Mitogen- and Stress-activated Protein Kinase 1 Mediates Activation of Akt by Ultraviolet B Irradiation*

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In this study, we investigated the mechanism by which UVB irradiation activates Akt (also known as protein kinase B (PKB)) in mouse epidermal JB6 cells. Treatment with a phosphatidylinositol 3-kinase inhibitor, LY 294002, or expression of a dominant negative mutant of p85 (regulatory component of phosphatidylinositol 3-kinase) inhibited UVB-induced Akt activation. Interestingly, Akt activation by UVB was attenuated by treatment with PD 98059, a specific mitogen-activated protein kinase/extracellular signal-regulated protein kinase (Erk) kinase 1 inhibitor, or SB 202190, a specific p38 kinase inhibitor. Furthermore, the expression of a dominant negative mutant of Erk2 or p38 kinase, but not that of c-Jun N-terminal kinase 1 (JNK1), blocked UVB-induced Akt activation. The expression of a dominant negative mutant of p85 or treatment with LY 294002 also inhibited UVB-induced Erk phosphorylation. The UVB-activated mitogen-activated protein kinase members, which were immunoprecipitated from cells exposed to UVB, did not phosphorylate Akt. Instead, Akt was phosphorylated at both threonine 308 and serine 473 and activated by UVB-activated mitogen- and stress-activated protein kinase 1 (Msk1). The expression of a Msk1 C-terminal kinase-dead mutant inhibited UVB-induced phosphorylation and activation of Akt. These data thus suggested that UVB-induced Akt activation was mediated through Msk1, which is a downstream kinase of the Erk and p38 kinase signaling pathways.

Ultraviolet irradiation, especially in the UVB range (290–320 nm), accounts for most of the harmful biological effects associated with sunlight, including cancer in mammals and malformations in amphibians (1, 2). UVB has been shown to damage biological macromolecules, including lipids, proteins, and nucleic acids, and the effects of UVB on DNA damage have been investigated in detail (3). UV irradiation has also been reported to activate various signal transduction pathways and to induce the expression of specific genes (4–6). UV irradiation triggers the activation of cell surface receptors, such as epidermal growth factor receptor and the insulin receptor (7), src family tyrosine kinases (8), and the small GTP-binding proteins Ras and Rac (9, 10). The serine-threonine kinase Akt (also known as protein kinase B (PKB)) is activated by a wide variety of growth stimuli, including epidermal growth factor and insulin through phosphatidylinositol 3-kinase (PI3-K) (11, 12). Ras activates the PI3-K/Akt signaling pathway by interacting directly with the p110 catalytic subunit of PI3-K (13). In addition, Kabuya et al. (14) reported that UVB irradiation activates PI3-K in human fibroblast cells. These findings suggest that UV irradiation induces the activation of Akt. In a wide range of cellular systems, Akt has been shown to control intracellular pathways responsible for preventing cell death in response to a variety of extracellular stimuli (15–17). Furthermore, recent work has shown that Akt is not only a “cell survival” kinase but it may play an important role in protein synthesis, glycosylation, and regulation of cell cycle progression (18, 19). In contrast, identification of the gene encoding Akt as a transforming oncogene that causes thymic lymphomas in mice suggests a role for Akt in tumorigenesis (20). Overexpression of Akt has been demonstrated in a large proportion of ovarian (21), pancreatic (22), and breast cancers (23) in humans. Mutations of tumor suppressor gene Phosphatase and Tensin (PTEN), which directly antagonizes PI3-K, have been observed in a number of human cancers (24, 25). The alteration of PTEN causes elevated phosphorylation of Akt (26–28). A part of the tumor-promoting effects of UV irradiation may occur through activation of Akt. UVB plays a major role in development of human skin cancer (29, 30), and UV irradiation has been shown to act both as a tumor initiator and a tumor promoter in animals (31, 32). However, whether UV irradiation activates Akt is unclear.

In quiescent or serum-starved cells, Akt resides within the cytosol in a catalytically inactive state. Following stimulation of cells extracellularly with (e.g.) growth factors or cytokines, Akt is recruited to the plasma membrane and catalytically activated by phosphorylation at threonine 308 and serine 473 (19, 33–35). Phosphorylation of Akt at threonine 308 is catalyzed by the ubiquitously expressed and constitutively activated phosphatidylinositol 3,4,5-trisphosphate-dependent protein kinase-1 (PKD-1) (34, 35). The kinase responsible for phosphorylation of Akt at serine 473 has not been established.

