Receptor-type Protein-tyrosine Phosphatase-κ Regulates Epidermal Growth Factor Receptor Function*

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Epidermal growth factor receptor (EGFR), the prototypic receptor protein tyrosine kinase, is a major regulator of growth and survival for many epithelial cell types. We report here that receptor-type protein-tyrosine phosphatase-κ (RPTP-κ) dephosphorylates EGFR and thereby regulates its function in human keratinocytes. Protein-tyrosine phosphatase (PTP) inhibitors induced EGFR tyrosine phosphorylation in intact primary human keratinocytes and cell-free membrane preparations. Five highly expressed RPTPs (RPTP-β, δ, κ, μ, and ξ) were functionally analyzed in a Chinese hamster ovary (CHO) cell-based expression system. Full-length human EGFR expressed in CHO cells, which lack endogenous EGFR, displayed high basal (i.e. in the absence of ligand) tyrosine phosphorylation. Co-expression of RPTP-κ, but not other RPTPs, specifically reduced basal EGFR tyrosine phosphorylation. RPTP-κ also reduced epidermal growth factor-dependent EGFR tyrosine phosphorylation in CHO cells. Purified RPTP-κ preferentially dephosphorylated EGFR tyrosines 1068 and 1173 in vitro. Overexpression of wild-type or catalytically inactive RPTP-κ reduced or enhanced, respectively, basal and EGF-induced EGFR tyrosine phosphorylation in human keratinocytes. Furthermore, siRNA-mediated knockdown of RPTP-κ increased basal and EGF-stimulated EGFR tyrosine phosphorylation and augmented downstream Erk activation in human keratinocytes. RPTP-κ levels increased in keratinocytes as cells reached confluency, and overexpression of RPTP-κ in subconfluent keratinocytes reduced keratinocyte proliferation. Taken together, the above data indicate that RPTP-κ is a key regulator of EGFR tyrosine phosphorylation and function in human keratinocytes.

Protein tyrosine phosphorylation is a reversible post-translational modification that modulates a variety of protein functions, including protein stability, protein-protein interaction, enzymatic activity, etc. Regulated reversible protein phosphorylation plays key roles in signal transduction pathways controlling many fundamental cellular processes, such as proliferation, differentiation, motility, metabolism, cytoskeletal organization, development, and cell-cell interactions (1, 2). The level of protein tyrosine phosphorylation is controlled by two classes of counteracting enzymes, namely protein-tyrosine kinases (PTKs) and protein-tyrosine phosphatases (PTPs). The number of genes in the human genome that encode active PTPs and active PTKs has recently been estimated to be very similar (3, 4). Emerging evidence indicates that, depending on the particular pathway, protein tyrosine dephosphorylation can be of equal or greater importance than protein tyrosine phosphorylation for the regulation of cellular function (5).

Epidermal growth factor receptor (EGFR, ErbB1) belongs to the receptor protein-tyrosine kinase (RPTK) superfamily. It is composed of an extracellular ligand binding domain, a single transmembrane domain, and an intracellular domain possessing PTK activity. Ligand binding to the extracellular domain of EGFR stabilizes homodimerization and heterodimerization with other ErbB members, which promotes trans tyrosine phosphorylation of the intracellular C-terminal domain. EGFR activation is synonymous with increased phosphorylation of specific tyrosine residues within its intracellular C-terminal domain. These phosphorylated tyrosines function as docking sites for a variety of signaling molecules that regulate membrane-proximal steps of signal transduction cascades that ultimately bring about cellular responses to EGFR ligands (6). Recent data suggest that EGFR not only participates in cognate ligand-induced signal transduction pathways but also plays important roles in diverse signal transduction pathways initiated by G protein-coupled receptors, cytokine receptors, integrins, ion channels, and stress responses (7–9).

Aberrant regulation of EGFR has been shown to promote multiple tumorigenic processes by stimulating proliferation, angiogenesis, and metastasis (10). EGFR and/or its ligands have a critical role in most common human epithelial cancers and many different types of solid tumors (11). The central role of EGFR in diverse signal transduction pathways dictates that its tyrosine phosphorylation must be strictly regulated. One potential mechanism for such regulation is through PTP-catalyzed dephosphorylation. The subfamily of “classical,” strictly tyrosine-specific PTPs contains 38 members, 21 of which are transmembrane receptor types and 17 of which are intracellular, non-receptor types (4). All classical PTPs contain a signature motif (HVCXXXXX(S/T)) within a catalytic domain of 250 amino acid residues (12). The cysteine residue in the PTP signature motif is absolutely required for catalytic activity (13). The receptor-type PTPs (RPTPs) are integral membrane proteins composed of extracellular adhesion molecule-like domains, a single transmembrane domain, and a cytoplasmic domain containing one or two catalytic domains.

