RhoB Protects Human Keratinocytes from UVB-induced Apoptosis through Epidermal Growth Factor Receptor Signaling*

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Exposure of the skin to UVB light results in the formation of DNA photolesions that can give rise to cell death, mutations, and the onset of carcinogenic events. Specific proteins are activated by UVB and then trigger signal transduction pathways that lead to cellular responses. An alteration of these signaling molecules is thought to be a fundamental event in tumor promotion by UVB irradiation. RhoB, encoding a small GTPase has been identified as a DNA damage-inducible gene. RhoB is involved in epidermal growth factor (EGF) receptor trafficking, cytoskeletal organization, cell transformation, and survival. We have analyzed the regulation of RhoB and elucidated its role in the cellular response of HaCaT keratinocytes to relevant environmental UVB irradiation. We report here that the activated GTP-bound form of RhoB is increased rapidly within 5 min of exposure to UVB, and then RhoB protein levels increased concomitantly with EGF receptor (EGFR) activation. Inhibition of UVB-induced EGF activation prevents RhoB protein expression and AKT phosphorylation but not the early activation of RhoB. Blocking UVB-induced RhoB expression with specific small interfering RNAs inhibits AKT and glycogen synthase kinase-3β phosphorylation through inhibition of EGF expression. Moreover, down-regulation of RhoB potentiates UVB-induced cell apoptosis. In contrast, RhoB overexpression protects keratinocytes against UVB-induced apoptosis. These results indicated that RhoB is regulated upon UVB exposure by a two-step process consisting of an early EGFR-independent RhoB activation followed by an EGFR-dependent induction of RhoB expression. Moreover, we have demonstrated that RhoB is essential in regulating keratinocyte cell survival after UVB exposure, suggesting its potential role in photocarcinogenesis.

Among solar UV radiation that reaches the surface of the earth, UVB wavelengths are the most energetic. Directly absorbed by DNA and proteins, they account for much of the damaging biological effects of UVB irradiation including premature skin aging and cancer (1). UVB acts as a carcinogen through both DNA damage and epigenetic effects. The initiation process in UVB-induced skin carcinogenesis involves UV light-induced DNA damage such as p53 or HA-ras mutations and represents an irreversible process (reviewed in Ref. 2). By contrast, UVB-mediated skin tumor promotion involves the clonal expansion of initiated cells through activation of numerous signal transduction pathways, such as the MAPK signaling cascades that coordinate cell cycle arrest, regulation of DNA repair, and apoptosis (3).

It appears evident that after UVB exposure, the cell needs to integrate two opposing responses, with the final fate of keratinocytes depending on the balance between pro- and anti-apoptotic pathways. On the one hand, eliminating cells with excessive DNA damage is essential to preserve the health of the skin. UV-induced apoptosis plays this role through a complex mechanism triggered in three independent ways, via DNA damage-induced activation of caspases 9 and 3, through the membrane death receptor CD95 activation of caspases 8 and 3, and via cytochrome c release induced by the generation of reactive oxygen species (4). On the other hand, since human skin is chronically exposed to UV irradiation, protection of suprabasal cells is critical for maintenance of epidermal integrity and barrier function. This is probably the reason why the UVB response also includes cell survival and proliferation. UVB triggers these processes by activating receptors to various growth factors and cytokines (5). In particular, UVB activates epidermal growth factor receptor (EGFR), which in turn activates AKT through a phosphatidylinositol 3-kinase (PI3K)-dependent pathway. The survival-promoting function of PI3K-AKT in human keratinocytes exposed to UV irradiation results from the inhibition of caspases-3, -8, and -9 (6) and from the inactivation of GSK-3β, which negatively regulates numerous transcriptional factors.

The influence of epigenetic effects of chronic UV exposure is of major significance since disruption of this equilibrium promotes skin cancer. Finding new regulators of this complex response is therefore essential and will likely be critical for the development of effective prevention and intervention strategies for human skin cancer.

