ERK promotes hydrogen peroxide-induced apoptosis through caspase-3 activation and inhibition of Akt in renal epithelial cells

Shougang Zhuang, Yan Yan, Rebecca A. Daubert, Jiahuai Han, and Rick G. Schnellmann

Departments of Pharmaceutical Sciences and Surgery, Medical University of South Carolina, Charleston, South Carolina; and Department of Immunology, The Scripps Research Institute, La Jolla, California

Submitted 16 May 2006; accepted in final form 24 July 2006

ERK promotes hydrogen peroxide-induced apoptosis through caspase-3 activation and inhibition of Akt in renal epithelial cells. Am J Physiol Renal Physiol 292: F440–F447, 2007. First published August 1, 2006; doi:10.1152/ajprenal.00170.2006.—Reactive oxygen species, including hydrogen peroxide (H2O2), are generated during ischemia-reperfusion and are critically involved in acute renal failure. The present studies examined the role of the extracellular signal-regulated kinase (ERK) pathway in H2O2-induced renal proximal tubular cells (RPTC) apoptosis. Exposure of RPTC to 1 mM H2O2 resulted in apoptosis and activation of ERK1/2 and Akt. Pretreatment with the specific MEK inhibitors, U0126 and PD98059, or adenoviral infection promotes hydrogen peroxide-induced apoptosis. In contrast, expression of constitutively active MEK1 enhanced H2O2-induced apoptosis. H2O2-induced activation of caspase-3 and phosphorylation of histone H2B at serine 14, a posttranslational modification required for nuclear condensation, which also were blocked by ERK1/2 inhibition. Furthermore, blockade of ERK1/2 resulted in an increase in Akt phosphorylation and blockade of Akt potentiated apoptosis and diminished the protective effect conferred by ERK inhibition in H2O2-treated cells. Although Z-DEVD-FMK, a caspase-3 inhibitor, was able to inhibit histone H2B phosphorylation and apoptosis, it did not affect ERK1/2 phosphorylation. We suggest that ERK elicits apoptosis in epithelial cells by activating caspase-3 and inhibiting Akt pathways and elicits nuclear condensation through caspase-3 and histone H2B phosphorylation during oxidant injury.

oxidative stress; renal proximal tubular cells; extracellular signaling-regulated kinase; phosphoinositide 3-kinase; histone

REACTIVE OXYGEN SPECIES (ROS) have been implicated in the pathogenesis of a variety of renal diseases, including ischemia-reperfusion and toxic acute renal failure, and cell death (7, 16, 28, 29, 33). ROS include superoxide anion, hydrogen peroxide (H2O2), and hydroxyl radical. Among them, H2O2 is a common mediator and is generated during hypoxia/reoxygenation and ischemia-reperfusion injury (18, 19, 28, 29). Therefore, understanding the signaling pathways of H2O2-induced cell death would provide important clues about the mechanisms of renal tubular cell injury and acute renal failure.

Caspases are a 12-member family of specific cysteine proteases. Among them, caspase-3 is a primary mediator in the effector phase of apoptosis induced by a variety of stimuli including H2O2 (8, 13, 22). Caspase-3 can be activated through intrinsic and extrinsic mechanisms. Intrinsic pathways are activated by some stress stimuli and lead to cytochrome c release from the mitochondria (13, 21). Released cytochrome c promotes the activation of caspase-9 through Apaf-1 and then activates caspase-3. Caspase-3 activation leads to cleavage and activation of caspase-activated DNase (CAD) and activated CAD is responsible for internucleosomal DNA fragmentation, a hallmark of apoptosis (40). Another hallmark of the terminal stages of apoptosis is chromatin condensation (10, 41). Although caspase-3 activation is also coupled to apoptotic nuclear condensation, the signaling molecules that transduce nuclear condensation remain poorly understood. Recently, histone modification, in particular, phosphorylation of histone H2B at serine 14, was associated with nuclear condensation in mammalian cells and yeast (1, 9). Caspase-3 induces phosphorylation of histone H2B in vitro and in vivo through cleavage of Mst1 kinase, a ubiquitously expressed serine/threonine kinase (9).

Exposure of cells to oxidant stress also induces activation of multiple members of the mitogen-activated protein kinases (MAPK) including extracellular signal-regulated kinase1/2 (ERK1/2), c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), and p38 kinase (35, 36), and the phosphoinositide 3-kinase (PI3K)/Akt pathway (42). Among these kinases, JNK and p38 pathways are frequently associated with induction of apoptosis, whereas the ERK and Akt pathways are thought to deliver survival signals that protect cells from apoptosis (42). However, increasing evidence reveals that ERK activation contributes to apoptosis in certain cell types and organs. For example, cisplatin induces ERK1/2 activation and inhibition of ERK-blocked apoptosis in renal epithelial cells (5, 23, 26). Furthermore, ERK inhibition by U0126 was renal protective in cisplatin-induced nephrotoxicity in mice (21).

