Inhibition of ERK activation by PD98059 attenuated LPS-induced serine phosphorylation of cortactin (Fig. 4C). These results indicate that LPS induces cortactin serine phosphorylation via enhancing ERK activity.
To investigate whether the serine phosphorylation regulates stability of cortactin, we first examined the role of serine phosphorylation in LPS-induced ubiquitination of cortactin. MLE12 cells were treated with LPS (20 μg/ml, 6 h) in the presence or absence of PD98059. Ubiquitination of cortactin was examined by immunoprecipitation with an anti-ubiquitin antibody, followed by immunoblotting with a cortactin antibody. Fig. 5A shows that LPS induced association of ubiquitin with cortactin, whereas PD98059 attenuated this interaction. Further, treatment with LPS induced cortactin degradation (Fig. 5B). Quantitative analysis from three independent experiments (mean ± S.D. (error bars)) is shown. *, p < 0.01 versus untreated cells.

**FIGURE 2. LPS induces cortactin degradation.** A, MLE12 cells treated with 20 μg/ml LPS for 0–16 h. Cell lysates were subjected to cortactin and β-actin immunoblotting. B, quantitative analysis from three independent experiments (mean ± S.D. (error bars)). *, p < 0.05 versus LPS 0 h. C, MLE12 cells treated with LPS (0–20 μg/ml) for 16 h. Cell lysates were subjected to cortactin and β-actin immunoblotting. D, quantitative analysis from three independent experiments (mean ± S.D.). *, p < 0.01 versus LPS 0 μg/ml. E, MLE12 cells grown on glass-bottom dishes treated with 20 μg/ml LPS for 16 h. Cells were fixed, and cortactin localization was determined by immunostaining with a cortactin antibody. Green, cortactin; red, nuclei. F, lung tissue lysates from intratracheal LPS (1 or 5 mg/kg body weight, 24 h) or PBS challenged mice subjected to cortactin and β-actin immunoblotting. G, quantitative analysis from three lung tissues/group (mean ± S.D.). *, p < 0.05 versus PBS control.

**FIGURE 3. Lys79 plays a critical role in cortactin stability.** A and C, MLE12 cells were transfected with myc-tagged cortactin wild type, cortactinK79R mutant, and a cortactinK314R mutant for 24 h, and then cells were treated with CHX (20 μg/ml, 8 h) (A) or LPS (20 μg/ml, 16 h) (C). Cell lysates were subjected to myc tag and β-actin immunoblotting. B and D, quantitative analysis from three independent experiments (mean ± S.D. (error bars)) is shown. *, p < 0.01 versus untreated cells.
we examined the effect of PD98059 on LPS-induced degradation of cortactin. PD98059 pretreatment attenuated the LPS-stimulated decrease in cortactin levels (Fig. 5, B and C) and cellular content as demonstrated by immunostaining (Fig. 5, D and E), whereas p38 MAPK inhibitor SB203580 pretreatment had no effect on cortactin level (Fig. 5, B and C). Ser405 and Ser418 are ERK-mediated phosphorylation sites on cortactin (24, 30). Furthermore, the serine phosphorylation sites mutant, cortactinS405A/S418A, were generated. CortactinS405A/S418A levels remained stable when measured in the CHX-chase assay (Fig. 5, F and G) and LPS treatment (Fig. 5, H and I). These results suggest that serine phosphorylation of cortactin by ERK promotes LPS-induced ubiquitination and degradation.

**β-Trcp Promotes Cortactin Degradation**—The SCF E3 ligase complex recognizes and binds specific substrates (43). A key subunit that recognizes substrates within the SCF complex is H9252-Trcp.
the F box protein. To investigate which F box protein targets cortactin for degradation, we overexpressed over 20 F box proteins and among them, only β-Trcp overexpression reduced cortactin expression (data not shown). In addition, overexpression of V5-tagged β-Trcp reduced cortactin expression in a dose-dependent manner (Fig. 6, A and B). To investigate the role of ERK-mediated phosphorylation of cortactin in β-Trcp-driven cortactin degradation, cells were transfected with V5-tagged β-Trcp plasmid, followed by incubation with PD98059 for additional 24 h. β-Trcp overexpression reduced immunoreactive cortactin levels, an effect antagonized by PD98059 treatment of cells (Fig. 6, C and D). Further, MLE12 cells were transfected with V5-tagged β-Trcp, and then cells were treated with PD98059 in the presence of MG-132. Cell lysates were subjected to immunoprecipitation (IP) with a cortactin antibody. The precipitates were analyzed by V5 tag and cortactin antibodies. F, MLE12 cells were transfected with V5-tagged β-Trcp, myc-tagged cortactin wild type, or cortactinS405A/S418A in the presence of MG-132. Localization of myc-tagged cortactin and V5-tagged β-Trcp was detected by immunofluorescence staining. Fluorescence intensity profiles are also shown. Green fluorescence is for cortactin-myc signal, red fluorescence is for β-Trcp-V5 signal, and blue fluorescence is for nuclei signal.
The association of overexpressed V5-tagged H9252-Trcp and cortactin was examined by co-immunoprecipitation. Fig. 6 shows that V5-tagged H9252-Trcp was bound to cortactin, whereas PD98059 attenuated the interaction. Because Ser405 and Ser418 are ERK-mediated phosphorylation sites, we investigated the interaction between V5-tagged H9252-Trcp and myc-tagged cortactinS405A/S418A in the presence of MG-132. Fig. 6 shows that V5-tagged H9252-Trcp interacted with cortactin wild type, but not with cortactinS405A/S418A variant. Furthermore, immunofluorescence staining shows that V5-tagged β-Trcp (red color) and myc-tagged cortactin wild type (green color), but not cortactinS405A/S418A (green color), co-localized in the cytoplasm of MLE12 cells (Fig. 6G). Thus, the results demonstrate that ERK activation regulates β-Trcp-mediated cortactin degradation.