RhoB is a member of the Rho family of small GTPases, including Cdc42, Rac, RhoA, and RhoC. These proteins influence several important processes in cancer, including cell transformation, survival, invasion, and metastasis, through their involvement in a wide spectrum of cellular processes such as regulation of cytoskeletal organization, adhesion, motility, vesicle transport, cell cycle progression, cytokinesis, and transcription regulation (7, 8). Although some GT-Pase family members, such as RhoA, Rac1, and Cdc42, promote oncogenesis, invasion, and metastasis, there is emerging evidence that points to a tumor-suppress-
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Loss of RhoB expression plays a role in several carcinoma models (11, 13, 15, 16, 27) and triggers the signaling cascade leading to activation of AKT and NFkB. However, little or nothing is known of the cellular consequences of the induction of RhoB in response to UV irradiation. Until now, the data available only partially address the question of RhoB regulation, specifically following environmentally relevant UVB irradiation. We have shown that, after such UVB exposure, RhoB is regulated by a two-step mechanism involving activation and expression of the RhoB protein. Moreover, RhoB is essential for UVB-induced cell survival and for the EGFR-mediated survival signaling pathway.

EXPERIMENTAL PROCEDURES

Cell Culture and UV Irradiation—Spontaneously immortalized human keratinocyte cells (HaCaT cells) were cultured routinely in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum (Invitrogen). For all experiments, cells were exposed to an UVB dose of 400 J/m², which corresponds to two minimal erythema doses and reproducibly induces relevant environmental conditions.

RhoB Activity Assay—RhoB activity was assayed using the RhoA activation assay of Ren and Schwartz (28) adapted for RhoB by Campell and Mellor (22). Briefly, the Rho binding domain of rothekin, an effector of Rho proteins that selectively binds to the GTP-loaded form, was expressed as a recombinant fusion with GST in Escherichia coli and purified through binding to GSH-Sepharose beads. At various times after exposure, cells were lysed by scraping on ice and vigorous mixing in lysis buffer (50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 10 mM MgCl₂, 1% Triton X-100, 10 mM dithiothreitol, 10 mM p-nitrophenyl phosphate, 2 mM Na₂VO₄, 20 mM NaF, 1× protease inhibitor mixture) for 30 min on ice and cleared by centrifugation (14,000 rpm, 10 min). Protein concentration was determined using the BCA assay (Pierce). Proteins were resolved on SDS-PAGE with the appropriate acrylamide concentration (7.5–12.5%). The antibodies against RhoB (119), RhoA (119), p38 (N-20), phospho-EGFR (Tyr-1173), and EGFR (1005) were obtained from Santa Cruz Biotechnology. The antibodies against PARP, phospho-AKT (Ser-472/Ser-473/ Ser-474), and AKT were obtained from BD Biosciences. The antibodies against phospho-p38 (Thr-180/Thr-182), phospho-GSK-3β (Ser-9), and GSK-3β were obtained from Cell Signaling Technology. The β-actin antibody was obtained for Chemicon. The RhoC antibody was a kind gift from Sophia D. Merajver (Ann Arbor, MI).

RNA Extraction and cDNA Synthesis—Cells plated in 35-mm dishes were grown until 80% confluent and UVB-irradiated. At various times after irradiations, total RNA was isolated by TRIzol reagent (Sigma) according to the manufacturer’s instructions. Extracted RNA was quantified using RNA 6000 Nano LabChip on 2100 Bioanalyzer platform (Agilent Technologies Inc., Palo Alto, CA). 2 μg RNA of each sample was then reverse-transcribed using random primers and SuperScript II reverse transcriptase kit (Invitrogen).

siRNA Treatment—Two siRNAs against human RhoB were designed using criteria developed by Reynolds et al. (30), synthesized as synthetic oligonucleotides (Dharmacon Research), and annealed to form a short double-stranded RNA with a 3′-dithymidine overhang: siRNA RhoB 1, 5′-CCGTCTTCGAGAACTATGdTdT-3′; and siRNA RhoB 2, 5′-CTATGTGGCCGACATTGAGdTdT-3′. As an siRNA control, we used nonspecific control VIII provided by Dharamcon Research. Transfections of siRNAs (10 nM) were performed using Oligofectamine™ and Opti-MEM media (Invitrogen) according to the manufacturer’s instructions on cells at 30–50% confluency in 60-mm cell culture dishes and incubated for 24 h prior to experiments.

