Inhibition of JNK by Cellular Stress- and Tumor Necrosis Factor α-induced AKT2 through Activation of the NFκB Pathway in Human Epithelial Cells*

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Previous studies have demonstrated that AKT1 and AKT3 are activated by heat shock and oxidative stress via both phosphatidylinositol 3-kinase 3-independent pathways. However, the activation and role of AKT2 in the stress response have not been fully elucidated. In this study, we show that AKT2 is activated by cellular stress- and tumor necrosis factor α (TNFα)-dependent pathways. AKT2 binds to and phosphorylates stress-activated p38. Furthermore, AKT2 inhibits JNK and p38 activities that are required for stress-induced apoptosis. In addition, AKT2 binds to and phosphorylates IκB, an event that results in the degradation of NFκB (TNFα), epidermal growth factor, and insulin-like growth factor 1 (IGF1), mediate stress-kinase activation (1–3). Recent studies suggest that nearly all stress stimuli activate phosphatidylinositol 3-kinase (PI3K), AKT is thought to play an essential role in the cellular response to stress signals. Therefore, AKT1 and AKT3 are activated by heat shock and oxidative stress, whereas expression of AKT2 is repressed by growth factors in a PI3K-dependent manner (4–9). AKT2 is activated by cellular stress and, when NIH 3T3 fibroblasts are stressed in a variety of ways (17, 18), based on data showing that PI3K inhibitors do not prevent stress-activated AKT2 activation was PI3K-independent. Other studies, however, found that PI3K activity was required for stress-induced AKT1 activation by heat shock or oxidative stress in Swiss 3T3 cells (19, 20). It has been suggested that certain cellular stresses activate AKT1 and AKT3 but not AKT2 (19), a finding that is consistent with the different functions of the AKTs as revealed by studies of mice lacking AKT1 or AKT2 (21–23). Nevertheless, activation of AKT2 by stress and the role of AKT2 in the stress response have yet to be fully explored. The data presented here show that AKT2 is significantly activated by stress stimuli (e.g. UV irradiation, heat shock, and hyperosmolarity) by TNFα in human epithelial cells but not in fibroblasts.

This article has been withdrawn by the authors. The same data were used to represent different experimental conditions. Specifically, lanes 3 and 4 from the PI3K assay shown in Fig. 2A were reused in lanes 5 and 6 of the same panel. The P-JNK panel from Fig. 3A, left, was reused as the p-JNK panel from Fig. 6, right. The total JNK immunoblot from Fig. 6, left, was reused as the total JNK immunoblot from Fig. 6, middle. The authors state that the overall conclusions are supported by the rest of the data.

Fas ligand (12–14). Via phosphorylation of IκB, AKT1 also activates NFκB, a transcription factor that has been implicated in cell survival (15, 16).

Two separate studies demonstrated that AKT1 is activated when NIH 3T3 fibroblasts are stressed in a variety of ways (17, 18). Based on data showing that PI3K inhibitors do not prevent AKT1 activation by stress, these studies concluded that stress-induced AKT1 activation was PI3K-independent. Other studies, however, found that PI3K activity was required for AKT1 activation by heat shock or oxidative stress in Swiss 3T3 cells (19, 20). It has been suggested that certain cellular stresses activate AKT1 and AKT3 but not AKT2 (19), a finding that is consistent with the different functions of the AKTs as revealed by studies of mice lacking AKT1 or AKT2 (21–23). Nevertheless, activation of AKT2 by stress and the role of AKT2 in the stress response have yet to be fully explored. The data presented here show that AKT2 is significantly activated by stress stimuli (e.g. UV irradiation, heat shock, and hyperosmolarity) by TNFα in human epithelial cells but not in fibroblasts. Stress-induced AKT2 activation in epithelial cells is completely blocked by inhibitors of PI3K. When activated by stress, AKT2 inhibits JNK and p38 activities that are required for stress-induced apoptosis. In addition, AKT2 binds to and phosphoryl-

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§ The abbreviations used are: JNK, c-Jun N-terminal kinase; TNFα, tumor necrosis factor α; IGF1, insulin-like growth factor 1; PI3K, phosphatidylinositol 3-kinase; HA, hemagglutinin; IKK, IκB kinase; NIK, NFκB-inducing kinase; GST, glutathione S-transferase; HIEK, human embryonic kidney; TUNEL assay, terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling assay.

