Stress-induced c-Jun Activation Mediated by Polo-like Kinase 3 in Corneal Epithelial Cells*

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Polo-like kinase 3 (Plk3) activation occurs after exposure to environmental or genotoxic stresses. Plk3 regulates cell fate through regulating cell cycle progression. UV irradiation is one of the major environmental stresses that affect corneal epithelial wound healing. In the present study, we report that UV irradiation activated Plk3 and that Plk3 interacts with AP-1 and c-Jun, which appears to be important to mediate corneal epithelial cell apoptosis after UV irradiation. Recombinant Plk3, as well as Plk3 immunoprecipitated from UV-irradiated cells, phosphorylated c-Jun in vitro. The phosphorylation of c-Jun by Plk3 immunoprecipitates was not altered by the pre-removal of JNK from the cell lysates. In addition, the effect of UV irradiation-induced phosphorylation of c-Jun and apoptosis were not significantly affected by knockdown of JNK mRNA. Co-immunoprecipitation reveals that Plk3 and c-Jun directly interacted with each other. Consistently, Plk3 co-localized with c-Jun to the nucleus after UV irradiation. Further, modulating Plk3 activities by overexpressing Plk3 or its mutants significantly affected UV irradiation-induced c-Jun activity and subsequent apoptosis. Our results thus provide for the first time that Plk3 mediates UV irradiation-induced c-Jun activation by phosphorylating c-Jun, suggesting that Plk3 plays an important role in mediating programmed cell death of corneal epithelial cells after UV irradiation.

UV irradiation is one of the environmental stresses that affect various cell types, including those of the corneal epithelial origin. UV irradiation-induced cellular responses share common signaling mechanisms with those induced by cytokine and other stresses, eventually modulating the programmed cell death (1). Several signaling components, including Ras-Raf, Src, and caspases, have been identified in response to UV irradiation. More importantly, exposure of mammalian cells to UV irradiation causes regulatory responses of various genes, including activation of transcription factors, AP-1 and NF-κB (2, 3), and of immediate early genes such as c-fos, c-jun, and jun-D (4–6). This activation, which is known as the UV response, is mediated by the activation of intracellular signaling pathways that are shared with growth factors and cytokines. It has been shown that Erk1/2,2 JNK/stress-activated protein kinase, and p38, which are the major players of the MAP kinase signaling pathways, mediating UV irradiation-induced corneal epithelial cell apoptosis (7–11). In addition, recent studies indicate that the stimulation of K+ channel activity causes the rapid loss of intracellular K+, which results in the activation of JNK and caspase cascades (11–14). In corneal epithelial cells, JNK1 activation is observed within 5–15 min after UV exposure, resulting in apoptosis. We have characterized the intermediary role mediated by the JNK kinase SEK, JNK, and p38 signaling pathways in this response in corneal epithelial cells (11, 15). UV-induced increases in the cell membrane K+ channel activity causes the activation of JNK kinase SEK, JNK, and caspase 3 (1).

In a previous study (16), we have found that the activation of NFκB by tumor necrosis factor-α affects cell survival in UV irradiation-induced corneal epithelial cells. Tumor necrosis factor-α induces NFκB nuclear translocation and increases the DNA binding activity of NFκB, but it does not elicit the activation of the JNK signaling pathway. The effect of increased NFκB activity in UV irradiation-induced corneal epithelial cell apoptosis is probably due to an increase in DNA repair. This study thus suggests a variation in stress-induced signaling processes in corneal epithelial cells. Polo-like kinases 3 (Plk3) is a multifunctional protein and involves stress-induced signaling pathways in fibroblasts, lymphoblastoids, and lung carcinoma cells (17–19). Plk3 is rapidly phosphorylated in response to various stresses, including ionizing radiation, reactive oxygen species, and methylmethane sulfonate. Plk3 is predominantly localized around the nuclear membrane and involved in the regulation of cell cycle progression (20). During mitosis, Plk3 appears to be localized to mitotic apparatuses such as spindle poles and mitotic spindles (29). Expression of a constitutively active Plk3 results in rapid cell shrinkage, frequently leading to the formation of cells with an elongated, unsevered midbody. Intriguingly, ectopic expression of both constitutively active Plk3 and kinase-defective Plk3K52R mutant induce apparent G2/M arrest followed by apoptosis (21, 22). In the present study, we found that UV irradiation-induced phosphorylation of c-Jun was partially mediated by Plk3. Plk3 and c-Jun proteins were co-local-

