CTEN Prolongs Signaling by EGFR through Reducing Its Ligand-Induced Degradation

Shiao-Ya Hong, Yi-Ping Shih, Tianhong Li, Kermit L. Caraway III, and Su Hao Lo

Abstract

Activation of EGF receptor (EGFR) triggers signaling pathways regulating various cellular events that contribute to tissue development and function. Aberrant activation of EGFR contributes to tumor progression as well as therapeutic resistance in patients with cancer. C-terminal tensin-like (CTEN; TNS4) is a focal adhesion molecule that is a member of the tensin family. Its expression is upregulated by EGF and elevated CTEN mediates EGF-induced cell migration. In the presence of CTEN, we found that EGF treatment elevated the level of EGFR protein but not mRNA. The extended half-life of activated EGFR sustained its signaling cascades. CTEN reduced ligand-induced EGFR degradation by binding to the E3 ubiquitin ligase c-Cbl and decreasing the ubiquitination of EGFR. The Src homology 2 domain of CTEN is not only required for binding to the phosphorylated tyrosine residue at codon 774 of c-Cbl, but is also essential for the tumorigenicity observed in the presence of CTEN. Public database analyses indicated that CTEN mRNA levels are elevated in breast, colon, lung, and pancreas cancers, but not correlated with EGFR mRNA levels in these cancers. In contrast, immunohistochemistry analyses of lung cancer specimens showed that CTEN and EGFR protein levels were positively associated, in support of our finding that CTEN regulates EGFR protein levels through a posttranslational mechanism. Overall, this work defines a function for CTEN in prolonging signaling from EGFR by reducing its ligand-induced degradation. Cancer Res; 73(16): 5266–76. ©2013 AACR.

Introduction

EGF receptor (EGFR) tyrosine kinase engages a vast array of signaling pathways to regulate tissue development and homeostasis (1, 2). EGFR signaling is normally induced by ligand binding (such as EGF), leading to receptor dimerization, autophosphorylation, activation of downstream signaling molecules, and cellular events such as proliferation, migration, and differentiation. Given its importance, EGFR signaling is tightly regulated. Ligand binding not only induces receptor activation but also triggers suppression mechanisms to ensure precise control of EGFR signaling output (3). One of the negative regulations is governed through ubiquitin-dependent EGFR degradation, which is largely mediated by the RING finger E3 ubiquitin ligase c-Cbl. c-Cbl binds to tyrosine-phosphorylated EGFR through its tyrosine kinase-binding (TKB) domain, allowing recruitment of ubiquitin-conjugating enzymes and transfer of ubiquitin to phosphorylated EGFR. Ubiquitination of EGFR then facilitates its internalization and degradation in lysosomes (4). Disruption of this negative regulatory system could trigger cellular events that contribute to tumor formation.

Focal adhesions connect extracellular matrix to cytoskeletal networks and play critical roles in cell adhesion, migration, proliferation, and survival. They are also involved in cross-talk with growth factor receptors, such as EGFR, to elicit a wider range of cellular responses. C-terminal tensin-like (CTEN) is the smallest protein in the tensin focal adhesion family (5) known to regulate cell adhesion and migration (6). Less amount of, if any, CTEN is expressed in normal tissues including breast, lung, ovary, and colon (7). However, its expression is profoundly increased when these tissues develop tumors. In colon cancer, 76% of patients' tumor samples exhibit significantly elevated CTEN protein levels (8), which is associated with poor prognosis (9). In invasive breast cancer, CTEN expression correlates with high EGFR and HER2 levels, as well as with metastasis to lymph nodes (6), and is associated with poor prognosis (10). Manipulation of CTEN protein levels alters cell invasion, epithelial–mesenchymal transition (EMT), and colony formation capacities of colon cancer cells (8, 11). Activation of EGFR by EGF leads to an expressional switch from tensin 3 (TNS3) to CTEN, and this upregulation of CTEN with reduced TNS3 contributes to EGF-promoted mammary cell migration (6). Mitogen-activated protein/extracellular signal–regulated kinase (MEK) activity is required for EGF-induced CTEN overexpression (6). Consistently, it is reported that gain-of-function mutations in K-RAS and B-RAF (both upstream molecules of MEK) are associated with CTEN overexpression (12). Altogether, these findings closely link CTEN to EGFR signaling cascades.