Study of RhoB Promoter Activity—The 1,876-bp MluI/Xhol fragment corresponding to RhoB promoter sequence was inserted into pGL3-Basic (Promega) upstream of the Firefly luciferase gene and co-transfected with a pRL-CMV vector (Promega) as an internal control (Renilla luciferase under the control of a cytomegalovirus (CMV) promoter) as described previously (31).

RhoB Overexpression Experiments—The coding region of RhoB was subcloned into pEYFP-C1 vector (Clontech) as a KpnI/BamH1 fragment. Empty pEYFP-C1 vector was used as a control. HaCaT cells were transfected with 4 μg of RhoB-YFP construct or empty vector using Lipofectamine 2000™ method (Invitrogen).

DAPI Staining—HaCaT cells were seeded on sterilized 22 × 22-mm coverslips in 35-mm dishes. At various times after irradiation, cells were fixed in a 0.3% formaldehyde solution for 20 min and then washed three times with phosphate-buffered saline. Next, coverslips were mounted on slides with Mowiol solution containing 4′,6-diamidino-2-phenylindole (DAPI). DAPI staining was detected by fluorescence microscopy.

Detection of Cell Death by ELISA—To evaluate the UVB-induced apoptosis, we used the cell death detection ELISA™PLUS kit (Roche Applied Science) according to the manufacturer’s instructions.
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RESULTS

UVB Induces RhoB GTP Binding and Expression in Human Keratinocytes—We investigated the effects of UVB irradiation on RhoB GTP binding by a pull-down assay. The level of GTP-bound RhoB increased rapidly, essentially doubling within 5 min of UVB exposure and achieving a 3.5-fold increase after 30 min, at which the level plateaued (Fig. 1, A and B). This high level of RhoB-GTP persisted until 16 h after irradiation and returned to basal levels after 24 h (data not shown). The GTP-RhoB/total RhoB ratio increased transiently, reaching a maximum between 30 and 60 min after UVB irradiation (Fig. 1B) and then returning to basal level 4 h later (data not shown). In addition, the RhoB protein expression was induced 1 h after UVB exposure (Fig. 1A), reached a maximum at about 4 h, and maintained this level until 16 h after irradiation before returning to basal levels by 24 h (Fig. 1, C and D).

Previous studies showed that RhoB induction after UVC irradiation was mediated through induction of its promoter activity (32) and through stabilization of its mRNA (25). After UVB exposure, we have shown an increase in RhoB mRNA level (a 3.5-fold maximal increase within 60 min) (Fig. 2A). This up-regulation of RhoB mRNA appeared to be triggered by both transcriptional and post-transcriptional mechanisms. On the one hand, UVB irradiation induced a 3.3-fold increase in RhoB promoter activity (Fig. 2B). On the other hand, the decay rate of RhoB mRNA under actinomycin D treatment (a transcription inhibitor) was slowed down after UVB irradiation. There was a greater than 4-fold increase in the half-life of RhoB, which increased from 60 min to more than 240 min when cells were UVB-irradiated as shown in Fig. 2C. Overall these data show that RhoB up-regulation after UVB exposure is driven by a two-step mechanism, the first step consisting of an early transient up-activation of the basal protein pool of RhoB followed by a second step involving a strong increase in RhoB protein expression mediated by both transcriptional and post-transcriptional mechanisms.