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2 The abbreviations used are: Erk1/2, extracellular signal-regulated kinases 1 and 2; JNK, c-Jun N-terminal kinase; MAP, mitogen-activated protein; Plk3, Polo-like kinase 3; HCE, human corneal epithelial; siRNA, small interference RNA; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TBS, Tris-buffered saline.
ized in the nucleus in the UV irradiation-stimulated corneal epithelial cells. Plk3 stimulated by UV irradiation directly phosphorylated c-Jun, resulting in apoptosis. Our study thus shows that Plk3 plays an important role in stress-induced c-Jun activation, in addition to its effect on the activation of the JNK signaling pathway.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfection**—Human corneal epithelial (HCE) cells were grown in Dulbecco’s modified Eagle’s medium/F-12 (1:1) culture medium containing 10% fetal bovine serum and 5 μg/ml insulin in an incubator supplied with 95% air and 5% CO₂ at 37 °C. The medium was replaced every 2 days, and cells were subcultured by treatment of cells with 0.05% Trypsin-EDTA.

Transfection of JNK1-specific siRNA (Qiagen, catalog no. SI02758637) was done by following an instruction manual provided by Qiagen. Briefly, cells were seeded at a density of 1.5 × 10⁴ per 35-mm dish in 1 ml of Dulbecco’s modified Eagle’s medium/F-12 medium. A transfection mixture was made by adding JNK1-specific siRNA (a final concentration of 25 nM) and 12 μl of HiPerFect (Qiagen, catalog no. 301705) in 100 μl of medium/F-12 medium without serum. Transfection complexes in the mixture were formed after 10 min incubation at 22 °C. The mixture was evenly dropped into cultured cells. Transfected cells were cultured in the normal growth condition for 48 h before performing experiments. Control cells were transfected with the non-silencing siRNA in the same way as described above. For UV-irradiation experiments, confluent corneal epithelial cells were placed in a tissue culture hood at a distance of 60 cm from the UV-C light source and exposed at an intensity of 45 μl/cm².

**Cell Death Detections**—An MTT assay was performed following an established protocol in our laboratory (24). Briefly, a colorimetric assay system was used to measure the reduction of a tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. The culture medium was replaced with 1 ml of serum free Dulbecco’s modified Eagle’s medium/F-12 (1:1) medium, 100 μl of MTT solution (5 mg/ml in phosphate-buffered saline) was added into each well, and the mixture was incubated for 1 h in a moisturized CO₂ incubator. Acidic isopropanol (0.4 ml of 0.04 M HCl in absolute isopropanol) was added to solubilize the colored crystals. All samples were placed into an enzyme-linked immunosorbent assay plate reader (Beckman DU-600, Spectrophotometer) at a wavelength of 570 nm with a background subtraction at 650 nm. Caspase 3 activity was determined by using the CaspACE™ assay system (Promega, Madison, WI). This system provides a highly sensitive and quantitative measurement of caspase-3 (DEVase) activity. DNA fragmentation experiments were performed to detect apoptosis, lysis buffer (200 mM Tris-HCl, pH 8.0, 100 mM EDTA, 1% SDS, and 100 μg/ml protease K) was added, and cells were then incubated for 4 h at 55 °C. The nuclear lysates were extracted twice with an equal volume of phenol and then extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25/24/1, v/v). DNA was precipitated with 0.05 volume of 5 M NaCl and 2.5 volumes of absolute ethanol, incubated overnight at −20 °C, and centrifuged at 13,000 × g for 10 min at 4 °C. The DNA pellet was dried and dissolved in TE buffer containing 20 μg/ml RNase A, and then incubated for 1 h at 37 °C. The DNA was extracted with an equal volume of phenol/chloroform/isoamyl alcohol. DNA samples were analyzed by electrophoresis in 1.5% agarose gels and visualized by staining with 1 μg/ml ethidium bromide.