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In the present study, we have identified a novel role for CTEN in EGFR regulation. Our data indicate that CTEN suppresses ligand-induced EGFR degradation by reducing receptor ubiquitination, which is accomplished via the ability of CTEN to interact with c-Cbl in a phosphotyrosine-SH2–dependent manner. Overexpression of CTEN enhances cancer cell migration, invasion, as well as colony formation activities, which can be reversed by EGFR inhibitor. In addition, the Src homology 2 (SH2) domain of CTEN is essential for promoting these tumorigenic properties. The clinical relevance of our findings is supported by the upregulation of CTEN mRNA in a variety of cancer types and the correlation of elevated CTEN and EGFR protein levels in lung cancer.

Materials and Methods

Reagents and plasmid constructs
Antibodies against EGFR, c-Cbl, ubiquitin (EMD Millipore), ERK1/2, pY845-EGFR, pY992-EGFR, pY1045-EGFR, pY1068-EGFR, pY1173-EGFR, phospho-tyrosine (Cell Signaling Technology), Flag (Sigma-Aldrich), and phospho-ERK1/2 (Santa Cruz Biotechnology) were purchased from indicated commercial suppliers. Recombinant human EGF was purchased from Promega Corporation. Lactacystin was purchased from Sigma-Aldrich, Cetuximab from ImClone System Incorporated. Flag-tagged CTEN full-length, N-terminal, C-terminal plasmids were constructed by PCR amplification of the EGFP-CTEN (13). PCR products were then directionally cloned into EcoRI and BamHI sites in the vector, pFLAG-CMV-2 (Sigma-Aldrich). Hemagglutinin (HA)-tagged c-Cbl mutants were constructed by site-directed mutagenesis with the HA-tagged wild-type (WT) c-Cbl.

Cell culture and DNA transfection
All cell lines were purchased from American Type Culture Collection and authenticated by this established provider through cytogenetic analysis. They were cultured and used within 6 months after resuscitation in appropriate media: Dulbecco’s Modified Eagle Medium (DMEM) 4.5 g/L glucose for HEK 293T, SW480, and A549 cells; RPMI-1640 for 5637 cells; McCoy’s 5A medium for HCT116 cells supplemented with 10% FBS (Sigma-Aldrich), 1% penicillin/streptomycin, and 2 mmol/L L-glutamine. All cells were maintained at 37°C in humid air with 5% CO2 condition. For the EGF-treatment experiments, cells were starved in serum-free media for 24 hours and then replaced with media containing EGF, prepared freshly from an aqueous 100 mg/mL recombinant human EGF solution. Transfections were carried out using Lipofectamine 2000 (Invitrogen) reagent according to the manufacturer’s protocol.