Reduced phosphorylation of EGFR has been associated with several different PTP activities (14–18). However, identification of RPTP(s) that directly dephosphorylate(s), and thereby regulate(s), EGFR function is lacking. Using an expression strategy in EGFR-lacking Chinese hamster ovary (CHO) cells, we have identified RPTP-κ as a specific EGFR PTP. We have further demonstrated that RPTP-κ regulates both basal and ligand-induced EGFR tyrosine phosphorylation and function.

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**EXPERIMENTAL PROCEDURES**

**Materials**—Adult human primary keratinocytes were obtained from Cascade Biologics Inc. (Portland, OR). CHO cells were purchased from American Type Culture Collection (Manassas, VA). EGFR, Erk, and RPTP-β antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). PD169540 was generously provided by Dr. David Fry from Pfizer (Ann Arbor, MI). Phospho-EGFR (pY-1068 and pY-992) and phospho-Erk antibodies were purchased from Cell Signaling Technology (Beverly, MA). Phospho-EGFR (pY-1173) was purchased from Upstate Biotechnologies (Lake Placid, NY). RPTP-μ antibody was purchased from Exalpha Biologicals, Inc. (Watertown, MA).

**Cell Culture**—Adult human primary keratinocytes were expanded in modified MCDB153 medium (EpiLife, Cascade Biologics, Inc.). CHO cells were cultured in Ham’s F-12 medium with 1.5 g/liter sodium bicarbonate supplemented with 10% fetal bovine serum.

**Preparation of Keratinocyte Membranes and EGFR Activation Assay**—Human keratinocytes were washed twice with ice-cold hypotonic buffer (20 mM Tris-HCl, pH 7.6 with 10 mM NaCl) supplemented with 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin A, and 1 mM phenylmethylsulfonyl fluoride. Cells were disrupted in a Dounce homogenizer. Lysates were centrifuged at 500 × g for 10 min, and the supernatant was centrifuged at 20,000 × g for 30 min. Membranes were extracted with 0.5 M NaCl to remove loosely associated proteins. Membrane suspension was then supplemented with 100 μM ATP, 0.2% β-mercaptoethanol and 30 mM MgCl2. Incubation with EGFR or phosphatase inhibitors was performed at room temperature. SDS sample buffer was added to stop the reactions. EGFR tyrosine phosphorylation was analyzed by Western blot using phospho-EGFR (pY-1068) antibody.

**Preparation of Whole Cell Lysates**—Human primary keratinocytes or CHO cells were washed twice with ice-cold phosphate-buffered saline, scraped from the culture plates in WCE buffer (25 mM Hepes, pH 7.2, 75 mM NaCl, 2.5 mM MgCl2, 0.2 mM EDTA, 0.1% Triton X-100, 0.5 mM dithiothreitol, 20 mM β-glycerophosphate) supplemented with 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin A, 1 mM phenylmethylsulfonyl fluoride, and 1 mM sodium orthovanadate. Following 10 min of incubation at 4 °C, cell homogenates were centrifuged at 14,000 × g for 10 min, and supernatants were collected and used as whole cell lysates.

**Protein Phosphatase Assay**—Phosphatase assay buffer (100 μl) containing 5 mg/ml p-nitrophenyl phosphate, 100 g/ml bovine serum albumin, 50 mM Tris, pH 7.6, 100 mM NaCl, and 10 mM EDTA was mixed with cell membrane extracts (50 μl) and incubated at 37 °C for 30 min in 96-well microtiter plates. Reactions were terminated by the addition of 13% K2HPO4 (15 μl). Hydrolysis of p-nitrophenyl phosphate was measured spectrophotometrically (405 nm) using a microtiter plate reader (TiterTek Multiskan MCC/340, EFLAB, Finland).