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Exposure of cells to environmental stress results in the activation of several signal transduction pathways including the MEKK4/MKK7/JNK1, MKK3/MKK6/p38, and IκB kinase (IKK)/IκB/NFκB cascades. Stress-induced clustering and internalization of cell surface receptors, such as those for platelet-derived growth factor, tumor necrosis factor α (TNFα), epidermal growth factor, and insulin-like growth factor 1 (IGF1), mediate stress-kinase activation (1–3). Recent studies suggest that nearly all stress stimuli activate phosphatidylinositol 3-kinase (PI3K), AKT is thought to play an essential role in the cellular response to stress signals. Therefore, AKT1 and AKT3 are activated by heat shock and oxidative stress, whereas expression of AKT2 is repressed by growth factors in a PI3K-dependent manner (4–9). Full activation of NFκB, an event that was originally designated as AKT, suppresses apoptosis induced by cellular stress. Stress-induced AKT2 activation in epithelial cells is completely blocked by inhibitors of PI3K. When activated by stress, AKT2 inhibits JNK and p38 activities that are required for stress-induced apoptosis. In addition, AKT2 binds to and phosphoryl-

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FIG. 1. AKT2 is activated by cellular stress and TNFα. A, in vitro kinase assay of AKT2 immunoprecipitates prepared from A2780 cells transiently transfected with HA-AKT2. Cells were exposed to 100 ng/ml IGF-1 (15 min), heat shock (45 °C for 20 min), 0.4 M NaCl (15 min), 40 J/m² UV-C (254 nm), or TNFα 20 ng/ml (15 min), and AKT2 activity was determined by in vitro kinase assay using histone H2B as substrate. B, OVCAR3 cells were treated with the indicated stimuli and immunoprecipitated with anti-AKT2 antibody. The immunoprecipitates were subjected to in vitro kinase assay (upper) and Western blotting analyses with anti-phospho-Ser473 AKT (middle), or anti-AKT2 (lower) antibody. The bottom panel shows relative AKT2 kinase activity quantified by phosphorimaging. Each experiment was repeated three times.
mM MnCl₂, and 1 mM dithiothreitol, all supplemented with 20 mM β-glycerol phosphate and 0.1 mM sodium vanadate. The immunoprecipitates were subjected to in vitro kinase assay or Western blotting analysis. Protein expression was determined by probing Western blots of immunoprecipitates or total cell lysates with the antibodies described above or with the appropriate antibodies as noted in figure legends. Detection of antigen-bound antibody was carried out with the ECL Western blotting Analysis System (Amersham Biosciences).

**In Vitro Protein Kinase Assay**—Protein kinase assays were performed as previously described (26, 27). Briefly, reactions were carried out in the presence of 10 Ci of [γ-³²P] ATP (PerkinElmer Life Sciences) and 3 μM cold ATP in 30 μl of buffer containing 20 mM Hepes (pH 7.4), 10 mM MgCl₂, 10 mM MnCl₂, and 1 mM dithiothreitol. Histone H2B was used as exogenous substrate. After incubation at room temperature for 30 min, the reaction was stopped by adding protein loading buffer, and proteins were separated on SDS-PAGE gels. Each experiment was repeated three times, and the relative amounts of incorporated radioactivity were determined by autoradiography and quantitated with a PhosphorImager (Molecular Dynamics).

**PI3K Assay**—PI3K was immunoprecipitated from the cell lysates with pan-p85 antibody (Santa Cruz Biotechnology). The immunoprecipitates were washed once with cold phosphate-buffered saline, twice with 0.5 M LiCl, 0.1 M Tris (pH 7.4), and finally with 10 mM Tris/100 mM NaCl/1 mM EDTA. The presence of PI3K activity in the immunoprecipitates was determined by incubating the beads in reaction buffer (10 mM HEPES [pH 7.4], 10 mM MgCl₂, 50 μM ATP) containing 20 μCi [γ-³²P] ATP and 10 μg 1,4,5-triphasphate (Bi- omol) for 20 min at 25 °C. The reactions were stopped by adding 100 μl of 1 N HCl. Phospholipids were extracted with 200 μl of CHCl₃/Methanol, and phosphorylated products were separated by thin-layer chromatography as previously described (24). The conversion of phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol 3,4,5-trisphosphate was detected by autoradiography and quantitated with a PhosphorImager.