**Immunocytochemistry Experiments**—For Western analysis, corneal epithelial cells (2 × 10⁶) were lysed in SDS-polyacrylamide sample buffer containing 62.5 mM Tris-HCl, pH 6.8, 2% w/v SDS, 10% glycerol, 50 mM dithiothreitol, 0.01% w/v bromphenol blue or phenol red. After denaturing, cell lysates were size-fractionated in 12% PAGE. Proteins were electrotransferred to polyvinylidene difluoride membranes. They were exposed to blocking buffer containing 5% nonfat milk in TBS-0.1% Tween 20 (TBS-T) for 1 h at 22 °C, and then incubated with the primary antibodies at 4 °C overnight. Horseradish peroxidase-conjugated secondary antibody was applied in TBS-T buffer for 1 h at 22 °C. Western blots were developed by an ECL Plus System (Santa Cruz Biotechnology, Santa Cruz, CA) and visualized by exposure of x-ray films. For immunostaining experiments, corneal epithelial cells were grown on glass slides and treated as indicated in the figures. The cells were permeated and incubated overnight at 4 °C with primary antibodies in phosphate-buffered saline-Tween 20 overnight. Slides were washed with phosphate-buffered saline and incubated with fluorescein isothiocyanate/Cy3-conjugated goat anti-mouse/rabbit IgG antibody. Immunoprecipitation experiments were carried out using corresponding antibodies for Plk3 (BD Pharmingen (25)) and c-Jun (Santa Cruz Biotechnology). Corneal epithelial cells (5 × 10⁴) were rinsed with phosphate-buffered saline and followed by incubation in 1 ml of lysis buffer (20 mM Tris, pH 7.5, 137 mM NaCl, 1.5 mM, 2 mM EDTA, 10 mM sodium pyrophosphate, 25 mM glycerophosphate, 10% glycerol, 1% Triton X-100, 1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin) on ice for 30 min. The cell lysates were spun at 13,000 × g for 10 min at 4 °C and incubated with corresponding antibodies at 4 °C overnight. The immunocomplexes were recovered by incubation with 50 μl of 10% protein A-Sepharose kinase assay buffer (expanded and stocked at 0.1 mg/ml in 20 mM HEPES, pH 7.5.) by conversion. Plk3 kinase assays were carried out by incubation of immunocomplex with casein or glutathione S-transferase-c-Jun fusion protein in 30 μl of kinase buffer (20 mM HEPES, pH 7.6, 20 mM MgCl₂, 25 mM glycerophosphate, 100 mM sodium orthovanadate, 2 mM dithiothreitol, 20 μM ATP, and 10 μCi of [γ-32P]ATP) for 30 min at room temperature. Equal volumes of samples were placed onto 12% SDS-PAGE and visualized by exposure on x-ray films.

**Electrophoretic Mobility Shift Assays**—Nuclear proteins were extracted by centrifugation at 18,000 × g for 20 min. The consensus oligonucleotide for AP-1 transcription factor (5’-CGCTTCATGAGTCAGCCGAA-3’) was 5’-end-labeled with [γ-32P]ATP by using T4 polynucleotide kinase. DNA probes were used to hybridize with extracted nuclear proteins. Reactions were conducted by adding 1 pm γ-32P-labeled DNA probe into each sample. DNA-protein complexes were displayed by electrophoresis on a 6% non-denaturing polyacryl-
amide gel. For the supershift assay, DNA-protein complexes were further incubated with a specific antibody against c-Jun. Specific competition experiments were performed by using unlabeled oligonucleotide to compete with the labeled oligonucleotide for AP-1. Nonspecific competition experiments were performed by using unlabeled AP1 oligonucleotides to interact with the γ-32P-labeled AP-1 oligonucleotides.

**Statistical Analysis**—For Western analysis, signals in the films were scanned digitally, and the optical densities were quantified with Image Calculator software. Data were shown as fractions of original values in MTT assay or relative optical density for Western blot experiments as mean ± S.E. Significant differences between the control group and treated groups were determined by one-way analysis of variance and Student’s t test at p < 0.05.

**RESULTS**

**UV-induced Activation of Plk3 and AP-1**—Plk3 is a multifunctional protein and involved in stress-induced signaling pathways. Early studies indicate that Plk3 is activated by various stresses, including reactive oxygen species and DNA-damaging drugs. We examined the role of Plk3 in UV irradiation-elicited stress responses by analyzing the activation of AP-1 in corneal epithelial cells. Human corneal epithelial cells were exposed to UV irradiation (42 μJ/cm²). Total Plk3 protein was immunoprecipitated with a monoclonal antibody and casein was used as an in vitro substrate for Plk3 kinase assays (Fig. 1A). Immunocomplex kinase assays revealed a marked increase in Plk3 activity shortly (15–60 min) following UV irradiation. Plk3 immunoprecipitated from H2O2-treated HCE cells served as the positive control. The role of Plk3 in UV irradiation-induced AP-1 activation was studied by transfecting HCE cells with cDNA encoding full-length Plk3. UV irradiation-induced AP-1 activation in the presence and absence of UV stimulation (Fig. 1B). The specificity of UV irradiation-induced AP-1 binding was competed by nuclear unlabeled AP1 oligonucleotides in an electrophoretic mobility shift assay. In addition, UV irradiation-induced c-Jun activity was identified by the supershift assay using anti-c-Jun antibodies. These studies suggest that Plk3 may be involved in UV-induced signaling pathways and, more specifically, that Plk3 may participate in activating c-Jun in the AP-1 complex.

