Oxidative Inhibition of Receptor-type Protein-tyrosine Phosphatase κ by Ultraviolet Irradiation Activates Epidermal Growth Factor Receptor in Human Keratinocytes*

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Ultraviolet (UV) irradiation rapidly increases tyrosine phosphorylation (i.e. activates) of epidermal growth factor receptors (EGFR) in human skin. EGFR-dependent signaling pathways drive increased expression of matrix metalloproteinases, whose actions fragment collagen and elastin fibers, the primary structural protein components in skin connective tissue. Connective tissue fragmentation, which results from chronic exposure to solar UV irradiation, is a major determinant of premature skin aging (photoaging). UV irradiation generates reactive oxygen species, which readily react with conserved cysteine residues in the active site of protein-tyrosine phosphatases (PTP). We report here that EGFR activation by UV irradiation results from oxidative inhibition of receptor type PTP-κ (RPTP-κ). RPTP-κ directly counters intrinsic EGFR tyrosine kinase activity, thereby maintaining EGFR in an inactive state. Reversible, oxidative inactivation of RPTP-κ activity by UV irradiation shifts the kinase-phosphatase balance in favor of EGFR activation. These data delineate a novel mechanism of EGFR regulation and identify RPTP-κ as a key molecular target for antioxidant protection against skin aging.

Skin is the largest human organ, and only organ directly exposed to the environment. Acute exposure to ultraviolet (UV) irradiation from the sun is harmful to skin, causing sunburn, immune suppression, DNA damage, and connective tissue degradation (1). Accumulated damage, resulting from chronic sun exposure, causes skin cancer and premature skin aging (photoaging) (2). Approximately one million individuals in the United States develop skin cancer each year, and essentially all people experience photoaging to some degree, depending on the amount of sun exposure and skin pigmentation (pigmentation is protective) (3).

Epidermal growth factor receptor (EGFR)2 is a ubiquitously expressed, cell surface, transmembrane receptor that possesses intrinsic protein-tyrosine kinase activity. Functional activation of EGFR results from increased phosphorylation of specific tyrosine residues in its C-terminal cytoplasmic domain. Tyrosine phosphorylation is catalyzed by intrinsic tyrosine kinase activity. Phosphotyrosine residues function as binding sites for assembly of protein complexes, which initiate downstream signaling pathways that regulate cellular function (4). EGFR is highly expressed in human skin cells (keratinocytes) in vivo and in vitro (5, 6). Emerging evidence indicates that EGFR is a critical functional mediator of cellular responses to a diverse array of extracellular stimuli, including ligands for other cell surface receptors (7, 8). Hypertyrosine phosphorylation (i.e. activation) of EGFR in response to non-cognate ligands involves release of surface membrane-bound EGF family ligands, as well as other mechanisms (9–15).

UV irradiation rapidly increases EGFR tyrosine phosphorylation in human keratinocytes in vivo and in culture (5). This EGFR activation is necessary for induction of signaling pathways (termed the mammalian UV response), including mitogen-activated protein kinases, phosphatidylinositol 3-kinase/Akt, and phospholipase C/protein kinase C (16, 17). These signaling pathways induce a variety of transcription factors and their target genes, including AP-1 and matrix metalloproteinases, respectively (17–22). AP-1 and matrix metalloproteinases play critical roles in development of skin cancer and photoaging (23, 24), indicating the importance of EGFR tyrosine phosphorylation in the pathophysiology of UV-induced human skin damage. In addition, EGFR activation protects against UV-induced apoptosis, through activation of the phosphatidylinositol 3-kinase/AKT pathway (16).