**NFκB Transcriptional Activation Analysis**—HEK293 cells were seeded in 60-mm dishes and transfected with 1.5 μg of NFκB reporter plasmid (pElam-luc), 0.8 μg of pSV2-β-gal, and different forms (wild type, constitutively active, or dominant-negative) of HA-AKT2 or vector alone. The total amount of DNA transfected was increased to 6 μg with empty vector DNA. After serum starvation overnight, the cells were treated with UV (40 J/m²) or TNFα (20 ng/ml) and lysed with 400 μl/dish of reporter lysis buffer (Tropix). The cell lysates were cleared by centrifugation for 2 min at 4 °C. Luciferase and β-galactosidase assays were performed according to the manufacturer’s procedures (Promega and Tropix, respectively). Each experiment was repeated three times.

**Terminal Deoxynucleotidyltransferase-mediated dUTP Nick End Labeling (TUNEL) Assay**—AKT2 stably transfected A2780 cells were seeded into 60-mm dishes and grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum for 24 h and pretreated with or without LY294002 for 2 h before exposure to UV, heat shock, NaCl, or TNFα. Apoptosis was determined by TUNEL using an in situ cell death detection kit (Roche Molecular Biochemicals). The cells were trypsinized, and cytosin preparations were obtained. Cells were fixed with freshly prepared paraformaldehyde (4% in phosphate-
buffered saline (pH 7.4). Slides were rinsed with phosphate-buffered saline and incubated in permeabilization solution followed by TUNEL reaction mixture for 60 min at 37 °C in a humidified chamber. After a rinse, the slides were incubated with converter-alkaline phosphatase solution for 30 min at 37 °C and then detected with alkaline phosphatase substrate solution (Vector Laboratories, Burlingame, CA) for 10 min at 25 °C. After an additional rinse, the slides were mounted and analyzed under a light microscope. These experiments were performed in triplicate.

RESULTS

AKT2 Is Activated by UV Irradiation, Heat Shock, Hyperosmolarity, and TNFα—Previous studies showed that stress activates AKT1 and AKT3 but not AKT2 in fibroblasts (19). It has also been shown that TNFα receptor mediates UV- and heat shock-induced stress signaling (1–3). In agreement with these studies, we found that exposure of NIH 3T3 fibroblasts to UV-C, heat, or hyperosmotic conditions did not result in AKT2 activation (data not shown). It is possible, however, that stress might activate AKT2 in epithelial cells due to the fact of frequent alterations of AKT2, but not AKT1 and AKT3, in human epithelial tumors (7, 24, 27). For this reason we examined the effects of stress on AKT2 activation in two ovarian epithelial cancer cell lines, A2780 cells, which were transiently transfected with HA-AKT2, and OVCAR3 cells, which express high levels of endogenous AKT2 (7). The cells were exposed to UV-C, heat shock (45 °C), 0.4 M NaCl, or 20 ng/ml TNFα. IGF1-stimulated cells were used as controls. As assessed by in vitro kinase and Western blot analyses of AKT2 immunoprecipitates, all the stimuli substantially increased AKT2 activity in both A2780 and OVCAR3 cells (Figs. 1, A and B). The levels of AKT2 activity induced by these agents, however, were variable. AKT2 activity induced by TNFα and UV was comparable with that stimulated by IGF-1, whereas the effect of heat shock and hyperosmolarity (NaCl) on AKT2 activity was relatively smaller (Fig. 1). Nevertheless, these findings suggest that stresses activate AKT2 in a cell type-specific manner.