**Generation of RPTP-κ Polyclonal Antibody**—A peptide with a unique sequence derived from the intracellular domain (RGHNESKADCLD-MDP KAPQH) with predicted high probability of surface exposure and high antigenic index was synthesized, conjugated to keyhole limpet hemocyanin, and injected into New Zealand White rabbits (Bethyl Laboratories, Inc., Montgomery, TX). After two booster injections, anti-RPTP-κ antibody was affinity-purified from hyperimmune serum. The antibody was tested for its performance in enzyme-linked immunosorbent assay, Western blot, and immunoprecipitation.

**In Vitro Dephosphorylation of Purified EGFR**—Purified full-length EGFR was purchased from BIOMOL (Plymouth Meeting, PA). EGFR was tyrosine-phosphorylated according to the manufacturer’s protocol and used as substrate for the RPTP-κ intracellular region GST fusion protein. Dephosphorylation reactions were terminated by the addition of SDS sample loading buffer, and the level of EGFR tyrosine phosphorylation was measured by Western analysis probed with phospho-EGFR antibody.

**Western Analysis Detection and Quantitation**—Western blots were developed and quantified using an enhanced chemifluorescence detection system (Amersham Biosciences). Immunoreactive fluorescent protein bands were detected by the STORM phosphor-imaging device using ImageQuant software (Molecular Dynamics, Sunnyvale, CA). Sample loads, antibody concentration, and incubation times were adjusted to yield fluorescent signals within the linear range of detection.

**Transient Transfection of CHO Cells**—Mammalian expression vectors for EGFR (pRK5 EGFR) and RPTP (pShuttle RPTP) coding sequences were transiently transfected into CHO cells using Lipofectamine 2000 (Invitrogen). Expression of EGFR and RPTP mRNAs and protein was confirmed by real-time reverse transcription (RT)-PCR (7700 Taqman Sequence Detector, Applied Biosystems, Foster City, CA) and Western analysis, respectively, 24 h after transfection.

**Association of EGFR with Wild-type and Trapping Mutant RPTP-κ**—Full-length EGFR and His6-tagged full-length wild-type or trapping mutant (D1051A) RPTP-κ were co-transfected into CHO cells. One day after transfection, the cells were treated with 50 ng/ml EGF for 10 min at 37 °C and subsequently lysed in TGH buffer (50 mM Hepes, pH 7.2, 20 mM NaCl, 10% glycerol, and 1% Triton X-100), and RPTP-κ protein complexes were purified using Dynabeads TALON (Dynal Biotech, Oslo, Norway) and analyzed by Western blot.

**Adeno-X Expression Vector Construction and Adenovirus Production**—Wild-type and catalytically inactive (2C/S) human RPTP-κ expression vectors were generated using the Adeno-X expression system (Clontech Laboratories, Inc., Palo Alto, CA). To facilitate detection of expressed RPTP-κ, a polyhistidine (His6) tag was inserted into the C terminus of RPTP-κ. HEK293 cells were used for adenovirus production and purification.

**Generation of RPTP-κ-GST Fusion Protein**—cDNA encoding the intracellular region of human RPTP-κ (RPTP-κ-IC, corresponding to residues 754–1414) was cloned into the XhoI (5’-AAG GTT TGC CGC TTC CTT CAG-3’) and NotI (3’-CAT TCG AGG CTC AAT GAT CCA-5’) sites of GST fusion protein expression vector pGEX-6P-3. GST-RPTP-κ-IC fusion protein was expressed in Escherichia coli strain BL21 and purified by GST affinity column according to the manufacturer’s protocol (Amersham Biosciences). Purity was at least 90%, as judged by SDS-PAGE.

**siRNA Silencing of RPTP-κ in Primary Human Keratinocytes**—A unique 21-mer RNA sequence derived from RPTP-κ coding sequence (5’-AAG GTT TGC CGC TTC CTT CAG-3’) was designed using Oligoengine software (Seattle, WA). Control siRNA contained a random sequence without homology to any known human gene. Double-stranded siRNA was synthesized by Xeragon Inc, (Valencia, CA). The synthetic siRNA was transfected into primary human keratinocytes using Amaxa Biosystems Nucleofector (Cologne, Germany). Expression of RPTP-κ in primary keratinocytes increased with cell confluency (see Fig. 10A). To achieve the maximal knockdown effect, siRNA-transfected keratinocytes were cultured to confluency for analysis.