Biological effects of UV irradiation occur as a consequence of absorption of electromagnetic energy by certain molecules within all cells. The excess energy is dissipated either by chemical modification of the absorbing molecule, or transfer of energy to an acceptor molecule. Molecular oxygen, which is in high concentrations in eukaryotic cells, can readily accept energy from UV-absorbing molecules (25, 26). This photochemical activation of molecular oxygen generates reactive oxygen species (ROS), which can oxidize cellular constituents including proteins, lipids, and nucleic acids (27–30). ROS are the major chemical initiators of UV irradiation-induced responses in human cells (21, 31–34). Whereas at high concentrations ROS can be cytotoxic, at low concentrations ROS appear to serve a physiological function as mediators of cellular response (35, 36). Recent studies have shown that activation of cell surface growth factors and cytokine receptors by UV results

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2 The abbreviations used are: EGFR, epidermal growth factor receptor; PTP, protein-tyrosine phosphatase; ROS, reactive oxygen species; RPTP-κ, receptor-type protein-tyrosine phosphatase-κ; CHO, Chinese hamster ovary; siRNA, small interfering RNA; ELISA, enzyme-linked immunosorbent assay; DTT, dithiothreitol; ERK, extracellular signal-regulated kinase.
RPTP-κ Regulates UV Irradiation Activation of EGFR

in the formation of ROS, which play a critical role in mediating downstream signaling pathways in cultured cells (37–39). Peus et al. (20) have reported that H2O2 generation precedes EGFR phosphorylation and ERK activation following UVB irradiation of human keratinocytes. All members of the protein-tyrosine phosphatase (PTP) family contain an active site cysteine residue, which is required for phosphohydrolase activity. This active site cysteine is highly susceptible to oxidation, particularly by hydrogen peroxide (H2O2) (40). The pKa of the cysteine within the active site is relatively low (5.5) at physiological pH, which promotes formation of the reactive thiolate form. The thiolate reacts readily with H2O2 to form a stable sulfenic acid (40), or sulfenyl-amide species (41), which renders the phosphatase catalytically inactive. Recent evidence indicates that reversible oxidative inactivation of PTP activities occurs as a consequence of ROS generated in response to growth factor and cytokine receptor activation, and regulates tyrosine phosphorylation-dependent signal transduction pathways (18, 38, 39, 43, 44).

Oxidative inhibition of PTP activity by ROS has been proposed as a mechanism for activation of EGFR by UV irradiation (45). Critical testing of this proposed mechanism has been hindered by lack of knowledge regarding phosphatases that directly regulate EGFR at the cell surface. We have recently identified receptor-type protein-tyrosine phosphatase κ (RPTP-κ) as a regulator of EGFR tyrosine phosphorylation, in human keratinocytes (6). RPTP-κ directly dephosphorylates EGFR in vitro, and functions in cells to maintain low levels of EGFR tyrosine phosphorylation in the absence of exogenous ligand. RPTP-κ counteracts EGFR intrinsic tyrosine kinase activity by preferentially dephosphorylating EGFR tyrosine residues 1068 and 1173 (6). We report here that activation of EGFR by UV irradiation is mediated by oxidative inhibition of RPTP-κ activity.

EXPERIMENTAL PROCEDURES

Materials—Adult human primary keratinocytes were purchased from Cascade Biologics Inc. (Portland, OR). Chinese hamster ovary (CHO) cells were obtained from ATCC. EGFR and phospho-EGFR (pY1068) antibodies used for Western analysis were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and Cell Signaling Technology (Beverly, MA), respectively. EGFR antibody for immunofluorescence was from Neomarkers (Fremont, CA). Neutralizing EGFR antibody LA1, which blocks ligand binding, was obtained from Upstate Biotechnologies (Waltham, MA). RPTP-κ antibody was generated and affinity purified from rabbits immunized with a peptide derived from the intracellular domain of human RPTP-κ, as described (6). Phosphotyrosine peptide derived from EGFR (Biotin-KGSTAENAE(pY)LRV-amide) was synthesized by New England Peptide, Inc. (Gardner, MA). PD169540 is a generous gift from Dr. David Fry (Pfizer Inc.). Oligonucleotide probes used for in situ hybridization were synthesized by GeneDetect (Bradenton, FL). Purified, full-length active human EGFR was obtained from BioMol (Plymouth Meeting, PA). The intracellular region of RPTP-κ was cloned into pGEX-6-P, and expressed as a His-tagged glutathione S-transferase fusion protein in BL21. Expressed RPTP-κ was purified by nickel chelate and glutathione affinity chromatography to a purity of greater than 90%, as judged by SDS-PAGE.