Stress Simulates PI3K That Mediates AKT2 Activation—To show that stress does indeed activate PI3K in epithelial cells, A2780 or HEK293 cells were exposed to UV irradiation, heat shock, and 0.4 M NaCl or TNFα, and cell lysates were immunoprecipitated with antibody to pan-p85, a regulatory subunit of PI3K. Assay of PI3K activity shows that these stress conditions as well as TNFα activated PI3K as efficiently as did IGF-1 (Fig. 2A). As described above, stress has been shown to activate AKT1 by both PI3K-dependent and -independent pathways.
To assess the role of PI3K in the stress-induced activation of AKT2, A2780 cells transfected with HA-AKT2 were exposed to LY294002, a specific PI3K inhibitor, for 30 min before stress or TNF treatment. LY294002 effectively inhibited stress- and TNF-induced AKT2 activation (Fig. 2B). These data provide direct evidence of stress-induced activation of AKT2 through a PI3K-dependent pathway in human epithelial cells.

Stress-induced AKT2 Activation Inhibits UV- and TNF-induced JNK and p38 Activities—Previous studies demonstrated that two groups of mitogen-activated protein kinases, the JNK and p38, are activated by environmental stress and TNFα (28). Therefore, we examined the effects of stress-induced AKT2 activation on the JNK and p38 to determine whether stress-induced AKT2 activation could target these two stress kinases. A2780 cells were transfected with constitutively active AKT2 or pcDNA3 vector alone. Thirty-six hours after transfection, cells were treated with TNFα or UV and analyzed by Western blot for JNK and p38 activation using anti-phospho-JNK and anti-phospho-p38 antibodies. Both JNK and p38 were activated by TNFα and UV irradiation. The maximal activation was observed at 10 min of stimulation. Expression of constitutively active AKT2, however, exhibited inhibitory effects on the activation of JNK and p38 that was induced by TNFα and UV irradiation. Notably, the activation of JNK and p38 in constitutively active AKT2-transfected cells does not significantly differ from that of the cells transfected with pcDNA3 vector at 10 min of TNFα treatment. However, the phosphorylation levels of JNK and p38 in the cells expressing constitutively active AKT2 declined much more than that of...
were transfected with different forms of AKT2 and treated with

These findings indicate that AKT2 constitutively

A ment of cells with PI3K inhibitor, wortmannin, or LY294002

induced JNK and p38 activities.

HEK293 cells were treated with or without TNF

HEK293 cells were transfected with the indicated plasmids. After treatment with or without 20 ng/ml TNF

phosphorylation (P-).

AKT2 phosphorylation sites in the IKK

AKT2 with IKK

antibody or vice versa. In both instances, the association of

GST-WT-IKKα + + + + + + 18RERLGT

GST-IKKαT23A - - + + + + 18RERLGA

pcDNA3-transfected cells after 30 min of stimulation (Fig. 3 and data not shown). We therefore conclude that the activation of AKT2 does not activate but rather inhibits TNFα- and UV-induced JNK and p38 activities.

AKT2 Interacts with and Phosphorylates IKKα, but Not NIK, Leading to IκBα Degradation and NFκB Activation—The capacity of both cellular stress and TNFα to activate the NFκB pathway is well documented (29). Previous studies also show that AKT1 induces activation of the NFκB by interaction with IKKα (13, 14). However, to date there are no reports addressing the potential role of AKT2 in the activation of the NFκB pathway.