RNA extraction and RT-PCR
Total cellular RNAs were extracted using TRIzol reagent (Invitrogen) according to the instructions of the user manual. cDNAs were generated from 2 μg RNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Real-time PCR (RT-PCR) analyses were conducted with SYBR Green PCR Master Mix using PCR primers on an ABI Prism H7900 Sequence Detection System (Applied Biosystems). EGFR prim-
Figure 1. CTEN attenuates EGF-induced EGFR downregulation in a transcription-independent manner. A, dose response of EGFR downregulation. Mock or Flag-CTEN–overexpressing HEK 293T cells were stimulated with indicated dosages of EGF for 1 hour. Left, representative immunoblot analyses; right, bar graphs showing the relative protein levels of EGFR. B, kinetics of EGFR protein levels. Mock or Flag-CTEN–overexpressing HEK 293T cells were treated with 10 ng/mL of EGF for indicated time. Left, immunoblot analyses with the relevant antibodies; right, quantification of the EGFR protein. The intensity of each band was quantified, and expressed as a percentage using the signal at dose 0 or time 0 as a reference. C, kinetics of pEGFR levels. The mock or transfected cells were stimulated with 10 ng/mL of EGF for indicated time. tEGFR were immunoprecipitated (IP) from equal amounts of cell lysis. D, relative EGFR mRNA expression.
Transwell. The experimental process is comparable with the Transwell migration assay described earlier, except that the membrane of the Transwell was precoated with 2 μg/well serum-reduced Matrigel (BD Biosciences), and invasion was assessed by cell counting after 48-hour incubation. For colony formation assay, cells (5 × 10^3/well) were seeded in 6-well plates and subjected to drug selection for 2 to 3 weeks, with G418 (400 μg/mL) selective medium refreshed every 3 days. The stable G418-resistant colonies were counted after staining with crystal violet, washed with water, and air-dried. For EGFR inhibition, 5 μg/mL of cetuximab was added to both upper and lower chambers in migration and invasion assay. In colony formation assay, 5 μg/mL of cetuximab was added to the selection medium to inhibit EGFR signaling.

**Microarray analysis**

The gene expression datasets were downloaded from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO). We chose four different types of cancer datasets that have balanced number of disease and control samples in each dataset. Figure 5 showed the gene expression datasets that were used in this study, including breast cancer (GSE15852), colon cancer (GSE10950), non–small cell lung cancer (GSE12236), and pancreatic ductal adenocarcinoma (GSE28735). Partek Genomics Suite (Partek software, version 6.6 Beta) was used to analyze gene expression values and to generate dot plots. Expression differences between tumor and adjacent normal tissues were assessed by one-way ANOVA. Statistical analysis was conducted using Student t test. A P value less than 0.05 was considered significant. All reported P values are two-tailed.

**Immunohistochemistry and evaluation**

Human lung cancer (8 small cell carcinomas, 13 adenocarcinomas, 18 squamous cell carcinomas, and 9 other type non–small cell carcinomas) and adjacent paired tissue microarray slides were purchased from Pantomics Inc. The formalin-fixed, paraffin-embedded tissue slides were first incubated at 65°C for 30 minutes. Then, the samples were deparaffinized and placed in a pressure cooker containing 10 mmol/L buffered sodium citrate solution (pH 6.0) for antigen retrieval. The slides were immersed in 3% hydrogen peroxide for 10 minutes to eliminate endogenous peroxidase activity. After that, the slides were blocked before being incubated overnight at 4°C with 1:50 diluted rabbit monoclonal anti-CTEN antibody (Spring Bioscience) or with mouse monoclonal anti-WT EGFR antibody (DAK-H1-WT from Dako). Detection was carried out on next day with streptavidin-biotinylated peroxidase-conjugated reagents with 3-amino-9-ethyl carbazole (AEC) as the chromogen (Vector Laboratories Inc.). The samples were observed using a Zeiss Axiosplan2 optical microscope imaging system with a real-color AxioCam high-resolution charge-coupled device camera (Carl Zeiss MicroImaging). Slides were evaluated and the staining intensity was ranked on a scale ranging from 0 to 3 (0, negative; 1, weak; 2, moderate; and 3, strong intensity staining). The score of staining more than 1 was identified as upregulated in CTEN or EGFR expression. Statistical analysis was conducted using GraphPad Prism (version 5.01, GraphPad Software Inc.). The statistical relationships between CTEN and EGFR of immunohistochemical (IHC) data were evaluated through the two-sided Fisher exact test. P values less than 0.001 were considered statistically highly significant.