**Effect of UV-induced Plk3 Activity on c-Jun Phosphorylation**—UV irradiation-induced activation of AP-1 involves several transcription factors, including c-fos and c-Jun. In HCE cells, UV irradiation-induced c-Jun phosphorylation was determined by using site-specific phospho-antibodies to c-Jun. UV irradiation-activated c-Jun involves phosphorylation of two serine residues (Ser-63 and Ser-73, Fig. 2A). The effect of Plk3 activation on c-Jun phosphorylation after UV irradiation was determined by the immunocomplex kinase assay. In the experiment, expressions of total c-Jun and β-actin were detected as the control. Plk3 was immunoprecipitated from UV irradiation-induced HCE cells using the antibody to Plk3; c-Jun fusion protein was used as a substrate in the assays (Fig. 2, B and C). Consistent with our prediction, Plk3 was able to effectively phosphorylate c-Jun 15 min after UV irradiation, and this phosphorylation activity lasted up to 60 min after UV treatment (Fig. 2C). As an alternative approach to confirm the phosphorylation of c-Jun by Plk3, the purified protein of constitutively activated Plk3 (Plk3a) was incubated with equal amounts of hypophosphorylated c-Jun fusion protein (2 μg per sample) in the kinase reaction mixture containing [γ-32P]ATP. There was a marked increase in c-Jun phosphorylation, as determined by fractional display in a PAGE gel, which was concentration-dependent (Fig. 2D). The specific effect of UV irradiation-induced Plk3 activation on c-Jun phosphorylation was further studied by a two-step immunoprecipitation process followed by immunocomplex kinase assays. First, the effect of UV irradiation-induced JNK-1 activation on c-Jun in HCE cells was analyzed by immunoprecipitation and in vitro kinase assays using c-Jun fusion protein as substrate. Second, Plk3 was further immunoprecipitated from the cell extracts that JNK-1 protein had...
have been depleted during the first immunoprecipitation. The precipitated Plk3 from the extracts was used to determine the effect of Plk3 activity after UV irradiation on phosphorylation of c-Jun fusion protein in immunocomplex kinase assays (Fig. 3A). The results indicate that UV stress activated Plk3, resulting in c-Jun protein phosphorylation in the absence of JNK activity. To further determine the effect of Plk3 activity on c-Jun phosphorylation, constitutively active and kinase-deficient Plk3 mutants were overexpressed in HCE cells, respectively. In the other group of experiments, HCE cells were transfected with wild-type Plk3 (Plk3<sup>wild</sup>), kinase-deficient mutant Plk3 (Plk3<sup>K52R</sup>), or the vector DNA. UV irradiation-induced Plk3 activation was determined by the immunocomplex kinase assay. There was an increased Plk3 activity in Plk3<sup>wild</sup>-transfected HCE cells. Expression levels of Plk3, JNK, and c-Jun proteins (Fig. 4C, right panel). In addition, nonspecific antibodies were used to test the specificity of c-Jun beads for control experiments (data not shown). Finally, immunostaining experiments were performed to investigate subcellular localizations of Plk3 and c-Jun in UV irradiation-induced HCE cells. Fluorescence microscopy revealed that Plk3 and c-Jun were co-localized in the nucleus in HCE cells 15 min after UV stimulation.