Cell Culture—Subcultures of adult human primary keratinocytes were expanded in modified MCDB153 media (EpiLife, Cascade Biologics, Inc.) at 37 °C under 5% CO2. CHO cells were cultured in Ham’s F-12 medium with 1.5 g/ml sodium bicarbonate, supplemented with 10% fetal bovine serum under 5% CO2 at 37 °C.

UV Source and Irradiation—Subconfluent cells in a thin layer of Tris-buffered saline were irradiated using a Durlay lamp apparatus containing six FS24T12 UVB-HO bulbs. A Kodacel TA401/407 filter was used to eliminate wavelengths below 290 nm (UVC) resulting in a UV spectrum consisting of 48% UVB, 31% UVAII, and 21% UVA1. The irradiation intensity was monitored with an IL1400A phototherapy radiometer and a SED240/UVB/W photodetector (International Light, Newbury, MA). Human subjects were phototested to determine the dose of UV irradiation that caused the skin to become slightly pink (minimal erythema dose). Subjects were exposed to twice this dose for studies. All procedures involving human subjects were approved by the University of Michigan Institutional Review Board and all subjects provided written informed consent.

Transient Transfection of CHO Cells—Mammalian expression vectors harboring EGFR (pRK5 EGFR) or RPTP-κ (pshuttle RPTP-κ) coding sequences were transiently transfected by the Lipofectamine 2000 method into CHO cells according to the manufacturer’s protocol (Invitrogen Corp.). siRNA Silencing of Endogenous RPTP-κ in Primary Human Keratinocytes—A 21-mer RNA sequence (5’-AAGGTTCGCGTTCCTTCAG-3’) derived from the RPTP-κ coding sequence was designed using Oligoengine’s software (Seattle, WA). Homology search was performed on this RNA sequence using BLAST to ensure it was not presented in any other known sequence in the data base. Double-stranded siRNA was synthesized by Qiagen-Xeragon Inc. (Valencia, CA). The synthetic siRNA was transfected into primary human keratinocytes using the Human Keratinocytes Nucleofector kit and device from Amaxa Biosystems (Cologne, Germany) according to the manufacturer’s protocol.

RPTP-κ Immunoprecipitation, Protein-tyrosine Phosphatase Assay, and EGFR Tyrosine Phosphorylation ELISA—Keratinocytes whole cell lysates were made in TGH buffer (50 mm Heps, pH 7.2, 20 mm NaCl, 10% glycerol, and 1% Triton X-100), supplemented with 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin A, and 1 mm phenylmethylsulfonyl fluoride, and were pre-cleared with normal rabbit IgG before incubation with RPTP-κ antibody for 3 h at 4 °C. For some assays, as indicated in the text, 10 mm iodoacetic acid was added to TGH buffer to irreversibly inhibit non-oxidized protein-tyrosine phosphatase activity (18). Protein A-conjugated agarose beads were then added, and further incubated at 4 °C for 2 h, followed by extensive washing. Washed immunoprecipitates were analyzed by Western blot, or assayed for protein-tyrosine phosphatase activity. For some assays, as indicated in the text, 10 mm dithiothreitol (DTT) was added to the assay buffer to reduce oxidized RPTP-κ (18). For measurement of protein phosphatase activity, tyrosine-phosphorylated peptide derived from EGFR was added to a final
concentration of 0.5 mM in 50 μl of PTP assay buffer (50 mM Tris, pH 7.6, 100 mM NaCl, 100 μg/ml bovine serum albumin). Reactions were terminated by addition of 100 μl of BIOMOL Green Reagent (BIOMOL, Plymouth Meeting, PA) and absorbance was measured at 620 nm. Human total EGFR and tyrosine 1068 phospho-EGFR were quantified by ELISA (BIOSOURCE International, Camarillo, CA).