**Results**

**CTEN expression modulates EGFR protein levels during EGF treatment**

It has previously been shown that EGF-induced CTEN upregulation promotes cell migration (6). To further investigate the potential role of CTEN in EGFR signaling and its regulation mechanism, recombinant CTEN was expressed in HEK 293T cells, which did not express endogenous CTEN. Then cells were treated with various concentrations of EGF for 1 hour. As shown in Fig. 1A, EGF-induced EGFR downregulation was significantly reduced in the presence of CTEN. At this 1 hour time point, maximal suppression of EGFR downregulation by CTEN was observed at 10 ng/mL EGF treatment. Consistently, the activation of downstream kinases, such as extracellular signal–regulated kinase (ERK1/2) (measured by its phosphorylation level), was markedly higher in the presence of CTEN (Fig. 1A). Time course experiments indicated that the half-life of EGFR was extended from 45 to 90 minutes in the presence of EGF when CTEN was overexpressed (Fig. 1B). Because EGFR tyrosine phosphorylation is an immediate event upon ligand binding and also represents the activated form of EGFR, the levels of tyrosine-phosphorylated EGFR were examined. As shown in Fig. 1C, total tyrosine phosphorylation levels of EGFR (pEGFR) were significantly enhanced in the presence of CTEN. The increase of pEGFR seemed to be a reflection of EGFR protein level. Once the pEGFR levels were normalized to total protein levels (tEGFR), there were no differences between cells with or without CTEN. By using phospho site-specific antibodies, higher phospho–Y845 (pY845), pY992, pY1045, pY1068, and pY1173 levels together with increased EGFR amounts were detected in CTEN transfectants than in mock cells (Fig. 1D). Altogether, these results showed that the presence of CTEN significantly diminished EGF-induced EGFR
downregulation, sustained activated receptor, and therefore prolonged EGFR signaling.

The effect of CTEN on EGFR protein and mRNA levels was also examined in colon (SW480), lung (A549), and bladder (5637) cancer cell lines that overexpress endogenous CTEN. Both basal and inducible downregulation of EGFR protein levels were significantly enhanced when CTEN expression was silenced by siRNA (Fig. 1E). Furthermore, EGFR mRNA levels were not changed after CTEN was silenced in these cell lines (Fig. 1F), indicating that the effect is independent of transcriptional control.

### CTEN reduces ligand-induced ubiquitination of EGFR and modulates c-Cbl–EGFR interaction

It is known that ubiquitination is a critical step for EGF-induced degradation of EGFR. To examine the effect of CTEN on EGFR ubiquitination, HEK 293T cells were transfected with CTEN and then treated with or without EGF. Equal amounts of EGFR were immunoprecipitated and their ubiquitination levels were measured. As shown in Fig. 2A, CTEN significantly reduced the ubiquitination of EGFR in the presence of EGF. This suggests that CTEN protects ligand-induced EGFR degradation through reducing its ubiquitination. c-Cbl is the primary E3 ubiquitin ligase that binds directly to pY1045 and indirectly to pY1068 sites of EGFR and then ubiquitinates the receptor upon ligand stimulation. Hence, the phosphorylation levels of both sites were examined to test whether CTEN might compromise c-Cbl–binding sites on EGFR. Equal amounts of EGFR were immunoprecipitated and their pY1045 and pY1068 levels were measured in a time course experiment (Fig. 2B). In agreement with our findings in Fig. 1C, no remarkable phosphorylation difference at these sites between control and CTEN transfectants was observed. In the same experiment, the interaction between c-Cbl and EGFR was analyzed. Overall, increasing amounts of c-Cbl were interacted with EGFR in control cells, whereas c-Cbl–EGFR interaction levels were reducing in CTEN transfectants during the time course (Fig. 2B). Nonetheless, significant more c-Cbl proteins were bound to EGFR in the present of CTEN before 15 minutes. After 15 minutes, more c-Cbl–EGFR interaction was detected in the control cells. These data strongly suggest that CTEN may regulate the dynamic of c-Cbl–EGFR interaction.