**FIGURE 3. Effect of UV irradiation-induced Plk3 activity on c-Jun phosphorylation.** A, effect of Plk3, purified from UV-induced and JNK removed HCE cells on c-Jun phosphorylation. UV irradiation-induced HCE cells were collected and immunoprecipitated with JNK-protein A-agarose to remove JNK protein. The supernatants precleared of JNK kinase were subjected to second immunoprecipitation with the specific antibody against Plk3. JNK and Plk3 immunoprecipitates were used in immunocomplex kinase assay, respectively. The c-Jun fusion protein was used as the substrate in in vitro kinase assays. B, effect of overexpression of Plk3 (Plk3<sup>wild</sup>) or kinase-deficient Plk3 mutant (Plk3<sup>K52R</sup>) on UV irradiation-induced c-Jun phosphorylation. Expression levels of Plk3 and phospho-c-Jun in Plk3<sup>wild</sup> and its mutant transfected HCE cells were detected by Western blot analysis using the anti-Plk3 and the anti-phospho-c-Jun antibodies. C, effect of suppressing JNK1 on UV irradiation-induced c-Jun phosphorylation. JNK1 mRNA was knocked down by using JNK1-specific siRNA. Phosphorylation of JNK and c-Jun were detected by antibodies against phospho-JNK and phospho-c-Jun, respectively. Total JNK and c-Jun expressions were detected by Western analysis.
Stress-induced Plk3 Activation and c-Jun Phosphorylation

Both Plk3 and c-Jun signals were enhanced 60 min after exposure of cells to UV irradiation (Fig. 4D). Thus, the results from studying protein-protein interaction and subcellular localization strongly suggest that Plk3 lies upstream of c-Jun and that it may directly regulate the c-Jun function by phosphorylation after UV irradiation.

Effect of UV-induced Plk3 Activation on Apoptosis—Previous studies demonstrate that apoptosis of HCE cells induced as a result of UV irradiation is mediated by the activation of JNK. Therefore, UV irradiation-induced apoptosis was further determined in JNK1 mRNA knockdown cells. Exposure of cells to UV irradiation decreased cell viability and increased DNA fragmentation in both of the control and JNK1 mRNA knockdown cells, respectively (Fig. 5, E and F). The results shown in Fig. 5 indicate that Plk3 activation and subsequent c-Jun phosphorylation by Plk3 plays an important role in UV stress-induced apoptosis in addition to the JNK signal pathway.

DISCUSSION

One important function of the cornea is to act as a barrier that protects the corneal interior from being damaged by noxious environmental hazards such as UV irradiation. Recent studies show that UV irradiation induces the activation of a Kv channel in the cell membrane and activates JNK cascades to phosphorylate c-Jun protein, resulting in corneal epithelial cell apoptosis (11, 26). The results from our present study are consistent with others that exposure of HCE cells to UV irradiation increased c-Jun phosphorylation at two residues proximal to the major transactivation domain, including serines 63 and 73 (Fig. 2). Phosphorylation of c-Jun is required for the efficient transactivation function, and the kinases responsible for this modification include MAP kinases, such as JNKs (27, 28). Interestingly, UV irradiation also activated Plk3 within 15 min of UV exposure. Plk3 is a protein serine/threonine kinase and involved in the regulation of a variety of events during the cell cycle (29). It is very likely that, in addition to JNK, Plk3 also participates in phosphorylation and activation of c-Jun in HCE cells, especially in response to UV irradiation. Indeed, direct phosphorylation of c-Jun by Plk3 was demonstrated in two independent experiments. First, UV-induced AP-1 activation was significantly enhanced by overexpression of wild-type Plk3 in HCE cells. Consistently, the effect of Plk3 on AP-1 activation was observed in cells transfected with a constitutively active Plk3 cDNA construct (Fig. 1B). Second, activated Plk3 immunoprecipitated from UV-induced HCE cells was able to directly phosphorylate c-Jun fusion protein in vitro (Fig. 2). The experiment that pre-depletes JNK also strongly supports the notion that JNK may not be the major contaminant in Plk3 immunocomplex kinase assays (Fig. 3). With pre-removal of JNK, Plk3 precipitated from the same cell extracts can still strongly phos-
Stress-induced Plk3 Activation and c-Jun Phosphorylation

A

|        | Plk3<sup>wild</sup> | Vector |
|--------|---------------------|--------|
| p-c-Jun| +                   | +      |
| c-Jun  | +                   | +      |
| β-actin| +                   | +      |

B

![Diagram showing survival cell index in response to UV light and Plk3 expression](image)

C

![Diagram showing survival cell index in response to JNK1 siRNA](image)

D

![Diagram showing caspase 3 activity in response to Plk3-PBD expression](image)

E

![Diagram showing survival cell index in response to UV light and JNK1 siRNA](image)