Western Blot Analysis of UV Irradiation-induced Oxidation of RPTP-ζ in Human Primary Keratinocytes—Human primary keratinocytes were mock irradiated or UV irradiated (90 mJ/cm²). Five minutes post-UV irradiation, cells were lysed in the presence of 100 mM iodoacetic acid, and RPTP-ζ was immunoprecipitated as described above. The immunoprecipitate was reduced by addition of 10 mM dithiothreitol in TGH buffer, containing protease inhibitors, for 30 min at 4 °C. The immunoprecipitate was washed three times, and then irreversibly oxidized by incubation with 2 mM pervanadate at 4 °C for 1 h. Oxidized RPTP-ζ was analyzed by Western blot probed with OXPTP antibody (a gift from Dr. Arne Ostman, Cancer Center Karolinska, Stockholm, Sweden) as described (47).

Western Analysis Detection and Quantitation—Western blots were developed and quantified using a chemifluorescent substrate (ECF Western blot Reagents, Amersham Biosciences). Detection of chemifluorescence was performed using a STORM PhosphorImager (GE Healthcare). Sample loads, antibody concentration, and incubator times were adjusted to yield fluorescent signals within the linear range of detection. Fluorescent intensity of protein bands were quantified by ImageQuant software, which is an integral application of the STORM.

Detection of UV Irradiation-induced DNA Fragmentation in Human Primary Keratinocytes—Human primary keratinocytes were infected with either empty or RPTP-ζ adenovirus. Cells were mock or UV irradiated 2 days post-infection. Six hours post-UV irradiation, cells were lysed, and DNA fragmentation was measured by Cell Death Detection ELISA according to the manufacturer's instructions (Roche Applied Science).

In Situ Hybridization—Hybridization buffer (4× SSC, 20% dextran sulfate, 50% formamide, 0.25 mg/ml salmon sperm DNA, 0.25 mg/ml yeast tRNA, 0.1 mM DTT, 0.5× Denhardt’s solution) with three fluorescein-conjugated sense or antisense DNA oligonucleotide probes, corresponding to nucleotides 1549–1596, 3440–3487, and 4290–4337 in the human RPTP-ζ mRNA sequence (GenBank™ accession number NM_002844), were incubated at 37 °C overnight. Sections were
RPTP-κ Regulates UV Irradiation Activation of EGFR