### CTEN interacts with the E3 ubiquitin ligase c-Cbl in a SH2-phosphotyrosine–dependent fashion

Because (i) EGF-induced EGFR ubiquitination level is compromised in the presence of CTEN, (ii) CTEN modulates the c-Cbl–EGFR interaction without altering pY1045 and pY1068...
levels, and (iii) c-Cbl protein levels are not altered by CTEN expression (Fig. 3), we investigated whether CTEN could interact with c-Cbl and therefore affect EGFR ubiquitination. To show the interaction between CTEN and c-Cbl, Flag-tagged full-length, N-terminus, or C-terminus of CTEN were transfected into HEK 293T cells (Fig. 3A). Recombinant CTEN and its
CTEN loss-of-function mutations (R474A for the SH2 domain and R650A for the PTB domain) to identify the c-Cbl–binding domain. As shown in Fig. 3C, SH2 mutant (R474A) of CTEN clearly lost the ability to interact with c-Cbl, whereas PTB mutant (R650A) sustained the binding to c-Cbl after EGF induction (Fig. 3C). These results indicate that the SH2 domain of CTEN is essential for ligand-induced binding to c-Cbl. We further confirmed the interaction between the CTEN SH2 domain and c-Cbl using a GST fusion pull-down assay. GST, GST–SH2, and GST–PTB fusion proteins were expressed in and purified from E. coli. The fusion proteins were incubated with cell lysates from EGF-treated or -untreated HEK 293T cells, and the pull-down assay was conducted as described in Materials and Methods. Indeed, CTEN GST–SH2, but not GST or GST–PTB, fusion proteins were able to pull down c-Cbl (Fig. 3D), suggesting that the SH2 domain of CTEN binds to tyrosine phosphorylated c-Cbl.

c-Cbl is a prominent substrate of EGFR tyrosine kinase and tyrosine 674 (AY700SLAR), 700 (JEY700MTPS), 731 (CTY731EAMY), and 774 (DGY774DVPK) residues are four major phosphorylation sites (15–17). To identify the potential phosphorylation-dependent binding sites on c-Cbl, we individually mutated tyrosine 674, 700, 731, and 774 to phenylalanine and examined their CTEN-binding activities. c-Cbl Y774F mutant seemed to lose binding to CTEN after ligand stimulation (Fig. 3E), indicating that tyrosine 774 of c-Cbl could be a crucial residue for EGF-induced phosphorylation-dependent interaction with CTEN. To verify the role of Y774 residue in CTEN binding, synthetic peptides containing the Y700 or Y774 sites with or without phosphorylation were used to compete with c-Cbl binding to GST–SH2 (Fig. 3F). Only phosphorylated Y774 peptide (774P) was able to block the interaction between CTEN and c-Cbl, further confirming that Y774 of c-Cbl is a specific phosphotyrosine residue for binding to SH2 domain of CTEN.

A functional SH2 domain of CTEN is essential for reducing EGF-induced EGFR ubiquitination/degradation and promoting CTEN-mediated tumorigenicity

Our results showing c-Cbl–CTEN interaction suggest that the effect of CTEN on EGF-induced EGFR downregulation may depend on CTEN SH2 domain binding to phosphorylated Y774 on c-Cbl. To test this possibility, EGFR ubiquitination in the presence of CTEN SH2 mutant (R474A) was analyzed. As shown in Fig. 2A, R474A no longer exhibits the potential to reduce the ubiquitination of activated EGFR. We further examined the effect of CTEN R474A on EGFR protein and ERK1/2 activation levels in the presence of EGF. Downregulation of EGFR in response to EGF was reduced in CTEN-transfected HEK 293T cells when compared with those of experimental groups that transfected with control vector and R474A mutant (Fig. 4A). Consistent with the diminished downregulation of EGFR, phosphorylation of the EGFR effector ERK1/2 in response to EGF was markedly increased in the presence of WT CTEN, but not R474A mutant (Fig. 4A). These results show that the effect of CTEN on EGF-induced EGFR ubiquitination, degradation, and signaling is primarily mediated through the CTEN SH2 domain.