+ The abbreviations used are: PKB, protein kinase B; CMV, cytomegalovirus; DN, dominant negative mutant; DTT, dithiothreitol; Erk, extracellular signal-regulated protein kinase; FBS, fetal bovine serum; JNK, c-Jun N-terminal kinase; MAP, mitogen-activated protein; MAP-KAP-K, MAP kinase-activated protein kinase; MEM, minimal essential medium; MOPS, 3-[N-morpholino]propanesulfonic acid; Msk, mitogen- and stress-activated protein kinase; PDK, phosphatidylinositol 3,4, 5-trisphosphate-dependent protein kinase; PI3-K, phosphatidylinositol 3-kinase; PTEN, Phosphatase and Tensin; Rsk, ribosomal S6 protein kinase.
definitively, although possible candidates have been proposed (36–38).

Mitogen-activated protein (MAP) kinases belong to a large family of serine/threonine protein kinases and include extracellular signal-regulated protein kinases (Erks), p38 kinase, and c-Jun N-terminal kinases (JNKs) (39–41). Generally, JNKs and p38 kinase are known to be activated by various forms of stress, such as UV irradiation, heat shock, and inflammation (40–43). Our studies and those of others have shown that Erks are critical for UV-induced signal transduction (44–46). Rane et al. (38) recently showed that the p38 kinase pathway regulates Akt activation in human neutrophils. The Erk signaling pathway also may be associated with anti-apoptotic effects (47, 48). Therefore, we investigated the involvement of the MAP kinase signaling pathways in UVB-induced Akt activation. In this study, we demonstrated that activation of Akt is induced by UVB irradiation and that the activation of Akt is mediated by Erks and p38 kinase through their downstream kinase, mitogen- and stress-activated protein kinase 1 (Msk1), in addition to the PI3-K/PDK pathway.

EXPERIMENTAL PROCEDURES

**Materials—** Fetal bovine serum (FBS) was from Gemini Bio-Product (Calabasas, CA); gentamicin was from BioWhittaker, Inc. (Walkersville, MD); Eagle’s minimal essential medium (MEM) and l-glutamine were from Life Technologies, Inc.; the MAP kinase/Erk kinase 1 specific inhibitor PD 98059 and the PI3-K inhibitor LY 294002 were from BIOMOL Research Laboratories, Inc. (Plymouth Meeting, PA); the p38 kinase inhibitor SB 202190 was from Calbiochem (La Jolla, CA); Akt fusion protein, radioactive and nonradioactive Akt immunoprecipitation kinase assay kit, anti-Msk1 antibody, anti-MAP kinase-activated protein kinase 1 (Msk1) antibody, PH domain antibody, and Akt substrate peptide were from BIOMOL Research Laboratories, Inc. (Plymouth Meeting, PA). All other reagents were of the highest grade commercially available.

**FIG. 1. Induction of Akt phosphorylation by UVB irradiation.** JB6 Cl 41 cells (80% confluence) were starved by replacing the medium with 0.1% FBS MEM and culturing for 48 h. The cells were then exposed to UVB (4 kJ/m²) or not and were subsequently cultured for the indicated time periods. The cells were lysed, and the phosphorylation levels of Akt at Thr-308 or Ser-473 were assessed as described under “Experimental Procedures.”