FIGURE 3. UV-induced EGFR tyrosine phosphorylation is ligand-independent and mediated by oxidative inhibition of RPTP-κ in primary human keratinocytes. A, primary human keratinocytes were treated with control IgG1, or EGFR antibody LA1 (1 μg/ml), which blocks ligand binding, as indicated. Cells were then treated with vehicle (CTRL) or EGF (10 ng/ml) for 10 min, or UV irradiated (50 mJ/cm²) and harvested 15 min post-irradiation. Whole cell lysates were subjected to Western analysis to determine total and tyrosine-phosphorylated EGFR levels. Levels of immunoreactive EGFR were quantified by chemifluorescent detection. Results are mean ± S.E. of three independent experiments; *, p < 0.05. Inset shows a representative image of chemifluorescent immunoreactive total and tyrosine-phosphorylated EGFR proteins. B, human keratinocytes were UV irradiated (50 mJ/cm²), and whole cell lysates were prepared at the indicated times. RPTP-κ and β-actin (internal control) were detected by Western blot, and quantified by chemifluorescent detection. Results are mean ± S.E. of three independent experiments; *, p < 0.05. Inset shows a representative image of chemifluorescent immunoreactive RPTP-κ and β-actin proteins. C, primary human keratinocytes were mock (No UV) or UV irradiated (50 mJ/cm²), and whole cell lysates were prepared 5 min post-UV irradiation. RPTP-κ was immunoprecipitated, and phosphatase activity was determined using a tyrosine-phosphorylated EGFR peptide as substrate. Phosphatase activity was measured after UV irradiation regulation of EGFR tyrosine phosphorylation, we initially examined the effects of ROS on purified RPTP-κ activity and EGFR tyrosine phosphorylation in vitro. Addition of hydrogen peroxide (H₂O₂) caused dose-dependent inhibition washed in 2× phosphate-buffered saline with 0.01% Tween 20, then 1× phosphate-buffered saline, 0.01% Tween 20. Washed slides were incubated with protein block (Biogenex, San Ramon, CA), biotin-labeled antibody, or EGFR antibody plus peptide used to raise the antibody, or preimmune serum. For negative control, staining was performed using RPTP-κ antibody plus peptide used to raise the antibody, or preimmune serum, instead of primary antibody. Staining was observed under a Zeiss microscope (Axioskop 2) and images were obtained with digital camera (SPOT2, Diagnostic Inc. Sterling Heights, MI). All reagents, except as noted, were from Biogenex.

RESULTS AND DISCUSSION

To investigate the role of RPTP-κ in UV irradiation regulation of EGFR tyrosine phosphorylation, we initially examined the effects of ROS on purified RPTP-κ activity and EGFR tyrosine phosphorylation in vitro. Addition of hydrogen peroxide (H₂O₂) caused dose-dependent inhibition...
FIGURE 4. Knockdown of RPTP-κ increases EGFR tyrosine phosphorylation in primary human keratinocytes. A, human keratinocytes were transfectected with scrambled control (CTRL) or RPTP-κ siRNA. Two days post-transfection, whole cell lysates were prepared and analyzed for RPTP-κ and β-actin (internal control) proteins by Western blot. Results are mean ± S.E. of three independent experiments; *, p < 0.05. Inset shows a representative image of chemiluminescent immunoreactive RPTP-κ and β-actin proteins. B, two days after transfection with control (CTRL) or RPTP-κ siRNA, keratinocytes were UV irradiated (50 mJ/cm²). Whole cell lysates were prepared 15 min post-UV irradiation and analyzed for total EGFR and tyrosine-phosphorylated EGFR Western blot. Results are mean ± S.E. of three independent experiments; *, p < 0.05. Inset shows a representative image of chemiluminescent immunoreactive total and phospho-EGFR (pY-EGFR) proteins. C, keratinocytes were transfectected with control (CTRL) or RPTP-κ siRNA and treated with control IgG or neutralizing anti-EGFR antibody. Two days post-transfection, whole cell lysates were prepared and analyzed for total and tyrosine-phosphorylated EGFR by ELISA. Results are mean ± S.E. of three independent experiments; *, p < 0.05.

of RPTP-κ activity, with 80% loss of activity observed at 100 μM (Fig. 1A). In the presence of ATP/Mg²⁺, purified EGFR was phosphorylated by its intrinsic tyrosine kinase activity. In contrast to RPTP-κ, H₂O₂ had no effect on tyrosine phosphorylation of purified EGFR, in vitro (Fig. 1B). Incubation of purified RPTP-κ and EGFR together resulted in a low level of steady state EGFR tyrosine phosphorylation, representing the balance between the rates of tyrosine kinase and tyrosine phosphatase activities. In the presence of H₂O₂, which inhibits RPTP-κ, EGFR tyrosine phosphorylation increased to the level observed in the absence of RPTP-κ (Fig. 1C). These data provide proof of concept for RPTP-κ-dependent regulation of EGFR tyrosine phosphorylation by ROS, in a cell-free system.