RhoB Is Up-regulated through Both EGFR-dependent and EGFR-independent Pathways—Since RhoB has been reported as being regulated by EGFR (20, 22), we investigated whether the known UVB-induced EGFR activation (33) might trigger RhoB activation and expression. Initially, we compared the kinetics of EGFR phosphorylation by UVB and by EGF in HaCaT cells. As expected, data shown in Fig. 3, A and B, indicate that EGF induced a strong activation of both EGFR and RhoB as rapidly as 15 min. These activations can be prevented by the potent and selective inhibitor of EGFR phosphorylation, ZD1839. In contrast, UVB...
induced a weak EGFR phosphorylation starting only 1 h after irradiation (Fig. 3, A and B), whereas they stimulated RhoB activation within 15 min (Figs. 1A and 3B). These observations suggest that the early UVB-induced activation of RhoB is independent of EGFR phosphorylation. We have confirmed this by showing that ZD1839 (20 \mu M) as well as AG1478 (10 \mu M), another specific inhibitor of EGFR phosphorylation, did not prevent the UVB-induced early activation of RhoB (Fig. 3B and supplemental Fig. 7). Interestingly, inhibition of EGFR by ZD1839, AG1478, or siRNA treatments fully inhibited the stimulation of RhoB expression 4 h after UVB exposure (Fig. 3C and Supplemental Fig. 8). These results suggest a critical role for EGFR activation in the regulation of RhoB expression following UVB exposure. These data therefore provide evidence that although the initial UVB-induced RhoB activation is independent of EGFR activation, the late protein induction is dependent on such activation.

RhoB Protects from UVB-induced Apoptosis—We addressed the consequences of RhoB up-regulation to the apoptotic response to UVB irradiation of HaCaT cells by specific down-regulation of protein expression using specific RNA interference. Initially, we assessed the efficiency of RhoB silencing. Following a 24-h treatment of HaCaT cells with 10 nM of RhoB siRNA, cells were UVB-irradiated, and subsequent RhoB expression was analyzed. As shown in Fig. 4A, RhoB siRNA fully abolished the UVB-induced RhoB expression, whereas neither RhoA nor RhoC expression is altered by RhoB siRNA. This demonstrates the high specificity of the RhoB siRNA used. Moreover, one can notice that UVB irradiation did not modify RhoA and RhoC expression, underlining the fact that, among other Rho proteins, only RhoB is a UVB-regulated gene. Moreover, RhoB siRNA treatment also prevented the early GTP-RhoB increase within 15 min of UVB exposure (Fig. 4B), consequently inhibiting its first activation step.

FIGURE 3. UVB induction of RhoB expression but not activity is EGFR activation dependent. A, HaCaT cells, serum-starved for 24 h, were treated with EGF (20 ng/ml) or UVB-irradiated. Cells were lysed in radioimmune precipitation buffer at the indicated times after treatment, and Western blots against EGFR (phospho-EGFR) and total were performed. B, ZD1839 (20 \mu M) was either added to or omitted from serum-starved HaCaT cells 15 min before UVB irradiation or EGF addition. 15 min later, cells were lysed, and GTP-RhoB and phospho-EGFR evaluations were performed as in Figs. 1A and 2A, respectively. Ctrl, control. C, serum-starved HaCaT cells were either left untreated or treated with ZD1839 and then UVB-irradiated or left unirradiated as described in the legend for Fig. 3B. 4 h later, total cell lysates were resolved on SDS-PAGE, and RhoB and EGFR (phospho and total) were detected as described above.