**FIG. 2. Inhibition of UVB-induced phosphorylation and activation of Akt by pretreatment of cells with LY 294002, PD 98059, or SB 202190.** A, JB6 Cl 41 cells (80% confluence) were starved by replacing the medium with 0.1% FBS MEM and culturing for 48 h. The cells were then pretreated with LY 294002, PD 98059, or SB 202190 for 1 h at the indicated concentrations. The cells were irradiated with UVB (4 kJ/m²) and subsequently cultured for 30 min. The cells were lysed, and the phosphorylation levels of Akt were assessed by immunoblotting. B, lysates were prepared from the cells treated as described above, and Akt was immunoprecipitated using anti-Akt1/PKBα, PH domain antibody. The activity of Akt was measured using Akt substrate peptide and [γ-32P]ATP. Columns and bars indicate the mean ± S.E. of at least three experiments. C, lysates were prepared from the cells treated as described above, and Akt was immunoprecipitated using anti-Akt1/PKBα, PH domain antibody. The activity of Akt was assessed using Bad fusion protein as a substrate, and the phosphorylation of Bad at Ser-136 was assessed by immunoblotting.
protein kinase (MAPKAP-K) 2 antibody, and anti-riboosomal S6 protein kinase kinase (Rck) 1 antibody were from Upstate Biotechnology, Inc. (Lake Placid, NY); Elk-1, c-Jun, and ATF-2 fusion protein, Akt antibody, phospho-specific Akt (threonine 380 or serine 473), Bad (serine 136), Elk-1 (serine 383), c-Jun (serine 63), and ATP-2 (threonine 71), PhosphoPlus p44/42 MAP kinase, p38 kinase, and JNKs antibody kits were from New England BioLabs, Inc. (Beverly, MA); phospho-JNK antibody (G-7) and agarose conjugated with monoclonal anti-phospho-specific Akt (threonine 308 or serine 473), Bad (serine 136), Elk-1 (serine 383), c-Jun (serine 63), and ATF-2 (threonine 71), PhosphoPlus p44/42 MAP kinase, p38 kinase, and JNKs antibody kits were from Santa Cruz Biotechnology (Santa Cruz, CA); and phosphorylinositol was from Sigma. Dominant negative mutants of Erk2, p38 kinase, or JNK1 were subcloned into a mammalian expression vector plasmid, CMV-neo. A dominant negative mutant plasmid of PI3-K p85 subunit or Msk1 C-terminal kinase-dead mutant plasmid was transfected or co-transfected with CMV-neo in JB6 Cl 41 cells by using LipofectAMINE (Life Technologies, Inc.) according to the manufacturer’s instructions. The stable transfectants were obtained by selection for G418 resistance (300 μg/ml) and further confirmed by assay of respective activities (44, 55–57).

**UV Irradiation**—UVB irradiation was performed on serum-starved monolayer cultures utilizing a transilluminator emitting UVB (54). The source of UVB was a bank of four Westhouse F520 Lamps (National Biological, Twinsburg, OH) at 6 J/cm2 light in the UVB range. Approximately 10% of the remaining radiation from the F520 lamp is in the UVB region (320 nm). Although almost no UVC leakage occurred, the UVB irradiation was carried out in a UVB exposure chamber fitted with a Kodak Kodak K6808 filter that eliminates all wavelengths below 290 nm. This lamp is one of the most frequently used UVB sources for the study of carcinogenesis. The International Agency for Research on Cancer refers to this lamp as a source emitting mainly UVB irradiation for the study of cancer induction in animals. UVB irradiation was measured using the UVX radiometer from UVF (UVX-31).

**Cell Culture**—The JB6 mouse epidermal cell line Cl 41 (JB6 Cl 41) and its stable transfectants, Cl 41 CMV-neo, Cl 41 DN-Erk2, Cl 41 DN-p85, Cl 41 DN-JNK1, Cl 41 DN-PI3-K, Cl 41 DN-p38, Cl 41 CMV5, and Cl 41 Msk1 C-dead, were grown at 37 °C in MEM supplemented with 5% heat-inactivated FBS, 2 mM l-glutamine, and 25 μg/ml gentamicin.

**Generation of Stable Cotransfectants**—The dominant negative mutants of Erk2, p38 kinase, or JNK1 were subcloned into a mammalian expression vector plasmid, CMV-neo. A dominant negative mutant plasmid of PI3-K p85 subunit or Msk1 C-terminal kinase-dead mutant plasmid or its control vector CMV5 plasmid was transfected or co-transfected with CMV-neo in JB6 Cl 41 cells by using LipofectAMINE (Life Technologies, Inc.) according to the manufacturer’s instructions. The stable transfectants were obtained by selection for G418 resistance (300 μg/ml) and further confirmed by assay of respective activities (44, 55–57).