To further investigate UV irradiation regulation of EGFR tyrosine phosphorylation by RPTP-κ, we used a model mammalian cell system. CHO cells do not express either EGFR or RPTP-κ. We have previously used this system to screen PTPs for EGFR phosphatase activity (6). Transient transfection of CHO cells with EGFR expression vector resulted in high level of constitutive (i.e. in the absence of ligand) EGFR tyrosine phosphorylation (Fig. 2). This constitutive EGFR tyrosine phosphorylation was abolished by specific EGFR kinase inhibitor PD169540, indicating tyrosine phosphorylation was due to intrinsic tyrosine kinase activity (data not shown). Exposure of EGFR-expressing CHO cells to UV irradiation did not further increase EGFR tyrosine phosphorylation. However, co-expression of EGFR with RPTP-κ substantially reduced EGFR tyrosine phosphorylation (Fig. 2). UV irradiation of CHO cells expressing both EGFR and RPTP-κ increased the level of EGFR tyrosine phosphorylation to the level observed in the absence of RPTP-κ (Fig. 2). These data demonstrate that RPTP-κ is required for UV irradiation induction of EGFR tyrosine phosphorylation, in the CHO cell model system.

We next investigated the role of RPTP-κ in UV irradiation regulation of EGFR tyrosine phosphorylation in human keratinocytes. In addition to expressing both EGFR and RPTP-κ, keratinocytes express several EGFR ligands, including transforming growth factor-α, amphiregulin, HB-EGF, betacellulin, and epiregulin. To examine potential involvement of ligand binding in UV irradiation induction of EGFR tyrosine phosphorylation, we used a neutralizing monoclonal antibody, which blocks ligand binding to EGFR. Whereas this antibody reduced EGFR-induced EGFR tyrosine phosphorylation to near basal levels, it had no significant effect on UV irradiation induction of EGFR.

FIGURE 5. RPTP-κ enhances UV irradiation-induced DNA fragmentation. Human primary keratinocytes were infected with either empty or RPTP-κ adenovirus. Cells were mock or UV irradiated 2 days post-infection. Six hours post-UV irradiation, cells were lysed, and DNA fragmentation was measured by ELISA. Results are mean ± S.E. of three independent experiments; *, p < 0.05 RPTP-κ versus empty vector.
RPTP-κ Regulates UV Irradiation Activation of EGFR

A

Sense
Antisense

B

Pre-immune
Peptide + RPTP-κ Ab
RPTP-κ Ab

C

D

E

RPTP-κ Protein (Fold Change)

No UV
UV

RPTP-κ Activity (Percentage)

No UV
UV

*
RPTP-κ Regulates UV Irradiation Activation of EGFR

We observed that expression of exogenous RPTP-κ conferred UV irradiation induction of EGFR tyrosine phosphorylation, in CHO cells (Fig. 2). Keratinocytes, however, express endogenous RPTP-κ. Therefore, we utilized siRNA-mediated knockdown to examine the role of RPTP-κ in UV irradiation regulation of EGFR tyrosine phosphorylation. Transient transfection of RPTP-κ siRNA caused 80 and 70% reduction of RPTP-κ mRNA and protein (Fig. 4A), respectively (6). Knockdown of RPTP-κ had no effect on gene expression levels of other related RPTPs expressed in keratinocytes (RPTP-μ, -β, -δ, or -ζ) (6). UV irradiation induced EGFR tyrosine phosphorylation nearly 5-fold in keratinocytes transfected with scrambled control siRNA (Fig. 4B), similar to that observed in non-transfected keratinocytes (Fig. 3). Knockdown of RPTP-κ increased EGFR tyrosine phosphorylation in non-irradiated keratinocytes nearly 4-fold. Exposure to UV irradiation further increased EGFR tyrosine phosphorylation only 20% (Fig. 4B). Addition of EGFR antibody that blocks ligand binding had no effect on increased EGFR tyrosine phosphorylation induced by RPTP-κ knockdown (Fig. 4C). These data indicate that normal levels of RPTP-κ function to maintain low basal EGFR tyrosine phosphorylation. In the presence of reduced levels of RPTP-κ, basal EGFR tyrosine phosphorylation is increased, and therefore can only be marginally further increased by UV irradiation. In the presence of normal levels of RPTP-κ, basal EGFR tyrosine phosphorylation is low, and oxidative inhibition of RPTP-κ by UV irradiation alters the EGFR tyrosine kinase/phosphatase balance to elevate EGFR tyrosine phosphorylation.