To determine whether AKT2 associates with IKKα, HEK293 cells were treated with or without TNFα, immunoprecipitated with anti-AKT2, and immunoblotted with anti-IKKα antibody or vice versa. In both instances, the association of AKT2 with IKKα was observed (Fig. 4A). Additional studies showed that AKT2-IKKα interaction was unaffected by treatment of cells with PI3K inhibitor, wortmannin, or LY294002 (Fig. 4A). These findings indicate that AKT2 constitutively associates with IKKα. In addition, we have identified putative AKT2 phosphorylation sites in the IKKα (18RERLGT) and in NFκB-inducing kinase (NIK, 360RSREPS) (bold residue letters represent Akt consensus sequence). To determine whether IKKα and/or NIK are phosphorylated by AKT2, A2780 cells were transfected with different forms of AKT2 and treated with LY294002 and TNFα. In vitro AKT2 kinase assays were performed using FLAG-IKKα or HA-NIK, purified from the transfected COS7 cells, as substrate. Repeated experiments show that TNFα-induced AKT2 and constitutively active AKT2 phosphorylated IKKα (Fig. 4B) but not NIK (data not shown). Phosphorylation of IKKα induced by TNFα was largely attenuated by PI3K inhibitor LY294002. Quantification analyses revealed that approximate 70% of TNFα-induced IKKα phosphorylation was inhibited by pretreatment with LY294002 (Fig. 4B). Furthermore, we assessed AKT2 to determine if it phosphorylates IKKα in vivo. COS7 cells were transfected with FLAG-IKKα together with either constitutively active or dominant-negative AKT2 or vector alone and labeled with [γ-32P]orthophosphate. IKKα immunoprecipitates prepared using anti-FLAG antibody were separated by SDS-PAGE and transferred to nitrocellulose. The phoshopho-IKKα was detected by autoradiography. As shown in Fig. 4C, IKKα was highly phosphorylated in cells expressing constitutively active AKT2 but not in the cells transfected with pcDNA3 and dominant-negative AKT2. Collectively, these data indicate that IKKα is an AKT2 physiological substrate.

Activation of NFκB requires its dissociation from its cytosolic inhibitor, IκB, a process dependent on the phosphorylation and consequent degradation of IκB by IκK. Thus, we next examined AKT2 to determine if it induces IκB degradation. Immunoblotting analyses revealed that constitutively active AKT2 significantly promoted IκB degradation (Fig. 4D). To assess the involvement of AKT2 in NFκB activation, HEK293 cells were co-transfected with a NFκB-luciferase reporter and either
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**Fig. 6.** AKT2 phosphorylation of IKKα is required for inhibition of TNFα-induced JNK activation.

Immunoblotting analyses of HEK293 cells transfected with indicated expression constructs and treated with TNFα (20 ng/ml). The blots were probed with anti-phospho-JNK (p-JNK; upper) and -total JNK (middle) antibodies. Results represent one of three independent experiments. The lower panel shows the quantification of phosphorylated JNK at the indicated time points.

vector alone, wild type, or constitutively active AKT2 was able to induce NFκB activity in cells transfected with IKKα, GST fusion proteins containing either wild type IKKα (IKKαRERLGT23), termed GST-WT-IKKα or mutant IKKα (IKKαRERLGA23), termed GST-IKKαT23A were prepared and used as substrates in in vitro AKT2 kinase assays. As seen in Fig. 5A, UV- and TNFα-activated AKT2 as well as constitutively active AKT2 phosphorylated GST-WT-IKKα but not GST-IKKαT23A. We next assessed the capacity of AKT2-induced IKKα to phosphorylate IκBα. Constitutively active AKT2 was expressed in HEK293 cells, and cell lysates were immunoblotted with an antibody that specifically recognizes phosphorylated IκBα at Ser21. The results of these experiments show that constitutively active AKT2 increased IκBα phosphorylation ~2-fold and that this increase was abolished by cotransfection of pcDNA3-IKKαT23A. Expression of IKKαT23A also blocked IκBα phosphorylation induced by TNFα or UV (Fig. 5B). Additional luciferase reporter experiments demonstrated that expression of IKKαT23A inhibited the TNFα- or constitutively active AKT2-induced NFκB activation (Fig. 5C). These data indicate that phosphorylation of IKKα at Thr23 is required for AKT2-mediated NFκB activation.

**IKKα Phosphorylation by AKT2 Is Required for Inhibition of JNK but Not p38 Activation**—Recent studies showed that NFκB exerts its cell survival function by inhibition of JNK activation in response to extracellular stress (30, 31). However, it is currently unknown whether AKT-induced NFκB activation results in inhibition of JNK. Therefore, we next attempted
DISCUSSION

In this report, we have provided evidence that AKT2 is activated by extracellular stress and TNFα through a PI3K-dependent pathway in human epithelial cells. Most importantly, the activation of AKT2 inhibits stress- and TNFα-induced JNK and p38 activities and activates the NFκB cascade, leading to protection of cells from stress- and TNFα-induced apoptosis.