**Immunoblotting**—Immunoblotting was carried out as described previously (44, 55, 56). In brief, JB6 Cl 41 cells and their stable transfectants were cultured to 80% confluence. The cells were starved in 0.1% FBS MEM for 48 h at 37 °C. Then, medium was changed to fresh 0.1% FBS MEM for 48 h at 37 °C. Then, medium was changed to fresh 0.1% FBS MEM for 48 h at 37 °C. Then, medium was changed to fresh 0.1% FBS MEM for 48 h at 37 °C.
UVB (4 kJ/m²) + + +
LY294002 (µM) 20 50

FIG. 4. Blocking of UVB-induced Erk phosphorylation by treatment with LY294002 or overexpression of dominant-negative mutant of PI3-K subunit p85. JB6 CI 41 cells and their transfectants, CI 41 DN-p85 (80% confluence), were starved by replacing the medium with 0.1% FBS MEM and culturing for 48 h. The cells were then irradiated with UVB (4 kJ/m²) and subsequently cultured for 30 min. The cells were lysed, and the phosphorylation levels of Erks, JNKs or p38 kinase were assessed by immunoblotting.

(Molecular Dynamics).

Assay for Erk, p38 Kinase, and JNK Activities—Cells were treated with UVB (4 kJ/m²) as described above. The cells were lysed in 400 µl of lysis buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1 mM Na₂VO₃, 1 mM β-glycerophosphate, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM phenylmethylsulfonyl fluoride, 1 µg/ml leupeptin, and 1 µM microcystin). The lysates were sonicated and centrifuged, and the supernatant fraction was incubated with the phospho-specific Erks, p38 kinase, or JNKs antibody with gentle rocking for 6–10 h at 4 °C. Then, the protein A/G plus agarose was added, and the incubation was continued for another 4 h. The beads were washed twice with 500 µl of lysis buffer and twice with 500 µl of kinase buffer (25 mM Tris, pH 7.5, 5 mM β-glycerophosphate, 2 mM dithiothreitol (DTT), 0.1 mM Na₂VO₃, 10 mM MgCl₂) containing 10 µM DTT. The kinase reactions were carried out in the presence of 200 µM ATP at 30 °C for 30 min using 5 µg of Elk-1, p38, or c-Jun as substrate for Erks, p38 kinase, or JNKs, respectively. The phosphorylated proteins were detected by immunoblotting using phospho-specific antibodies.

PI3-K Assay—PI3-K activity was carried out as described previously (57). In brief, cells were treated with UVB (4 kJ/m²) as described above. The cells were lysed in 400 µl of lysis buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM MgCl₂, 10% glycerol, 1% Nonidet P-40, 1 mM DTT, 1 mM sodium orthovanadate, 1 mM phenethylmethylsulfonyl fluoride, 10 µM aprotinin, 10 µM leupeptin). The lysate was sonicated and centrifuged, and the supernatant fraction was incubated with 20 µl of agarose conjugated with monoclonal anti-phosphotyrosine antibody PY20 with gentle rocking overnight at 4 °C. The agarose beads were washed twice with each of the following buffers: 1) PBS with 1% Nonidet P-40, 1 mM DTT, 0.1 mM sodium orthovanadate; 2) 10 mM Tris-HCl, pH 7.6, 0.5 mM LiCl, 1 mM DTT, 0.1 mM sodium orthovanadate; and 3) 10 mM Tris-HCl, pH 7.6, 0.1 mM NaCl, 1 mM DTT, 0.1 mM sodium orthovanadate. The beads were incubated for 5 min in ice in 20 µl of Buffer 3, and then 20 µl of 0.5 mg/ml phosphatidylinositol (previously sonicated in 50 mM Hepes, pH 7.6, 1 mM EGTA, 1 mM Na₂PO₄) was added. After 5 min at room temperature, 10 µl of the reaction buffer was added (50 mM MgCl₂, 100 mM Hepes, pH 7.6, 250 mM ATP containing 10 µCi of [γ-³²P]ATP), and the beads were incubated for an additional 15 min. The reaction was stopped by the addition of 15 µl of 4 N HCl and 130 µl of chloroform/methanol/NH₄OH/H₂O (60:47:2:11.3) and dried at room temperature. Radiolabeled spots were quantified using the Storm 840 scanner (Molecular Dynamics).