UV irradiation can damage skin cells, and with sufficient damage, induce apoptosis (48). In human keratinocytes, EGFR protects against UV-induced apoptosis, primarily through activation of the phosphatidylinositol 3-kinase/ATK pathway (16). Therefore, we examined whether overexpression of RPTP-κ could modulate UV irradiation-induced DNA fragmentation, a marker of apoptosis, in human keratinocytes. At a dose of 50 ml/cm², UV irradiation did not cause significant DNA fragmentation, compared with mock irradiation, in keratinocytes infected with control vector (Fig. 5). In contrast, this dose of UV irradiation causes a significant increase of DNA fragmentation of keratinocytes overexpressing RPTP-κ (Fig. 5). Higher doses of UV irradiation (70–90 ml/cm²) caused increased DNA fragmentation in both control and RPTP-κ overexpressing cells. However, increased expression of RPTP-κ caused increased levels of DNA fragmentation, at all doses of UV irradiation.

Finally, we examined RPTP-κ expression and regulation by UV irradiation in human skin in vivo. Epidermis primarily consists of stratified layers of keratinocytes. The lowest layer of keratinocytes (basal keratinocytes) undergoes cell division. Daughter cells (suprabasal keratinocytes) migrate upward to differentiate into stratum corneum. UV irradiation can induce defective keratinocyte differentiation, which is characteristic of skin aging. We observed that expression of exogenous RPTP-κ conferred UV irradiation induction of EGFR tyrosine phosphorylation, in both human skin (Fig. 3A). We conclude that ligand binding has little, if any, role in activation of EGFR by UV irradiation. This conclusion is consistent with data demonstrating that the EGFR ligand-binding domain is not required for UV irradiation induction of EGFR tyrosine phosphorylation in NIH 3T3 cells (45).

We next examined whether UV irradiation altered RPTP-κ expression in human keratinocytes. We found no change of RPTP-κ protein levels following UV irradiation (Fig. 3B). We next examined the effect of UV irradiation on RPTP-κ activity in human keratinocytes. For these studies, keratinocytes were mock-exposed or exposed to UV irradiation (50 ml/cm²), and harvested in lysis buffer 5 min post-UV irradiation. RPTP-κ was immunoprecipitated, and its activity measured by dephosphorylation of a phosphotyrosine-containing synthetic peptide substrate, derived from the amino acid sequence of the EGFR (amino acids 1164–1176). UV irradiation reduced RPTP-κ activity in human keratinocytes more than 60%, compared with mock-irradiated cells (Fig. 3C). It has recently been reported that UV irradiation can reduce protein levels of PTP 1B and LAR, in certain cell types, through activation of proteolytic cleavage (46). However, no reduction of the RPTP-κ protein level in human keratinocytes was observed within 90 min following UV irradiation (data not shown).

The data above indicate that UV irradiation inhibits RPTP-κ activity, in human keratinocytes. To determine whether inhibition results from oxidation, iodoacetate was included in the lysis buffer that was used to harvest cells following mock or UV irradiation. Iodoacetate forms a stable adduct with non-oxidized, but not with oxidized, cysteine thiols. Therefore non-oxidized RPTP-κ is irreversibly inhibited by iodoacetate, whereas oxidized RPTP-κ is not (18). The activity of oxidized, but not acetylated, RPTP-κ can be restored by reduction with DTT (18). Immunoprecipitates from mock-irradiated keratinocytes, prepared in the presence of iodoacetate, and treated with DTT, contained four times less RPTP-κ activity, compared with immunoprecipitates from UV-irradiated cells (Fig. 3D). These data indicate that UV irradiation caused oxidation of RPTP-κ, which protected it against acetylation, in human keratinocytes.