Cortactin Stability Regulates Lung Epithelial Barrier Function—Cortactin is critical for maintaining endothelial barrier integrity because its depletion results in a marked loss of cell-to-cell integrity (8, 14). Here, we investigated the role of cortactin in lung epithelial integrity. Dextran leaks, TER, and E-cadherin accumulation at cell-cell contacts were determined as the index of lung epithelial barrier integrity. Down-regulation of cortactin by shcortactin increased dextran leak (Fig. 7A), as well as reduced TER (Fig. 7B). Overexpression of ubiquitin acceptor site mutant cortactinK79R rescued the effects of shcortactin (Fig. 7, A and B). The effect of shcortactin on cortactin

**FIGURE 7.** β-Trcp attenuates cortactin-mediated lung epithelial barrier integrity. A, MLE12 cells were transfected with shcortactin or shcortactin + cortactinK79R for 24 h and then were subcultured on permeable inserts containing 0.4-μm pores. After the addition of FITC-dextran (4 kDa) into the upper chamber for 20 h, leaks of dextran from upper chamber to the bottom chamber were measured by fluorescence microplate reader as indicated under "Experimental Procedures." *, p < 0.05 versus vector control; **, p < 0.01 versus shcortactin-transfected cells. B, MLE12 cells were transfected with shcortactin or shortcortactin + cortactinK79R for 24 h, and then cells were subcultured on permeable inserts containing 0.4-μm pores for an additional 24 h. TER were measured by EVOM-Epithelial Voltohmeter. *, p < 0.01 versus vector control; **, p < 0.01 versus shcortactin-transfected cells. C, cell lysates from control cells and shortcortactin-transfected cells were analyzed by Western blotting with cortactin and β-actin antibodies. Quantitative analysis from three independent experiments (mean ± S.D. (error bars)) is shown. *, p < 0.01 versus control. D, MLE12 cells were transfected with myc-tagged cortactin wild type, a cortactinK79R variant with or without V5-tagged β-Trcp for 24 h, and then cells were subcultured on permeable inserts containing 0.4-μm pores. Dextran leak was measured as described above. *, p < 0.01 versus vehicle; **, p < 0.01 versus cortactin wild type transfected cells. E, MLE12 cells grown on glass-bottom dishes were transfected with myc-tagged cortactin wild type, cortactinK79R variant with or without V5-tagged β-Trcp for 72 h, and then cells were fixed. E-cadherin localization was examined by immunofluorescence staining with an anti-E-cadherin antibody. F, MLE12 cells were transfected with shβ-Trcp and then were subculutred on permeable inserts containing 0.4-μm pores. Cells were treated with LPS (20 μg/ml, 16 h). Dextran leak was measured as described above. *, p < 0.01 versus vector control; **, p < 0.05 versus LPS-treated control cells. G, MLE12 cells were transfected with shβ-Trcp for 72 h, and then cells were fixed. E-cadherin localization was examined by immunofluorescence staining with an anti-E-cadherin antibody.
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expression was confirmed by Western blotting (Fig. 7C). Overexpression of cortactin reduced the dextran leak in MLE12 cells (Fig. 7D) and increased E-cadherin accumulation at cell-cell contacts (Fig. 7E), suggesting that the overexpression of cortactin increases lung epithelial integrity. Co-transfection of a cortactin plasmid with V5-tagged β-Trcp attenuated cortactin-mediated lung epithelial barrier enhancement (Fig. 7, D and E). Further, overexpression of a plasmid encoding cortactinK79R produced similar effects as wild type cortactin by enhancing lung epithelial integrity; importantly, co-overexpression of β-Trcp had no effect on cortactinK79R-mediated lung epithelial barrier function (Fig. 7, D and E). We have shown that LPS treatment reduces lung epithelial integrity (40, 41). Here, we showed that LPS increased dextran leak in MLE12 cells (Fig. 7F). Furthermore, down-regulation of β-Trcp by shβ-Trcp attenuated LPS-induced dextran leak (Fig. 7F) and E-cadherin mislocalization in the cytoplasm of MLE12 cells (Fig. 7G). These results suggest a regulatory role for SCFβ-Trcp in modulating cytoskeletal function by targeting cortactin for degradation.