Previous studies show that stress activates cell membrane
receptors, including those for epidermal growth factor, platelet-derived growth factor, and IGF. As a result, receptors associate with numerous proteins that activate downstream signaling molecules (1–3). One such protein is PI3K, which has been implicated in the regulation of nearly all stress signaling pathways (1). Because the AKTs are major downstream targets of PI3K, their role in the stress response has been recently investigated. In Swiss 3T3 cells, both oxidative stress and heat shock were shown to induce a marked activation of AKT1 and AKT3 but not AKT2 (19). AKT1 activation by hyperosmotic stress in COS7 and NIH 3T3 cells has also been demonstrated (17). In this study, we show that AKT2 is activated by different stress conditions including UV irradiation, hyperosmolality, and heat shock as well as by TNFα in several human epithelial cell lines.

Three isoforms of AKT display high sequence homology and share similar upstream regulators and downstream targets as identified so far. However, there are clear differences between them in terms of biological and physiological function. In addition to the more prominent role of AKT2 in human malignancy and transformation (7, 32), the expression patterns of AKT1, AKT2, and AKT3 in normal adult tissues as well as during development are quite different (4, 8, 33). Recent studies suggest that AKT1, AKT2, and AKT3 may interact with different proteins and, thus, may play different roles in signal transduction. For instance, the Tcl1 oncoprotein preferentially binds to and activates AKT1 but not AKT2 (34). Gene knockout studies revealed that AKT1-deficient mice display defects in both fetal and postnatal growth but, unlike AKT2−/− mice, do not exhibit a type II diabetic phenotype; these differences suggest that the functions of AKT1 and AKT2 are non-redundant with respect to organismic growth and insulin-regulated glucose metabolism (21–23). It has been also shown that AKT2 but not AKT1 plays a specific role in muscle differentiation (35). In this study, we demonstrated that AKT2 is activated by a variety of stress conditions in human epithelial cells but not in fibroblasts, suggesting that activation of different isoforms of AKT is cell type-specific in response to extracellular stress.

It is controversial whether stress-induced AKT1 activation is mediated by the PI3K pathway (17–19). Two previous reports showed that PI3K inhibitors did not block heat shock- or H2O2-induced activation of AKT1 and, thus, suggested that stress (unlike growth factors) activates AKT1 in a PI3K-independent manner (17, 18). However, the opposite results were observed by other groups (19, 20). Konishi et al. also provide evidence of AKT1 activation by H2O2 and heat shock through both PI3K-dependent and -independent pathways (18). We previously demonstrated that activation of AKT2 by growth factors required PI3K activity, whereas both PI3K-dependent and -independent pathways contributed to AKT2 activation by Ras (26). In this report, we show that PI3K inhibitors completely block AKT2 activation induced by UV-C, heat shock, and hyperosmolality, indicating that stress activates AKT2 via the PI3K pathway.

JNK and p38 are stress mitogen-activated protein kinases that are activated by cytokines and a variety of cellular stresses (28). Like the classical mitogen-activated protein kinase kinase (MEK), direct activators for JNK and p38 have been identified. JNK is activated by phosphorylation of tyrosine and threonine by the dual specificity kinases, MKK4/SEK1 and MKK7. Similarly, p38 is activated by MKK3 and MKK6. However, biochemical studies have documented the existence of other JNK

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and p38 activators or inhibitors in cells stimulated by a variety of cellular stresses (28). Although previous reports showed that AKT, JNK, and p38 are downstream targets of PI3K and represent parallel pathways in response to stress (17–20, 37, 38), the data presented in this study indicate that stress- and TNFα-induced activation of AKT2 inhibits the JNK and p38 activities, suggesting that AKT2 cross-talks with JNK and p38 stress pathways.