FIGURE 4. SiRNA inhibition of UVB-induced RhoB protein expression increases apoptotic cell death in HaCaT cells. A, cells treated with siRNA RhoB 1 or control siRNA were exposed or not exposed to UVB. 4 h later, cells lysates were immunoblotted with anti-RhoB, anti-RhoA, or anti-RhoC antibodies. Ctrl, control; siRhoB #1, siRNA RhoB 1. B, cells treated with siRNA RhoB 1 or control siRNA were UVB-irradiated 15 min later. Cells were lysed, and a RhoB activation assay was performed as described under “Experimental Procedures” and in the legend for Fig. 1A. C, cells treated with siRNA RhoB 1 or control siRNA were UVB-irradiated 4 h after UVB exposure. Cells were fixed and stained with DAPI. D, apoptosis was performed as described under “Experimental Procedures” and under “Materials and Methods.” E, cells were treated with siRNA RhoB 1 or control siRNA, exposed or not to UVB. At various times later, cells were lysed in radioimmune precipitation buffer. Cell lysates were resolved on SDS-PAGE and immunoblotted with the anti-PARP antibody. F, cell lysates were analyzed for nucleosomes in cytoplasmic fractions by the cell death detection ELISA kit. Data from a representative experiment are shown. The enrichment factor represents the absorbance measured at 405 nm of treated cells divided by that of the corresponding untreated cells. Values represent the means of triplicate determinations. Error bars reflect the standard deviations of the mean.
Apoptotic cell death following UVB irradiation was analyzed by DAPI staining of nuclei. As shown in Fig. 4, C and D, whereas inhibition of RhoB expression in control cells did not induce any effects, inhibition of RhoB up-regulation after UVB irradiation by siRNA induced a 3-fold increase in nuclear DNA condensation observed 16 h following UVB exposure. RhoB siRNA treatment also significantly accelerated PARP cleavage induced by UVB, which was detectable as early as 4 h after irradiation and reached a maximum at 16 h (Fig. 4E). These results have been consolidated using a method measuring nucleosomal particles produced by endonuclease cleavage of DNA during apoptosis (34). RhoB siRNA treatment significantly increased the release of oligonucleosomes detected 16 h after UVB exposure (Fig. 4F). Each of the above experiments has been confirmed using the second siRNA sequence against RhoB described under “Experimental Procedures” (supplemental Fig. 9).

To confirm the involvement of RhoB in the UVB response, we analyzed the effect of RhoB overexpression on UVB-induced apoptosis. HaCaT cells were transiently transfected either with the pEYFP-C1 vector encoding YFP as a control or with the pEYFP-RhoB plasmids in fusion with YFP. Then, apoptosis was analyzed by DAPI staining 16 h after UVB irradiation of the transfected cells. The rate of apoptosis was specifically quantitated in green fluorescent cells and compared with that of mock-transfected cells. As shown in Fig. 5, A and B, whereas expression of YFP did not modify the rate of UVB-induced apoptosis, expression of YFP-RhoB significantly reduced the number of apoptotic cells 16 h after UVB exposure from 20% ± 2.2 to 5% ± 0.9 cells. Overall these results demonstrate that RhoB is crucial to protect human keratinocytes from UVB-induced apoptotic cell death and suggest that one of the consequences of RhoB up-regulation may be to increase the survival of irradiated cells.

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RhoB Controls UVB-induced Phosphorylation of AKT through EGFR Expression—We examined the involvement of RhoB in p38 and AKT UVB-induced phosphorylation. Fig. 6A shows that siRNA down-regulation of RhoB had no effect on the UVB-induced phosphorylation of p38 at either of the time points studied. In contrast, RhoB down-regulation prevented UVB-induced AKT phosphorylation at 4 h, although not at 15 min, after irradiation. We then examined the phosphorylation of GSK-3β, which is a substrate for activated AKT. As expected, RhoB silencing prevented GSK-3β phosphorylation on Ser-9 in the same way. These data demonstrate that UVB exposure induces an early RhoB- and EGFR-independent AKT activation (Figs. 3B and 6) and a late RhoB- and EGFR-dependent AKT activation (Figs. 3C and 6 and supplemental Fig. 10). We assessed whether RhoB controls the EGFR-dependent AKT activation through EGFR regulation. As shown in Fig. 6B, down-regulation of RhoB expression prevents both UVB-induced EGFR phosphorylation and protein expression. The ratio of phosphorylated-EGFR/total-EGFR was not modified by siRNA treatment (0.85 versus 0.84 at 4 h and 0.71 versus 0.77 at 6 h), suggesting that phosphorylated EGFR decrease is due to the down-regulation of the protein expression.