Akt Phosphorylation Assay—Cells were treated with UVB (4 kJ/m²), lysates were prepared from the cells, and the immunoprecipitation was carried out using the phospho-specific Erk, p38 kinase, or JNKs antibody by replacing the medium with 0.1% FBS MEM and culturing for 48 h. The cells were then irradiated with UVB (4 kJ/m²) and subsequently cultured for 30 min. Lysates were prepared from the cells, and the immunoprecipitated (IP) Msk1 and MAPKAP-K2 were subjected to a phosphorylation assay by adding the Akt fusion protein. The phosphorylation levels of Akt were assessed as described under "Experimental Procedures." B, cells were treated as described above and lysed. The immunoprecipitated MAPKAP-K2 (IP-MAPKAP-K2), Msk1 (IP-Msk1), and Rsk1 (IP-Rsk1) from the lysates were subjected to a phosphorylation assay by adding the Akt fusion protein. The phosphorylation levels of Akt were assessed as described under "Experimental Procedures."
concentrations. The cells were irradiated with UVB (4 kJ/m²) and LY 294002. The activity of Msk1 was determined as described under inhibition of UVB-induced Msk1 activation by PD 98059, SB 202190, or Msk1 antibody.

The cells were irradiated with UVB (4 kJ/m²) and subsequently cultured for the indicated periods or 30 min. Lysates were prepared from the cells, and Msk1 was immunoprecipitated using anti-Msk1 antibody. A, time course of UVB-induced Msk1 activation. B, inhibition of UVB-induced Msk1 activation by PD 98059, SB 202190, or LY 294002. The activity of Msk1 was determined as described under “Experimental Procedures.” Columns and bars indicate the mean ± S.E. of at least three experiments.

Activation of Akt by UVB-activated Msk—Cells were treated with UVB (4 kJ/m²), lysates were prepared from the cells, and the immunoprecipitation was carried out using 4 μg of anti-Mak1 or anti-MAPKAP-K2 antibodies as described above. The enzyme immune complex was added to 12.5 μl (250 ng) of Akt in assay dilution buffer, 10 μl (3 μg) of Bad protein, and 50 μl of magnesium/ATP mixture (75 mM MgCl₂ and 500 μM ATP in assay dilution buffer). The reaction was incubated for 30 min at 30 °C and centrifuged, and then 20 μl of the supernatant fraction was used as a sample of immunoblotting. Immunoblot analysis was performed by using the phospho-specific Bad (serine 136) antibody. Antibody-bound proteins were detected by chemiluminescence (ECF from Amersham Pharmacia Biotech) and analyzed using the Storm 840 scanner (Molecular Dynamics).

RESULTS

Induction of Akt Phosphorylation by UVB Irradiation—Because the phosphorylation of threonine 308 and serine 473 is a prerequisite for the catalytic activity of Akt (19, 33–35), we first investigated whether exposure of cells to UVB irradiation results in phosphorylation of Akt. As shown in Fig. 1, phosphorylation of Akt at both threonine 308 and serine 473 occurred 5 min after cells were exposed to UVB irradiation (4 kJ/m²) and reached a maximum at 30 min.

Inhibition of UVB-induced Phosphorylation and Activation of Akt by Pretreatment of Cells with LY 294002, PD 98059, or SB 202190—Akt is an established downstream target of PI3-K that is activated following stimulation of cells with growth factors (11, 12, 15–17). UVB-induced phosphorylation and activation of Akt were blocked by pretreatment with LY 294002, a PI3-K inhibitor (Fig. 2), showing that UVB-induced Akt activation is mediated through the PI3-K pathway. UV irradiation also leads to activation of the MAP kinase superfamily, composed of Erks, JNKs, and p38 kinase (9, 40–46). Therefore, we investigated the possible role of the MAP kinase family in mediating this process. To do this, we examined the influence of specific chemical inhibitors of MAP kinases on UVB-induced phosphorylation and activation of Akt. Interestingly, UVB-induced phosphorylation and activation of Akt were impaired by pretreatment of cells with PD 98059, a specific MAP kinase/Erk kinase 1 inhibitor, and/or SB 202190, a specific p38 kinase inhibitor (Fig. 2). These data suggest that both Erks and p38 kinase may be involved, in addition to the PI3-K pathway, in UVB-induced Akt activation.