NFκB is another critical stress response pathway (29). Activation of NFκB is achieved through the signal-induced proteolytic degradation of IkB, which is associated with and inhibits the activity of NFκB in the cytoplasm. The critical event that initiates IkB degradation is the stimulus-dependent activation of the IkB kinases IKKα and IKKβ, which phosphorylate IkB at specific N-terminal serine residues (Ser32 and Ser36 for IkBα; Ser19 and Ser23 for IkBβ). Phosphorylated IkB is then selectively ubiquitinated by an E3 ubiquitin ligase and degraded by the 26S proteosome, thereby releasing NFκB for translocation to the nucleus where it initiates the transcription of target genes (29). Moreover, two mitogen-activated protein kinase kinase (MAPKKK) members, NIK and MEKK1, have been reported to enhance the activity of the IKKs and consequently trigger the phosphorylation and destruction of the IkBs and induce the activation of the NFκB pathway (29). Recent studies also showed that AKT1 induces the NFκB cascade through activation of IKK and degradation of IkB (13, 14). In this report, we show that AKT2 physically binds to and phosphorylates IKKα but not NIK even though NIK contains an AKT2 phosphorylation consensus sequence. When activated by stress, stress or TNFα, AKT2 degrades IkB and activates NFκB-mediated transcription, indicating that stress-activated AKT2 targets the NFκB pathway.

Importantly, we have provided evidence that AKT2 induced by stress and TNFα through activation of the NFκB pathway is apoptotic in response to the stimuli that the AKT2 pathway is implicated in malignant transformation (7, 16, 17, 19). We show that cells expressing constitutively active AKT2 are resistant to stress- and TNFα-induced apoptosis, whereas cells injected with dominant-negative AKT2 and LY294002 or cells to stress- and TNFα-induced programmed cell death. These findings indicate that stress-induced AKT2 activation promotes cell survival. Among the stress-activated kinases is JNK; recent studies demonstrated that activation of JNK and p38 plays an important role in triggering apoptosis in response to extracellular stress and TNFα (36, 39–41), whereas activation of NFκB protects cells from programmed cell death (29). Although a number of downstream targets of AKT2 have been identified, our data indicate that AKT2-inhibited JNK and p38 activities and AKT2-induced NFκB activation could play, at least in part, an important role in the AKT2 pathway that protects cells from stress- and TNFα-induced apoptosis. Recent reports demonstrate that NFκB-up-regulated Gadd45α and Xiap inhibited JNK activation and abrogated TNFα-induced programmed cell death (30, 31). Our cDNA microarray experiments showed that constitutively active AKT2 induces Xiap. Thus, AKT2 inhibition of JNK activity could be due to up-regulation of Xiap by NFκB pathway (Fig. 8). Further studies are required to characterize the mechanism of inhibition of p38 stress pathway by AKT2 and involvement of Xiap in AKT2/NFκB inhibition of the JNK activation.