To confirm that UV irradiation leads to oxidation of RPTP-κ in human keratinocytes, we utilized an antibody that specifically recognizes the oxidized active site of protein-tyrosine phosphatases (47). RPTP-κ was immunoprecipitated from keratinocytes following mock exposure or exposure to UV irradiation. Immunoprecipitated RPTP-κ was analyzed for active site oxidation by Western analysis. The level of oxidized RPTP-κ was increased 3-fold in UV-irradiated, compared with non-irradiated keratinocytes (Fig. 3E).
RPTP-κ Regulates UV Irradiation Activation of EGFR

toward the surface, and, as they migrate, undergo a coordinated, complex program of maturation. Suprabasal keratinocytes normally do not proliferate. RPTP-κ mRNA was expressed predominantly in suprabasal keratinocytes (Fig. 6A). A similar pattern of expression was observed for RPTP-κ protein (Fig. 6B). In contrast, EGFR protein, the substrate for RPTP-κ, was expressed throughout the epidermis, in both basal and suprabasal keratinocytes (Fig. 6C). ERK mitogen-activated protein kinase is a major EGFR effector in many cells, including human keratinocytes. UV irradiation activates ERK1/2 in human keratinocytes in skin in vivo, and this activation is dependent on EGFR (49). Interestingly, the localization of activated ERK closely coincides with that of RPTP-κ in UV-irradiated human skin (5). This observation is consistent with inhibition of RPTP-κ leading to EGFR-dependent ERK activation of suprabasal keratinocytes in human skin in vivo. In addition, EGFR is a major mitogenic pathway in basal keratinocytes. Therefore, predominant expression of RPTP-κ in non-proliferating suprabasal keratinocytes is consistent with its role in limiting EGFR tyrosine phosphorylation. Our observation that overexpression of RPTP-κ in cultured basal keratinocytes completely inhibits proliferation provides support for this notion (6).

We have previously shown that exposure of human skin in vivo to UV irradiation increases EGFR tyrosine phosphorylation (42). Increased tyrosine phosphorylation was maximal (5-fold) 30 min after exposure. To determine the effect of UV irradiation on RPTP-κ, sun-protected buttock skin of adult subjects was exposed to UV irradiation, and skin samples were obtained 30 min post-exposure. UV irradiation had no effect on RPTP-κ protein level (Fig. 6D), in human skin in vivo, as was also observed in cultured keratinocytes (Fig. 3B). In contrast, UV irradiation inhibited RPTP-κ activity more than 60% (Fig. 6E). These results are similar to those obtained in cultured keratinocytes, and provide support for RPTP-κ as a critical regulator of EGFR tyrosine phosphorylation, in UV-irradiated skin in vivo.

Activation of signal transduction cascades and concomitant alterations in gene expression that occur in skin cells in response to UV irradiation are largely dependent on increased EGFR tyrosine phosphorylation. In human skin, EGFR-dependent responses are critical elements in the pathophysiology of UV irradiation-induced cancer and aging. Currently, with the exception of sunscreens, there are no effective measures for prevention of these serious solar UV irradiation-induced skin conditions. Our data demonstrate that oxidative inhibition of RPTP-κ is a central mechanism by which UV irradiation activates EGFR in human skin. Anti-oxidants, as topical preparations or dietary supplements, have gained popular attention with claims for a multiplicity of health benefits. However, these claims have been difficult to substantiate. One reason for this difficulty is lack of specific molecular targets for assessment of antioxidant effect. Our findings identify RPTP-κ as a key molecular target for antioxidant action for prevention of the primary manifestations of solar UV-induced skin damage.

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