F

![Diagram showing survival cell index in response to UV light and JNK1 siRNA](image)

FIGURE 5. Increased c-Jun phosphorylation and apoptosis in UV-stimulated cells by overexpression of Plk3. A, effect of overexpression of Plk3<sup>wild</sup> on UV irradiation-induced c-Jun phosphorylation. B, effect of overexpression of Plk3<sup>wild</sup> on UV irradiation-induced decreases in cell viability detected by MTT assays. C, effect of overexpression of constitutively active Plk3-PBD on UV irradiation-induced decreases in cell viability detected by MTT assays. D, effect of overexpression of constitutively active Plk3-PBD on UV irradiation-induced cell death determined by measuring caspase 3 activity. E, effect of suppressing JNK1 on UV irradiation-induced decreases in cell viability detected by MTT assays. F, effect of suppressing JNK1 on UV irradiation-induced cell death determined by measuring DNA fragmentation. JNK1 activity was suppressed by knockdown of JNK1 mRNA with JNK1-specific siRNA. HCE cells were collected 1 day after transient transfection of cDNAs encoding full-length Plk3<sup>wild</sup> and Plk3-PBD. Cells were lysed for Western analysis after 1-h incubation in culture medium or collected for MTT assay after a 12-h incubation period. *, statistical significance (n = 4, p < 0.05).

Stress-induced Plk3 Activation and c-Jun Phosphorylation

phorylate c-Jun fusion protein in vitro, suggesting that the effect of Plk3 on c-Jun phosphorylation is rather specific.

The regulatory relationship between Plk3 and c-Jun in HCE cells is also confirmed by protein-protein interaction studies. Both Plk3 and c-Jun proteins are capable of pulling down each other. Intriguingly, the phospho-specific anti-c-Jun antibody, but not the general c-Jun antibody, precipitated Plk3. This suggests that a much stronger interaction exists between Plk3 and phosphorylated c-Jun (Fig. 4B). On the other hand, it is also possible that the c-Jun antibody may disrupt the interaction between c-Jun and Plk3 through competing for the same binding site on c-Jun or through inducing a conformational change. It is known that phosphorylation of c-Jun determines its function (28). Thus, it is tempting to speculate that phosphorylation of c-Jun by Plk3 also leads to down-regulation of c-Jun function.

During interphase, a low level of Plk3 signals can be detected throughout the cell; during mitosis, Plk3 appears to be localized to mitotic apparatuses, such as spindle poles and mitotic spindles (21, 22). Our fluorescence microscopy experiments show that little nuclear Plk3 is detected in untreated control cells. Following UV exposure, Plk3 appears to accumulate in the nucleus, forming visible foci in HCE cells and co-localizing with c-Jun (Fig. 4D). These subcellular localization studies thus also strongly suggest that Plk3 directly interacts with c-Jun and plays a role in stress-induced signaling pathways to regulate cell cycle arrest and apoptosis (30).

Our results indicate that Plk3 involves the regulation of UV irradiation-induced HCE cell apoptosis, and overexpression of Plk3<sup>wild</sup> increased UV irradiation-induced HCE cell apoptosis (Fig. 5). The effect of Plk3 on cell viability was further confirmed by expression of a constitutively active Plk3-PBD cDNA construct. Transient expression of Plk3-PBD in HCE cells significantly reduced viability in the absence of UV stimulation and remarkably enhanced UV irradiation-induced apoptosis (Fig. 5). These results are consistent with results of previous studies. Plk3 is identified as an immediate early response gene product and involves growth factor-elicited cell cycle regulation and stress-induced DNA damage responses (18, 20, 23, 31). Ectopic expression of Plk3 and its mutants result in dramatic morphological changes, G2/M arrest, and apoptosis (21, 22). In our recent reports, exposure of corneal epithelial cells to UV irradiation stimulates JNK activities, and this activation is associated with UV irradiation-evoked increases in cell membrane K<sup>+</sup> channel activity (26). Activation of the JNK signaling pathway by UV irradiation finally results in corneal epithelial apoptosis (11). Now, we provide new evidence for a functional role of Plk3 in UV stress-induced AP-1 and c-Jun activation. In addition to activation of the JNK signaling pathway, UV irradiation-activated Plk3 interacts with c-Jun and modulates c-Jun by protein phosphorylation, subsequently resulting in corneal epithelial cell apoptosis.

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