UVB-induced Phosphorylation and Activation of Akt in Cells Expressing a Dominant Negative Mutant of p85, Erk2, p38 Kinase, or JNK1—To directly investigate the role of PI3-K and the MAP kinase family in UVB-induced Akt activation, we used dominant negative mutant transfectants of Erk2 (DN-Erk2) (44), p38 kinase (DN-p38) (56), JNK1 (DN-JNK1) (55), or PI3-K p85 subunit (DN-p85) (57). Overexpression of DN-Erk2, DN-p38, or DN-JNK1 specifically blocked UVB-induced Erk, p38 kinase, or JNK activities, respectively (Fig. 3A, right panel) (44, 55, 56). We also confirmed that expression of DN-p85 inhibits UVB-induced PI3-K activity (Fig. 3A, left panel). UVB-induced phosphorylation and activation of Akt was blocked by the expression of DN-p85 (Fig. 3, B and C). The expression of DN-Erk2 or DN-p38 attenuated UVB-induced phosphorylation and activation of Akt, whereas its phosphorylation and activation were not inhibited in cells expressing DN-JNK1 (Fig. 3, B and C). The difference in the strength of inhibition observed between the chemical inhibitor and DN-p38 cells for Akt activation may be caused by an inferior dominant negative effect due to loss of the transfection efficacy of the cDNA.

Erk Activation by UVB Is Mediated by PI3-K—Several studies have shown that PI3-K inhibitors repress Erk activation (58, 59). We assessed the effect of LY 294002 on UVB-induced phosphorylation of MAP kinases. The results showed that LY 294002 blocked UVB-induced Erk phosphorylation but not JNK or p38 kinase phosphorylation (Fig. 4). Moreover, cells expressing DN-p85 also blocked only UVB-induced Erk phosphorylation (Fig. 4). Thus, these results indicated that in UVB signaling, the Erk pathway is mediated by PI3-K.

Erks and p38 Kinase Regulate the Activation of Akt by UVB through Msk1—To test whether Erks and p38 kinase are direct mediators of UVB-induced Akt activation, we carried out immune complex phosphorylation assays of UVB-activated Erks, p38 kinase, or JNKs, which were immunoprecipitated from cells exposed to UVB, using an inactive Akt fusion protein as the substrate. In these experiments, the Akt protein was not phosphorylated by UVB-activated Erks, p38 kinase, or JNKs (Fig. 5A). Among the substrates of MAP kinases are members of a second family of protein-serine/threonine kinases, including Raks, Msks, and MAPKAP-Ks (MAPKAP-K2 and MAPKAP-K3). Raks are activated by Erks (60), Msks are activated by Erks and p38 kinase (53, 61), and MAPKAP-Ks are activated by p38 kinase (62, 63). These protein-serine/threonine kinases (Rsk1, Msk1, and MAPKAP-K2) are also activated in JB6 Cl 41 cells by UVB irradiation (data not shown and Figs. 7 and 8). We therefore determined whether Akt is activated by these protein-serine/threonine kinases. As shown in Fig. 5B,
UVB-activated Msk1 phosphorylated Akt at both threonine 308 and serine 473, whereas MAPKAP-K2 phosphorylated Akt at only serine 473, as previously demonstrated by Alessi et al. (64). Using the specific substrate Bad with the Akt fusion protein, UVB-activated Msk1 (but not UVB-activated MAPKAP-K2) was also observed to induce the activation of Akt (Fig. 6). Thus, phosphorylation of both sites may be necessary for Akt activation. These results suggest that Akt is, at least in part, phosphorylated via Msk1.

UVB-induced Msk1 activity was at a maximum at 30 min, corresponding to UVB-induced Akt phosphorylation (Fig. 7A), and the activity was inhibited by pretreatment of cells with PD 98059 and/or SB 202190 (Fig. 7B). Furthermore, LY 294002, which blocks UVB-induced Erk activation, also partially impaired UVB-induced Msk1 activity (Fig. 7B). Thus, Erk and p38 kinase pathways were confirmed to mediate UVB-induced Msk1 activation.