**RESULTS**

**Inhibition of Protein-Tyrosine-Phosphatase Activity Is Associated with Increased EGFR Tyrosine Phosphorylation in Primary Human Keratinocytes**—Treatment of intact primary adult human keratinocytes with two nonspecific PTP inhibitors, H2O2, and pervanadate, caused substantial and rapid tyrosine phosphorylation of EGFR (Fig. 1A). The
profile of RPTPs in EGFR-expressing Human Keratinocytes—To investigate the above possibility, we first assessed which of 21 known RPTPs in the human genome are expressed in human keratinocytes. Specific PCR primers for each of the 21 human RPTPs were designed and tested for specificity using cloned cDNAs as templates. Each of the 21 PCR products generated from cloned templates was authenticated by DNA sequencing. RT-PCR was used to detect mRNA expression of each of the 21 RPTPs in adult human keratinocytes and adult human skin. RT-PCR reactions for 13 RPTPs yielded products of the expected size (data not shown). Each product was cloned and verified by DNA sequencing. RPTPs β, δ, κ, μ, and ξ were predominantly expressed and therefore chosen for further study.

Dephosphorylation of EGFR by RPTP-κ Transiently Expressed in CHO Cells—To determine whether any of the five candidate RPTPs is able to regulate EGFR tyrosine phosphorylation, we employed a transient transfection system using CHO cells, which do not express EGFR. Transient transfection of CHO cells with EGFR expression vector alone resulted in a high level of basal (i.e., in the absence of ligand) EGFR tyrosine phosphorylation (Fig. 2A). Treatment of EGFR-expressing CHO cells with EGF modestly increased tyrosine phosphorylation of EGFR (Fig. 2A). Tyrosine phosphorylation of EGFR in CHO cells was completely blocked by treatment of cells with EGFR tyrosine kinase inhibitor PD169540, demonstrating that tyrosine phosphorylation of EGFR in CHO cells was due to intrinsic EGFR tyrosine kinase activity (data not shown).

To examine the ability of the RPTPs to dephosphorylate EGFR, cDNAs encoding human RPTP-β, δ, κ, μ, and ξ were co-expressed with EGFR in CHO cells. To verify that each RPTP was expressed, we determined their mRNA levels using real-time RT-PCR. In vector control-transfected CHO cells, no mRNA for any of the five RPTPs was detectable. In RPTP-transfected CHO cells, the mRNA level of each of

RPTP-κ Dephosphorylates EGFR

magnitude of EGFR tyrosine phosphorylation was similar to that induced by EGF (20 ng/ml) (Fig. 1A). For these, an antibody that specifically recognizes phosphorylated tyrosine 1068 in EGFR was used. This approach allowed us to detect phosphorylated EGFR in lysates without the need for immunoprecipitation prior to Western blotting. The finding that PTP inhibitors cause accumulation of EGFR tyrosine phosphorylation suggests the potential importance of PTP activity in the regulation of EGFR function.

This potential importance was further investigated in cell-free, EGFR-enriched membrane fractions from human keratinocytes. Treatment of membranes in the presence of Mg²⁺/ATP with PTP inhibitors hydrogen peroxide, pervanadate, or orthovanadate significantly induced phosphorylation of EGFR tyrosine residue 1068 (Fig. 1B). The magnitude of tyrosine 1068 phosphorylation following treatment with either PTP inhibitor was 3–5-fold greater than treatment of membranes with EGF (20 ng/ml). As expected, treatment of keratinocyte membranes with hydrogen peroxide, pervanadate, or orthovanadate (but not EGF) inhibited endogenous membrane-associated PTP activity (Fig. 1C). These data suggest the possible involvement of an integral membrane PTP activity in the regulation of EGFR tyrosine phosphorylation.
RPTP-κ Dephosphorylates EGFR

FIGURE 3. Co-expression of RPTP-κ with EGFR reduces EGFR tyrosine phosphorylation in CHO cells. A, CHO cells were co-transfected with EGFR and the indicated RPTP expression vectors. Whole cell lysates were prepared and analyzed for EGFR phosphotyrosine 1068 (pY-1068) and total EGFR by Western blot. Inset shows representative Western blots and corresponding quantification. B, CHO cells were co-transfected with EGFR and either empty or RPTP-κ expression vectors. Cells were treated with vehicle (Veh) or EGF (10 ng/ml) for 5 min at 37 °C. Whole cell lysates were prepared for EGFR phosphotyrosine 1068 (pY-1068), total EGFR, and RPTP-κ by Western blot. Results are means ± S.E. of fluorescent band intensities quantified by STORM as described under "Experimental Procedures." n = 5; *, p < 0.05 versus empty vector. Inset shows representative Western blots. Results are means ± S.E. of fluorescent band intensities quantified by STORM as described under "Experimental Procedures." n = 4; *, p < 0.05 versus empty vector. Inset shows representative Western blots, s, p < 0.05 versus 0 min.