DISCUSSION

Cortactin is a key structural element and protein kinase substrate that regulates cytoskeletal arrangement (1, 2). Cortactin activity controlled by post-translational modifications, such as phosphorylation (23, 24) and acetylation (44), has been studied extensively; however understanding of cortactin protein stability is limited. Here, we provide evidence suggesting that cortactin degradation is regulated by the β-Trcp and the ubiquitin-proteasomal system. Lys79 within cortactin is essential in regulating cortactin degradation. Serine phosphorylation of cortactin by ERK facilitated cortactin ubiquitination and degradation. Further, the results support a role for the ERK-SCFβ-Trcp, ubiquitin proteasomal pathway in modulating lung epithelial barrier integrity. These results suggest that future interventions such as use of small molecular agonists or antagonists within the ubiquitin system (e.g. SCFβ-Trcp) could perhaps be employed to regulate E3 ligase activity, thereby impacting cell motility.

Protein degradation is an important aspect of cell regulation. Two major systems (proteasome and lysosome systems) contribute to the vast majority of intracellular protein degradation (45). Cortactin binds to filamentous actin to act as a regulator of cytoskeleton dynamics (1, 2, 6), thus understanding cortactin stability is essential in regulating cell motility. Prior studies have demonstrated that calpain 2 mediates proteolysis of cortactin in fibroblasts (37) and platelets (38). Perrin et al. showed that calpain 2 cleaves cortactin between the actin-binding repeats and the α-helical domain (37). The current study for the first time attempts to demonstrate that cortactin is ubiquitinated and degraded in the proteasome system, but not in the lysosomal pathway. Actin-binding proteins are not stable under certain biological conditions. Alanine-rich kinase substrate (MARCKS) and fascin, two actin-binding proteins, are ubiquitinated, displaying a rapid turnover rate after preconditioning ischemia (46). Here, cortactin displayed extended in vivo stability consistent with half-lives of structural and regulatory proteins, but destabilized in the presence of LPS. Our mutational studies suggest that Lys79 as an acceptor site within the first actin binding repeat appears to play a critical role in regulating cortactin stability. In contrast to cortactin wild type and other Lys to Arg mutants, the cortactinK79R variant displayed significant proteolytic resistance in the CHX chase assay or in response to LPS treatment. β-Trcp, a well studied E3 ligase subunit, regulates cell functions by mediating intracellular protein degradation (47). β-Trcp targets phosphorylated IкB for its degradation (48), a protein also regulated by LPS. Our prior work demonstrates that β-Trcp also targets acyl-CoA:lysophosphatidylcholine acyltransferase 1 (Lpcat1), a phospholipid-remodeling enzyme, for disposal in lung epithelial cells (36). Hence, these observations suggest that SCFβ-Trcp appears to exert diverse effects on fundamental processes within lung epithelium.

Protein phosphorylation provides a phosphodegron molecular signal for recruiting E3 ligase-associated proteins for ubiquitination of molecular targets (34–36). For example, β-Trcp targets phosphorylated IκB (48) and phosphorylated β-catenin (49). We have shown that Lpcat1 is serine-phosphorylated by GSK3β, and subsequently the phosphoenzyme is targeted by β-Trcp for disposal (36). Serine phosphorylation of cortactin on Ser405 and Ser418 by ERK has been well demonstrated (21, 23, 24); however, its link to cortactin stability has not been investigated. ERK regulates degradation of several intracellular proteins via the ubiquitin-proteasome system, such as dual-specificity phosphatase 1 (50) and GATA3 (51). In response to LPS treatment, cortactin was serine- (not tyrosine) phosphorylated, ubiquitinated, and degraded. ERK activity plays a critical role in these reactions because inhibition of ERK activity blocked cortactin phosphorylation, ubiquitination, and subsequent degradation. Serine-phosphorylated cortactin is localized in the sites of dynamic actin assembly in tumor cells (30). It is likely that with many proline-directed kinases, ERK assemblies within a scaffolding complex with multiple effectors at a docking domain or region within sites of cortactin (52). This assembly of kinases and regulatory scaffolding molecules might then provide a phosphodegron-like signature for recruitment of SCFβ-Trcp. Serine phosphorylation sites Ser405 and Ser418 and ubiquitin acceptor site Lys79 are localized at the N terminus and the C terminus of cortactin, separately. Hence, the molecular interplay between ERK and SCFβ-Trcp within these regions of cortactin could serve as a feedback signal to control cortactin steady-state levels to dynamically regulate barrier integrity.

As the role of cortactin in controlling lung epithelial barrier function is still unknown and SCFβ-Trcp has multiple targets, our results do not exclude a more complex model where ERK and the ubiquitin apparatus jointly modulates multiple targets to disrupt the epithelial lining during LPS-mediated inflammation. One such potential target is E-cadherin, which interacts with cortactin, and might also be modulated by the SCF complex. Future work will focus on related cell adhesion proteins that influence stability of the epithelial barrier.

REFERENCES

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