Accumulating reports have indicated that CTEN overexpression may be correlated to various types of cancer development (8, 10, 18, 19). Our results show that the interaction between the SH2 domain of CTEN and pY774 c-Cbl is important in attenuating EGFR degradation and prolonging EGFR signaling, which might play important roles in tumor progression. Thus, we set up a series of experiments to examine the potential role of CTEN SH2 domain in tumorigenic properties. Ectopic expression of CTEN in HCT116 (low CTEN) colon cancer cells significantly promoted cell migration, invasion, and colony formation activities (Fig. 4C–E). In contrast, no enhancement effects on these activities were observed in cells transfected with CTEN SH2 mutant (R474A), showing the essential role of the SH2 domain on CTEN-mediated tumorigenicity in cancer cells. To further validate the involvement of EGFR signaling in these CTEN-mediated phenotypes, the experiments were repeated in the presence of cetuximab, an anti-EGFR antibody. Although cetuximab had no effect on HCT116 cell migration and invasion, it suppressed HCT116 colony formation activity (Fig. 4C–E). More importantly, the effects of CTEN overexpression were reversed by cetuximab, implicating a direct link between CTEN and EGFR signaling in these processes.

CTEN mRNA levels are upregulated in a variety of cancers and its protein expression is correlated with that of EGFR in human lung samples

Several lines of evidence have indicated that CTEN mRNA expression is upregulated in various cancers (8, 10, 18, 19). To extend these findings, we took advantages of four microarray datasets available from GEO and analyzed CTEN and EGFR expression profiles using the Partek Genomics Suite software. Compared with paired normal tissues, we found that CTEN mRNA expression is significantly upregulated in tumors from breast (GSE15852; ref. 20), colon (GSE10950; ref. 21), lung (GSE12236; ref. 22), and pancreas (GSE28735; Fig. 5; ref. 23). Nonetheless, EGFR mRNA levels were downregulated with statistical significance in breast cancer, but not in colon, lung, and pancreas datasets. These results show that CTEN transcript was increased, but not correlated with EGFR mRNA levels in these cancers. Because our cell culture studies suggested that CTEN expression altered EGFR protein but not mRNA levels, we analyzed the relationship between CTEN and EGFR protein levels by IHC staining in normal and lung cancer consecutive tissue samples. To rule out the possibility that truncated variants of EGFR such as EGFR-vIII may escape from ligand-induced downregulation, we limited our analysis to WT EGFR in these samples by using an anti-WT EGFR antibody (DAK–H1-
These lung samples, supporting the cell culture finding that CTEN upregulation increases EGFR protein level. WT). Figure 6A shows representative IHC staining images of CTEN and EGFR. In a total of 96 cases, CTEN and EGFR are coexpressed in 50 (52.1%) and 53 (55.2%) samples, respectively (Fig. 6B). Among them, 39 (40.6%) samples show both elevated CTEN and EGFR IHC staining, whereas 32 (33.3%) samples display no change or downregulation on CTEN and EGFR (Fig. 6B). The statistical analyses indicate a strongly positive correlation between CTEN and EGFR protein levels in these lung samples, supporting the cell culture finding that CTEN upregulation increases EGFR protein level.