FIGURE 4. RPTP-κ preferentially dephosphorylates EGFR tyrosine 1068 and 1173 in vitro. Autophosphorylated EGFR was incubated with purified RPTP-κ or GST at room temperature. At the indicated times, reactions were analyzed for autophosphorylated EGFR tyrosine residues 1068, 992, and 1173. Results are means ± S.E. of fluorescent band intensities quantified by STORM as described under "Experimental Procedures." n = 3.

The five RPTPs was readily detectable (Fig. 2B). We also determined that transfection resulted in detectable RPTP-β, -κ, and -μ protein expression (we could not obtain useful antibodies for RPTP-δ and -ζ) (Fig. 2C). Importantly, only RPTP-κ (but not RPTP-β, -δ, -κ, -μ, and -ζ) was able to significantly reduce constitutive tyrosine phosphorylation of EGFR in CHO cells (Fig. 3A). In addition to the reduction of constitutive EGFR tyrosine phosphorylation, expression of RPTP-κ reduced EGF-stimulated EGFR tyrosine phosphorylation in CHO cells (Fig. 3B). These results indicate that RPTP-κ is capable of reducing EGFR intrinsic tyrosine kinase-catalyzed phosphorylation when co-expressed in CHO cells.

RPTP-κ Directly Dephosphorylates EGFR in Vitro—To determine whether RPTP-κ can directly dephosphorylate EGFR, we constructed, expressed, and purified catalytically active human RPTP-κ intracellular region GST fusion protein (GST-RPTP-κ-IC). GST-RPTP-κ-IC was incubated with autoprophosphorylated purified full-length human EGFR, and the rate of tyrosine dephosphorylation was monitored by Western analysis using antibodies specific for phosphotyrosine residues 1068, 992, and 1173. RPTP-κ rapidly dephosphorylated EGFR tyrosine 1068 and 1173 (Fig. 4). EGFR tyrosine 992 was dephosphorylated at a substantially slower rate than tyrosine 1068 (Fig. 4). In subsequent experiments, tyrosine phosphorylation at residue 1068 was chosen to monitor EGFR dephosphorylation by RPTP-κ.

Association of Substrate-trapping Mutant RPTP-κ and EGFR in Intact Cells—To further investigate dephosphorylation of EGFR by RPTP-κ, we performed substrate-trapping studies. Mutation of an aspartic acid (Asp-1051 for RPTP-κ), which is conserved in the active site of protein-tyrosine phosphatases, to alanine prevents completion of phosphate ester hydrolysis and therefore causes the formation of a stable enzyme-substrate complex (14). We co-expressed full-length human EGFR with His-tagged full-length wild-type or D1051A mutant RPTP-κ in CHO cells. Following expression, RPTP-κ was captured on nickel-coated beads and analyzed for its association with EGFR by Western analysis. Although wild-type RPTP-κ catalyzes the dephosphorylation of EGFR in vitro (Fig. 4), it does not appear to form a stable complex with EGFR, as expected, when co-expressed in cells (Fig. 5). In contrast, D1051A RPTP-κ does form a stable complex with EGFR, which can be readily detected by Western analysis (Fig. 5). These data demonstrate that EGFR is a substrate for RPTP-κ in intact cells.