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REFERENCES

1. Ronai, Z. (1999) Oncogene 18, 6084–6086
2. Rosette, C., and Karin, M. (1996) Science 274, 1194–1197
3. Karin, M. (1998) Annu. Rev. Biochem. 67, 139–146
4. Bellacasa, A., Testa, J. R., Staal, S. F., and Tsichlis, P. N. (1991) Science 254, 274–277
5. Jones, P. F., Jakubowicz, T., Pitossi, F. J., Maurer, F., and Hemmings, B. A. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 4171–4175
6. Jones, P. F., Jakubowicz, T., and Hemmings, B. A. (1991) Cell. Regul. 2, 1001–1009
7. Cheng, J. Q., Godwin, A. K., Bellacasa, A., Taguchi, T., Franke, T. F., Hamilton, T. C., Tsichlis, P. N., and Testa, J. R. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 9267–9271
8. Nakatani, K., Sakaue, H., Thompson, D. A., Weigel, R. J., and Roth, A. (1999) Biochem. Biophys. Res. Commun. 257, 906–910
9. Chan, T. O., Rittenhouse, S. E., and Tsichlis, P. N. (1999) Biochim. Biophys. Acta 1421, 282–286
10. Datta, S. R., Dudek, H., Tao, X., Masters, S., Fu, H., Gotoh, Y., and Greenberg, M. E. (1997) Cell 91, 231–241
11. Cardone, M. H., Roy, N., Stennicke, H. R., Salvesen, G. S., Franke, T. F., Stanbridge, E., Frisch, S., and Reed, J. C. (1998) Science 282, 1318–1321
12. Brunet, A., Bonni, A., Zigmond, M. J., Lin, M. Z., Joo, P., Hu, L. S., Anderson, M. J., Arden, K. C., Gius, D., and Greenberg, M. E. (1999) Cell 96, 857–868
13. Ozes, O. N., Mayo, L. D., Ony, Z., Pfeffer, S. R., Pfeffer, L. M., and Donner, D. B. (1999) Nature 401, 759–762
14. Medema, R. H., Kops, G. J., Bos, J. L., and Burgering, B. M. (2000) Nature 404, 759–762
15. Medema, R. H., Kops, G. J., Bos, J. L., and Burgering, B. M. (2000) J. Biol. Chem. 275, 1136–1141
16. Gesbert, F., Sellers, W. R., Signoretti, S., Loda, M., and Griffin, J. D. (2000) Cancer Res. 60, 6861–6867
17. Sato, K., Fujisawa, T., Kobayashi, A., and Kikkawa, U. (1999) J. Biol. Chem. 274, 10225–10231
18. Cho, H., Mu, J., Kim, J. K., Thorvaldsen, J. L., Chu, Q., Crenshaw, E. B. 3rd, Arden, K. C., Blenis, J., and Greenberg, M. E. (1999) J. Biol. Chem. 274, 35968–35973
19. Roninson, I., Weng, W., Suzuki, R., Tobe, K., Kadowaki, T., and Hay, N. (2001) Genes Dev. 15, 2203–2208
20. Cho, H., Thorvaldsen, J. L., Chu, Q., Feng, F., and Birrar, M. J. (2001) J. Biol. Chem. 276, 38349–38352
21. Jiang, K., Coppola, D., Crespo, N. C., Nicosa, S. V., Hamilton, A. D., Sebit, S. M., and Cheng, J. Q. (2000) Mol. Cell. Biol. 20, 139–148
22. Shain, K. H., Jove, R., and Oshawa, N. E. (1999) J. Cell. Biochem. 73, 237–247
23. Liu, A.X., Testa, J. R., Hamilton, T. C., Jove, R., Nicosa, S. V., and Cheng, J. Q. (1998) Cancer Res. 58, 2973–2977
24. Yuan, Z., Sun, M., Feldman, R. I., Wang, G., Ma, X. L., Jiang, C., Coppola, D., Nicosa, S. V., and Cheng, J. Q. (2000) Oncogene 19, 2324–2330
25. Fuchs, S. Y., Fried, V. Z., and Ronai (1998) Oncogene 17, 1485–1490
26. Mercurio, F., and Manning, A. M. (1999) Oncogene 18, 6163–6171
27. De Smaele, E., Fazzeroni, F., Papa, S., Nguyen, D. U., Jin, R., Jones, J., Cong, R., and Francke, G. (2001) Nature 414, 308–313
28. Tan, G., Minemoto, Y., Dibbing, B., Pureell, N. H., Li, Z., Karin, M., and Lin, A. (2001) Nature 414, 313–317
29. Cheng, J. Q., Altmann, D. A., Klein, M. A., Lee, W.-C., Mysliwietz, T., Lisse, N. A., and Testa, J. R. (1997) Oncogene 14, 2793–2801
30. Altmann, D. A., G.E. Lyons, Y. Mitsuhashi, J. Q. Cheng, and Testa, J. R. (1998) Oncogene 16, 2407–2411
31. Park, H. S., Lee, J. S., Huh, S. H., Seo, J. S., and Choi, E. J. (2001) EMBO J. 20, 446–456
32. Ortiz, M. A., Lopez-Hernandez, F. J., Bayon, Y., Pühl, M., and Piedrafita, F. J. (2001) Cancer Res. 61, 8504–8512

3 M. Sun and J. Q. Cheng, unpublished data.
Inhibition of JNK by Cellular Stress- and Tumor Necrosis Factor α-induced AKT2 through Activation of the NFκB Pathway in Human Epithelial Cells
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