**Insulin-induced Akt Activation Is Independently Stimulated with Msk1**—Akt is known to be activated by treatment with insulin or growth factor (11, 12). To confirm whether insulin-induced Akt phosphorylation is also mediated through Msk1, we investigated the involvement of Msk1 on insulin-induced Akt1 phosphorylation. Phosphorylation of Akt was induced by the treatment with insulin for 5 min (Fig. 8A). Msk1 was only weakly activated by the treatment with insulin and the activation was a maximum by the treatment with insulin for 30 min (Fig. 8B). Insulin-induced Msk1 activation was inhibited by the pretreatment with PD 98059 (Fig. 9C), whereas insulin-induced phosphorylation and activation of Akt did not markedly change by PD 98059 (Fig. 9, A and B). On the other hand, insulin-induced phosphorylation and activation of Akt were completely blocked by the pretreatment with LY 294002 (Fig. 9, A and B). These results suggested that Msk1 does not mediate insulin-induced Akt activation.

**Msk1 Mediates UVB-induced Phosphorylation and Activation of Akt**—To test the requirement for Msk1 in UVB-induced Akt activation, we established a stable transfectant with a Msk1 C-terminal kinase-dead mutant (53). The expression of a Msk1 C-terminal kinase-dead mutant (Msk1 C-dead) inhibited UVB-induced Msk1 activity (Fig. 10A). UVB-induced phosphorylation and activation of Akt was attenuated in the Msk1 C-terminal kinase-dead mutant transfectant cells (Fig. 10, B and C). These results further supported the hypothesis that Msk1 mediates UVB-induced Akt activation.

**DISCUSSION**

Akt is a multifunctional kinase that appears to have numerous substrates in vivo, and it promotes cell survival by inhibiting apoptosis in response to growth factors and cytokines (11, 12, 15–17, 19, 65). Several studies have shown that acute metabolic effects, such as protein synthesis and glycogenesis, and cell cycle regulation are also associated with activation of Akt (18, 19). The activation of Akt requires phosphorylation at two sites, threonine 308 and serine 473. A constitutively active kinase, PDK-1 (34, 35), has been shown to phosphorylate threonine 308, but the kinase (PDK-2) responsible for phosphorylating serine 473 has not been identified. In this study, we demonstrated that Akt is phosphorylated at both threonine 308 and serine 473 and activated in response to UVB irradiation. Furthermore, we found that Erk- and p38 kinase-dependent Msk1 activation, in addition to the PI3-K/PDK-1 pathway, is required for the UVB-induced Akt activation.

Exposure of cells to UV irradiation elicits a complex set of acute cellular responses called “UV responses.” The initial signal triggering the UV response is in large part independent of DNA damage, but it instead appears to be mediated by a membrane-associated component of the Ras pathway and activation of MAP kinases (7–10, 66, 67). Upstream signaling path-
ways leading to Akt activation include PI3-K and Ras (11–13), and Akt is believed to be a multifunctional mediator of PI3-K-dependent signaling (11–13, 15–17, 19). UVB-induced Akt activation was also mediated by the PI3-K pathway. Akt has been shown to be overexpressed in ovarian, prostate, breast, and pancreatic cancers in humans and is associated with a poor prognosis and increased tumorigenicity (21–23). Mutation or down-regulation of PTEN, which directly antagonizes PI3-K, is frequently observed in a number of cancers (24, 25), and the mutation is associated with Akt activation (26–28). In addition to an inhibitory effect on apoptosis, Akt is known to regulate cell cycle progression (18, 19). Therefore, Akt activation induced by UVB may be implicated in tumorigenesis by its effects on growth progression and promotion of survival of cells damaged by UVB irradiation. In the present study, we found that Erks and p38 kinase, but not JNKs, mediate Akt activation. PI3-K inhibitors repressed Erk activation in several cell types after various modes of stimulation, as previously described (58, 59, 68). Overexpression of DN-p85 also impaired UVB-induced Erk phosphorylation, although the blocking of Erk activation has been suggested to be independent of PI3-K activity (69, 70). Thus, in the present study, Erk activation by UVB was shown to be mediated via PI3-K.