Overall these data showed that RhoB plays a critical role in the UVB-induced AKT survival pathway by controlling EGFR expression. Consistent with this, inhibition of EGFR phosphorylation by ZD1839 in RhoB down-regulated HaCaT cells did not increase UVB-induced apoptosis (data not shown).
**DISCUSSION**

This study has provided evidence that the small GTPase RhoB is strongly up-regulated in human keratinocytes by environmentally relevant UVB irradiation through a two-step process consisting of an early EGFR-independent RhoB activation followed by an EGFR-dependent induction of RhoB expression. Beyond these observations, this work highlighted the protective role of this UVB-induced RhoB up-regulation in human keratinocytes, through a regulatory loop between EGFR and RhoB, which mediates AKT survival pathway.

The first step of RhoB regulation starts within a few minutes after irradiation and consists of a 3-fold increase in the RhoB-GTP/RhoB ratio. Although regulation of RhoB expression has been largely documented, this classical mode of Rho-GTPase regulation has never been described for RhoB in response to genotoxic stress. Rho-GDP/GTP cycling notably depends on the activity of Rho-GEF proteins. Among the 70 members of the Rho-GEF family, only Vav2 and XPLN have been shown to activate RhoB in vitro and in vivo (22, 35). Nevertheless, little or nothing is known about the regulation of Vav2 or XPLN by DNA-damaging agents such as UVB. Experiments are under way to determine which of the GTP/GDP cycling regulators is involved in this process.

In the second step of RhoB regulation, our results suggested that the long lasting maintenance of the RhoB-GTP abundance is allowed only by the increase in RhoB expression that is activated, most likely, by a basal cycling of GDP and GTP independently of EGFR. Indeed, we and others have previously reported that the sole constitutive expression of wild type RhoB is able to induce cellular responses such as tumor suppressive activity (9) or repression of NFkB signaling (32).

Moreover, the expression of RhoB mRNA is rapidly elevated by UVB light in human HaCaT keratinocytes as a result of transcriptional up-regulation and mRNA stabilization. Previously, transcriptional activation of RhoB by UV light was shown to be regulated via a CCAAT element in murine NIH-3T3 cells. Recently we have cloned the human RhoB promoter (31) and found numerous consensus sites with notably a CCAAT sequence. Thus one can suppose that this site is involved in UVB-induced RhoB transcription in HaCaT cells. Besides its activity on the rhoB promoter, UVB radiation also induced a significant stabilization of RhoB mRNA in HaCaT cells in a way comparable with the observed induction after UVC exposure of murine NIH-3T3 fibroblasts or normal human epidermal keratinocytes (25). This stabilization could be processed by the rhoB 3’-untranslated region that displays AU-rich elements known to be stabilized in response to UV (36). Westmark et al. (25) described that the mouse rhoB 3’-untranslated region is implicated in UV-induced stabilization of mRNA, binding HuR, a shuttle protein that stabilizes RNA containing AU-rich elements.

EGFR phosphorylation is a well established response to UV exposure as described for many cell types (for example, A431, Her14, or primary human keratinocytes). Although EGFR led within a few minutes to a potent activation of EGFR, the EGFR phosphorylation in response to UVB was detected only after 1 h and was of lower intensity. Furthermore, we have shown that the induction of RhoB protein expression is fully dependent on UVB-induced EGFR activation since inhibition of EGFR activity by specific inhibitors (ZD1839 and AG1478) or by RNA interference impeded this process. Moreover, our data suggested that UVB-induced EGFR activation induces only RhoB expression but not its activation. Indeed, the demonstration that UVB-induced RhoB activation is independent of EGFR activation is supported by three lines of evidence. i) The early UVB-induced activation of RhoB occurred in the absence of EGFR phosphorylation; ii) ZD1839 did not prevent the early RhoB activation; and iii) AG1478 treatment 3 h after UVB exposure did not affect the RhoB-GTP level (supplemental Fig. 11). These results therefore demonstrated that EGFR activation by different stimuli, e.g. EGF or UVB, involves distinct aspects of RhoB regulation, either protein expression or GTP binding.