Discussion

Previous studies have shown that CTEN expression is induced by EGF treatment in a variety of normal and cancer cells, including MCF10A, RWPE-1, HeLa, and SW480 cells (6, 24). Uregulated CTEN disrupts the interactions between integrin receptors and actin cytoskeleton (6) and promotes cancer cell migration, invasion, and colony formation (6, 8, 9, 11, 24). In this report, we have identified a novel function of CTEN in negatively regulating c-Cbl-mediated EGFR degradation. By binding to activated c-Cbl, CTEN is able to decrease EGFR ubiquitination and degradation. At this point, we do not know exactly how c-Cbl–CTEN interaction leads to the reduction of EGFR ubiquitination. It is possible that, by binding to c-Cbl, either CTEN sequesters c-Cbl away from activated EGFR or/and reduces its E3 ligase activity. The former seems to be supported by our results showing that c-Cbl–EGFR interaction was markedly reduced in the presence of CTEN after 15-minute EGF treatment. Because c-Cbl–CTEN interaction depends on c-Cbl tyrosine phosphorylation, which leads to the reduction of EGFR ubiquitination. It is possible that, by binding to activated EGFR, this may explain the delay disruption of c-Cbl–EGFR interaction by CTEN. Nonetheless, CTEN could somehow transiently (≤15 minutes) but robustly enhance c-Cbl–EGFR interaction. How CTEN can accomplish these dynamic processes remains to be investigated. The function of c-Cbl in promoting EGFR degradation is known to be suppressed in several ways (25), including (i) dephosphorylation of crucial phosphotyrosine sites on c-Cbl by SH2-containing phosphatase-1 (SHP1), (ii) targeting of c-Cbl for degradation by the E3 ligase atrophin-1–interacting protein-4 (AIP4), (iii) binding of c-Cbl to Sprouty 1/2 pTyr 55 sites that compete with the pTyr 1045 site on EGFR, and (iv) the formation of a complex between c-Cbl and PAK-interacting exchange factor (IPX1). PAK1 and Cdc42. Our study presents a
novel mechanism in modulating EGFR protein levels and provides additional layer of feedback control in EGFR signaling. In addition to EGFR, c-Cbl contributes to ubiquitination and downregulation of receptors for platelet-derived growth factor, VEGF, fibroblast growth factor, hepatocyte growth factor, macrophage colony-stimulating factor 1, neutrophin, and insulin, as well as members of non–receptor tyrosine kinase Src family (26). It will be interesting to examine whether CTEN may regulate the stabilities of these proteins through binding to c-Cbl.

We have shown that a functional SH2 domain is essential for CTEN's role in promoting cancer cell migration, invasion, as well as colony formation, and that CTEN SH2 domain binds to c-Cbl and prolongs EGFR signaling. These results indicate that the prolonged EGFR signaling is responsible for transformation properties, which is supported by the fact that EGFR inhibitor could reverse CTEN-mediated phenotypes. Moreover, SH2 domains of tensin members also interact with phosphoinositide 3-kinase (PI3K), p130Cas, focal adhesion kinase, phosphoinositide-dependent kinase-1 (PDK-1), and downstream of tyrosine kinase 2 (Dok-2; refs. 27–29). These binding partners may all contribute to CTEN-enhanced cancer phenotypes. On the other hand, SH2 domains of CTEN and tensins bind to the tumor suppressor DLC1 (deleted in liver...
cancer 1) in a phosphotyrosine-independent manner, which is required for DLC1’s tumor suppression activity (14). The function of recruiting DLC1 to focal adhesion for preventing cell transformation seems contradictory to the phenotypes observed in Fig. 4. One likely reason is that DLC1 is down-regulated or absence in many cancer cell lines including HCT116 cells used in this study. Nonetheless, these findings also implicate the complexities of CTEN regulatory networks in normal cells.

Upregulation and aberrant activation of EGFR are highly associated with tumor progression as well as therapeutic resistance in human patients with cancer (30, 31). EGFR-targeted therapies are used to treat several types of cancers including colorectal, breast, and lung cancer. Although showing promising effects in some patients, many other patients still suffer from side effects and resistance associated with anti-EGFR agents. The reasons that underline the anti-EGFR resistance have not yet been clarified, but may be related to much higher EGFR levels/activities and RAS/RAF mutations in some patients (32). Considering that CTEN (i) regulates activated EGFR levels, (ii) is a downstream of the RAS/RAF/MEK pathway, (iii) plays roles in tumor migration/invasion, and (iv) is expressed at very low levels in most normal tissues, targeting CTEN along with EGFR might be a more efficient approach for cancer therapies.
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S.-Y. Hong, T. Li, S.H. Lo
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Writing, review, and/or revision of the manuscript: S.-Y. Hong, Y.-P. Shih, T. Li, K.L. Carraway III, S.H. Lo

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S.-Y. Hong

Study supervision: S.H. Lo

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