Dephosphorylation of Endogenous EGFR by RPTP-κ in Primary Human Keratinocytes—We next examined the effect of RPTP-κ on tyrosine phosphorylation of endogenous EGFR in primary human keratinocytes. Adenovirus-mediated overexpression of RPTP-κ in primary human keratinocytes significantly reduced EGF-induced EGFR tyrosine 1068 phosphorylation (Fig. 6). Expression of catalytically inactive 2C/S-RPTP-κ, with cysteine to serine mutation in both PTP catalytic domains, increased both basal and EGF-induced EGFR tyrosine 1068 phosphorylation (Fig. 6). This increased EGFR tyrosine phosphoryla-
**RPTP-κ Dephosphorylates EGFR**

FIGURE 6. Overexpression of wild-type RPTP-κ reduces EGF-induced EGFR tyrosine phosphorylation in human keratinocytes. Primary human keratinocytes were infected with either empty (Ept), wild-type (κ-wt), or catalytically inactive mutant His-tagged RPTP-κ (κ-mt) adenovirus. Two days after infection, cells were treated with vehicle (−, Veh) or EGF (+, 25 ng/ml) for 10 min at 37 °C. Whole cell lysates were analyzed for EGFR phosphotyrosine 1068 (pY-1068), total EGFR, and His-RPTP-κ by Western blot. Data are means ± S.E. of five independent experiments. *p < 0.05 versus control siRNA. CTRL-RPTP-κ knockdown resulted in a further 2-fold increase of p44 and p42 Erk. In primary human keratinocytes, the rate of proliferation determined. Both empty and RPTP-κ adenovirus-treated keratinocytes displayed slow growth during the first two days after treatment, which is typical for human keratinocyte seeded at low density. Empty adenovirus-treated keratinocytes exhibited accelerated growth during days three and four post-treatment, reaching 80–90% confluency at day four (Fig. 10B). In contrast, RPTP-κ adenovirus-treated keratinocytes showed no significant proliferation during days three and four post-treatment (Fig. 10B). During the course of the experiment, keratinocyte

SECTION A

Reduction of endogenous RPTP-κ by siRNA significantly potentiated EGF-stimulated EGFR tyrosine 1068 phosphorylation (Fig. 9B).

**RPTP-κ Knockdown Potentiates EGF-induced Erk Activation in Primary Human Keratinocytes**—One of the major downstream effectors of EGFR is Erk mitogen-activated protein kinase. Because reduction of endogenous RPTP-κ potentiates basal and EGF-induced EGFR activation, we investigated the effect of RPTP-κ knockdown on Erk activation. siRNA-mediated RPTP-κ knockdown resulted in a 2-fold increase of basal phosphorylation of p44 and p42 Erk, and the rate of proliferation determined. Both empty and RPTP-κ adenovirus-treated keratinocytes displayed slow growth during the first two days after treatment, which is typical for human keratinocyte seeded at low density. Empty adenovirus-treated keratinocytes exhibited accelerated growth during days three and four post-treatment, reaching 80–90% confluency at day four (Fig. 10B). In contrast, RPTP-κ adenovirus-treated keratinocytes showed no significant proliferation during days three and four post-treatment (Fig. 10B). During the course of the experiment, keratinocyte

SECTION B

Reduction of endogenous RPTP-κ by siRNA significantly potentiated EGF-stimulated EGFR tyrosine 1068 phosphorylation (Fig. 9B).

**RPTP-κ Inhibits Keratinocyte Growth**—In culture, growth of human keratinocytes slows and eventually stops as the cells reach confluence. Because keratinocyte growth is EGFR-dependent (19, 20), we examined RPTP-κ expression as a function of culture confluency. We found that RPTP-κ mRNA expression was relatively low in subconfluent keratinocytes and increased markedly when keratinocytes became confluent (Fig. 9C). Similarly, RPTP-κ knockdown resulted in a further 2-fold increase of EGF-induced phosphorylation of Erk (Fig. 9C).

**siRNA-mediated Knockdown of RPTP-κ Increases EGFR Tyrosine Phosphorylation in Primary Human Keratinocytes**—Transfection of RPTP-κ siRNA was analyzed to determine the effect of reduced RPTP-κ on EGFR tyrosine phosphorylation. RPTP-κ siRNA specifically reduced RPTP-κ mRNA by 60% (Fig. 8A), whereas it did not reduce mRNA levels of related RPTPs, RPTP-β, RPTP-δ, RPTP-μ, and RPTP-ζ in confluent primary human keratinocytes (Fig. 8A). The RPTP-κ protein level was reduced by 70% (Fig. 8B). Reduction of endogenous RPTP-κ by siRNA increased EGFR basal tyrosine phosphorylation in a dose-dependent manner (Fig. 9A). In addition, reduction of endogenous RPTP-κ by siRNA significantly potentiated EGF-stimulated EGFR tyrosine 1068 phosphorylation (Fig. 9B).