In response to UV light, numerous signal transduction pathways are activated in keratinocytes, such as the MAPK pathways, including ERK, p38 (37), and PI3K/AKT (38). Although ERK is mostly activated by mitogenic signals, p38 is activated by stress, provided by DNA-damaging agents such as UV radiation. We have shown here that RhoB is dispensable for the UVB-induced p38 activation since RhoB down-regulation did not impair p38 phosphorylation. Moreover, it has been reported that UVB-induced p38 activation is independent of EGFR phosphorylation (39). Overall these data suggested that p38 UVB activation should be independent of the EGFR/RhoB pathway. Concomitantly with these effects on p38, UVB exposure induced an early and strong activation of AKT, which persisted for at least 4 h. Surprisingly, we observed that the early UVB-induced AKT phosphorylation was independent of EGFR and RhoB activation. In contrast, the late AKT phosphorylation and the subsequent GSK-3β inactivation were fully dependent of EGFR activation and RhoB expression. AKT and GSK-3β are involved in the control of many signaling pathways in response to UV irradiation such as c-Fos, cAMP-response element-binding protein (CREB), and Cox2 (40). These results underlined the critical role of RhoB in the UVB-induced AKT/GSK-3β pathway. Interestingly, UVB-induced RhoB expression is needed to sustain EGFR expression and phosphorylation. These results are in concordance with previous reports that show that RhoB stabilizes EGFR in regulating its endosomal transport (41). This suggested that, in response to UVB, RhoB controls AKT phosphorylation through the EGFR/PI3K pathway. We hypothesized that UVB-activated EGFR induces RhoB protein expression, which, subsequently, is necessary to maintain the high level of phosphorylated EGFR needed for AKT activation. These results were consistent with the role of RhoB in a positive feedback regulatory mechanism of the EGFR.

By examining the down-regulation and overexpression of RhoB, we have clearly demonstrated that RhoB is necessary for human keratinocytes survival after UVB exposure. Likewise, it has been shown in other cellular settings that RhoB protects primary endothelial cells from cell death (42). A pro-survival role of RhoB have also been described in response to γ-radiation. Indeed, the constitutive expression of RhoB in NIH3T3 cells-induced resistance (43) and inhibition of RhoB by a dominant negative strategy induced a radiosensitization of glioma cells (44). Moreover, overexpression of RhoB was shown to inhibit apoptosis in NIH3T3 cells after UVC irradiation (13). In this study, we have provided evidence that RhoB is required by EGFR to exert its survival function in cellular response to UV irradiation. Overall these data suggested that RhoB controls keratinocyte cell survival after UVB exposure through EGFR and AKT/GSK-3β pathway.

UVB-induced activation of EGFR promotes skin tumorigenesis by suppressing cell death and promoting cell proliferation. By contrast inhibition of EGFR suppressed proliferation, increased apoptotic cell death, and limited the onset of epidermal hyperplasia (45). Our work identifying RhoB as a critical regulator of EGFR in the UVB response suggested a definite involvement of RhoB in the promotion phase of carcinogenesis. However, in human keratinocytes, the pro-survival role of RhoB is a double-edged sword since in addition to preserving the pool of proliferative cells, it may foster survival of cells harboring oncogenic mutations and thereby promote skin carcinogenesis. Limiting RhoB expression could be a valuable target for preventing the deleterious effects of cell survival triggered by UVB exposure.
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