Erks are involved in survival signaling in response to a variety of growth factors (47, 48, 71), whereas activation of JNKs or p38 kinase is suggested to play decisive roles in the control of cell death (71). The activation of JNKs and p38 kinase and overexpression of MAP kinase kinase 6, an upstream kinase of p38 kinase, also have been reported to protect cells from apoptosis (72, 73). However, little direct evidence has been obtained to show that the MAP kinase family regulates Akt. Therefore, the results of our study indicating that Erks and p38 kinase mediate Akt activation suggest a novel role for MAP kinases in signal transduction. In the present study, members of the MAP kinase family did not directly phosphorylate Akt. Activated MAP kinases are translocated to the nucleus, where they phosphorylate several different transcription factors (41, 53, 74–79). In addition to phosphorylating nuclear proteins, several cytoplasmic proteins (e.g., Rsk and Msks), phospholipase A2, and the epidermal growth factor receptor have been shown to be substrates for Erks (41, 53, 60, 61, 74, 80), and p38 kinase has been shown to phosphorylate cytoplasmic proteins (e.g., MAPKAP-Ks and Msks) (55, 62, 63). In contrast to Erks and p38 kinase, which appear to have
substrates outside the nucleus, substrates for JNKs are believed, to date, to be transcription factors exclusively. Our results indicate that UVB-activated Msk1 phosphorylated Akt at both threonine 308 and serine 473, whereas UVB-activated MAPKAP-K2 phosphorylated Akt at only serine 473, as previously demonstrated by Alessi et al. (64). UVB-activated Msk1 phosphorylation and activation of Akt by overexpression of Msk1 C-terminal kinase-dead. A, the stable transfectants of JB6 Cl 41, Cl 41-CMV5 and Cl 41 MSK-C-dead (80% confluence), were starved by replacing the medium with 0.1% FBS MEM and culturing for 48 h. The cells were then irradiated with UVB (4 kJ/m²) and cultured for 30 min. Lysates were prepared from the cells, and Msk1 was immunoprecipitated using anti-Msk1 antibody. Msk1 activity was determined as described under “Experimental Procedures.” Columns and bars indicate the mean ± S.E. of at least three experiments. B, the transfectant cells were treated as described above and lysed. The levels of phosphorylation and activation of Akt were assessed by immunoblotting. C, the transfectant cells were treated as described above and lysed. Akt was immunoprecipitated from the lysates using anti-Akt1/PKBα, PH domain antibody. The activity of Akt was determined as described under “Experimental Procedures.” Columns and bars indicate the mean ± S.E. of at least three experiments.
stimulated Akt activation, and overexpression of a Msk C-terminal kinase-dead mutant inhibited UVB-induced Akt activation. Although MAPKAP-K2 has been suggested to function as PDK-2 for Akt in human neutrophils (38), UVB-activated MAPKAP-K2 did not stimulate Akt activation. A previous report suggested that phosphorylation of Akt at serine 473 may occur through an autophosphorylation mechanism following PDK-1-dependent phosphorylation of Akt at threonine 308 (37). Thus, the activation of Akt is suggested to require its phosphorylation at threonine 308 or at both threonine 308 and serine 473. Our results demonstrated that Erks and p38 kinase mediate UVB-induced Akt activation via MsK1. On the other hand, Akt activation by MsK1 may be specific in the UVB response, because PD 98059, which inhibited insulin-induced MsK1 activation, did not block insulin-induced phosphorylation and activation of Akt. Although combined addition of PD 98059 and SB 202190 completely inhibited UVB-induced MsK1 activation, the combined addition partially blocked UVB-induced Akt activation. In addition, partial inhibition of UVB-induced MsK1 activity was observed in comparison to the complete inhibition of Akt activity by a PI3-K inhibitor, suggesting that other kinase pathways, such as the PI3-K/PDK-1 pathway, are also involved in Akt activation in UVB signaling.

Taken together, the data demonstrate that Akt activation induced by UVB irradiation is mediated by the signaling of Erks and p38 kinase and their downstream protein-serine/threonine kinase, MsK1. Because Akt is a multifunctional kinase related to cell survival, cell cycle regulation, and tumorigenesis, these results provide important information regarding signal transduction and mechanisms associated with UV responses and tumor promotion.

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