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SECTION C

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We tested the five most abundantly expressed RPTPs for their ability to reduce basal EGFR tyrosine phosphorylation. Among the five RPTPs tested, only RPTP-κ was able to significantly reduce basal EGFR tyrosine phosphorylation. It is possible that other RPTPs, which we did not study, act in concert with RPTP-κ to reduce EGFR phosphorylation. We found that RPTP-κ preferentially dephosphorylated tyrosines 1068 and 1173, compared with tyrosine 992. This observation further supports the concept that EGFR is acted upon by multiple RPTPs. In this regard, both RPTP LAR, and RPTP-σ have been shown to reduce EGFR phosphorylation (18, 23). However, whether either RPT acts directly on EGFR, or which phosphorysine residue(s) either RPT affects remains to be determined.

Human keratinocytes, similar to many other cell types in culture, cease proliferation in response to cell-cell contacts. Although the detailed mechanism of contact inhibition remains elusive, interactions among adhesion molecules on the surface of adjoining cells plays a pivotal role. The extracellular domains of many PTPs contain adhesion molecule-like sequences, leading to the proposal that PTP functions may be regulated, at least in part, by cell-cell contacts (24). In fact, RPTP-κ and RPTP-μ have been shown to possess adhesion properties that can mediate cell-cell and cell-matrix communication (25, 26).

Interestingly, membrane-associated PTP activity is increased up to 10-fold in contact-inhibited cells and harvested at high density, compared with proliferating cells at low density, whereas tumor cells, which are not subjected to contact-inhibition, do not show density-dependent increase in membrane-associated PTP activity (27, 28). Furthermore, RPTP-μ, RPTP-β, and DEP-1 have been shown to be up-regulated as a function of increased cell density in culture (24, 28, 29).

In the current study, we demonstrated that RPTP-κ levels also increase as a function of confluence in cultured human keratinocytes. This finding is consistent with our previous observations demonstrating that the level of RPTP-κ expression increases in confluent human keratinocytes (15, 21). It is possible that other RPTPs, which we did not study, act in concert with RPTP-κ to reduce EGFR phosphorylation. We found that RPTP-κ preferentially dephosphorylated tyrosines 1068 and 1173, compared with tyrosine 992. This observation further supports the concept that EGFR is acted upon by multiple RPTPs. In this regard, both RPTP LAR, and RPTP-σ have been shown to reduce EGFR phosphorylation (18, 23). However, whether either RPT acts directly on EGFR, or which phosphorysine residue(s) either RPT affects remains to be determined.
RPTP-κ Dephosphorylates EGFR

with the reported increased expression of RPTP-κ with increased confluence in SK-BR-3 human mammary carcinoma cells (30).

In human keratinocytes, overexpression or reduced expression of RPTP-κ decreased or elevated EGFR tyrosine phosphorylation, respectively. These changes in EGFR tyrosine phosphorylation were mirrored by alterations in the level of Erk activation. Thus, RPTP-κ appears to regulate a key downstream EGFR effector through regulation of EGFR tyrosine phosphorylation. Erk is a critical mediator of keratinocyte proliferation and survival (31). Therefore, the ability of increased RPTP-κ levels to reduce Erk activation is expected to reduce keratinocyte growth. Indeed, we found that raising the level of RPTP-κ in subconfluent keratinocytes resulted in near complete inhibition of growth. Under these conditions, increased expression of RPTP-κ did not alter the level of EGFR. These data suggest that the ratio of RPTP-κ to EGFR, rather than the absolute level of either RPTP-κ or EGFR, is an important determinant of EGFR functionality. A number of epithelial tumors are characterized by hyperactivation of EGFR. Hyperactivation can result from EGFR mutation or overexpression. However, our data suggest that reduced expression of RPTP-κ also allows hyperactivation of EGFR in the absence of any alteration of EGFR itself. Whether reduced expression of RPTP-κ occurs in any human epithelial cancers is currently under investigation.

RPTP LAR and RPTP DEP-1 have been shown to regulate insulin receptor and hepatocyte growth factor receptor signaling, respectively (32, 33). DEP-1 has also been described (35). The transforming growth factor-β (TGF-β) growth inhibition exerted by TGF-β also allows hyperactivation of EGFR in association with growth inhibition in the H9260/H9252 human mammary carcinoma cells (30).

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