Although ERK is activated in other forms of acute tubular cell injury such as renal ischemia-reperfusion injury in animals (31), the role of ERK in the pathogenesis remains to be established. With the use of in vitro models of ischemia-reperfusion injury, conflicting evidence exists concerning the role of ERK in apoptosis. Arany et al. (6) and Di Mari et al. (11) reported that ERK activation is required for cell survival in mouse renal proximal tubular cells (RPTC) exposed to H2O2. Other studies showed that ERK activation contributes to either apoptosis in OK cells following H2O2 treatment (25) or necrotic cell death in LLC-PK1 cells exposed to 2,3,5-tris-(glutathione-S-yl) hydroquinone, which induces toxicity via ROS generation and H2O2 (12, 32). Consequently, additional studies are needed to clarify the role of ERK in oxidant injury-induced apoptosis and the signaling thereof.

We previously made improvements to the culture of rabbit RPTC such that the degree of differentiation and similarity to...
RPTC found in vivo is greater than other models of RPTC (27). Using this model and pharmacological and molecular approaches, we examined the role of ERK1/2 in H₂O₂-induced apoptosis and found that ERK is necessary for H₂O₂-induced apoptosis of RPTC. Furthermore, we provide evidence that ERK1/2-mediated apoptosis is through activation of caspase-3 and inactivation of PI3K/Akt pathway following oxidant injury and that nuclear condensation is the result of caspase-3-mediated histone H2B phosphorylation.

MATERIALS AND METHODS

Chemicals and reagents. Z-DEVD-FMK, Z-VAD-FMK, U0126, SB203580, SP600125, and PD98059 were obtained from Calbiochem (San Diego, CA). Rabbit anti-phospho-ERK1/2, anti-phospho-p38, anti-ERK1/2, anti-p38, anti-phospho-H2B-S14, anti-H2B, and anti-active caspase-3 were purchased from Cell Signaling (Boston, MA). All other chemicals were from Sigma (St. Louis, MO). All antibodies were used at 1:1,000 for immunoblot analysis.

Results

Role of MAPK activation in H₂O₂-induced apoptosis. Since both pro- and anti-apoptotic roles for ERK have been reported in renal epithelial cells following oxidant injury (6, 30), we further evaluated the role of ERK activation in H₂O₂-induced cell death using U0126 and PD98059, two specific inhibitors of the ERK upstream kinase MEK1/2 (3, 14). Treatment of RPTC with H₂O₂ for 5 h resulted in apoptotic nuclear changes in 49 ± 7% of the cells (Fig. 3A). Inhibition of ERK using U0126 or PD98059 decreased RPTC apoptotic nuclear changes to 11 ± 1% (Fig. 3B). These data reveal that the time courses for activation of these kinases are different following oxidant injury in RPTC and may be associated with different functions.

H₂O₂-induced apoptosis in RPTC. Primary cultures of RPTC were exposed to 1 mM H₂O₂, and cell viability was examined using the MTT assay. Cell viability was not changed during the initial 3 h after H₂O₂ exposure but decreased to 63 ± 4% at 4 h, 55 ± 8% at 5 h, and 52 ± 3% at 6 h (Fig. 1A). The 5-h time point was used for most of the subsequent studies. H₂O₂ exposure resulted in cell shrinkage, chromatin condensation, and increased caspase-3 activity, hallmarks of apoptosis (Fig. 1B and see Fig. 5A). With the use of Trypan blue staining as a marker of necrotic cell death, less than 5% of cells were necrotic during our experimental period (data not shown).

H₂O₂-induced activation of ERK, p38, and Akt in a time-dependent manner. Our previous studies showed that RPTC exposure to H₂O₂ resulted in ERK1/2, p38, and Akt activation, but not JNK (44, 45). Because these kinases are involved in the regulation of apoptosis and cell survival, we examined their activation profiles over longer time points. ERK1/2 was constitutively phosphorylated under basal conditions, and phosphorylation increased within 10 min following H₂O₂ exposure and persisted for 60 min (Fig. 2). The level of ERK1/2 phosphorylation began to decrease at 120 min and returned to basal levels at 180 min. p38 phosphorylation also increased within 10 min following H₂O₂ exposure and then decreased gradually over 240 min (Fig. 2). Total expression level of all these proteins did not change (Fig. 2). These data reveal that the time courses for activation of these kinases are different following oxidant injury in RPTC and may be associated with different functions.

Caspase-3-like activity assay. Caspase activity was measured by a colorimetric assay following the manufacturer’s instructions (BioVision, Palo Alto, CA). Cells were lysed in 0.1 M HEPES buffer (pH 7.4) containing 2 mM dithiothreitol, 0.1% 3-(3-cholamidopropyl)dimethylammonio)-1-propanesulfonate, and 1% sucrose. Cell lysates were incubated with a colorimetric substrate, Ac-DEVD-pNA, for 30 min at 30°C. The release of the chromophore p-nitroanilide was measured using a fluorescence spectrophotometer.

Statistical analysis. Data are presented as means ± SD and were subjected to one-way ANOVA. Multiple means were compared using Tukey’s test. P < 0.05 was considered a statistically significant difference between mean values. Renal proximal tubules isolated from an individual rabbit represent a single experiment (n = 1) consisting of data obtained from three wells.

RESULTS

H₂O₂-induced apoptosis in RPTC. Primary cultures of RPTC were exposed to 1 mM H₂O₂, and cell viability was examined using the MTT assay. Cell viability was not changed during the initial 3 h after H₂O₂ exposure but decreased to 63 ± 4% at 4 h, 55 ± 8% at 5 h, and 52 ± 3% at 6 h (Fig. 1A). The 5-h time point was used for most of the subsequent studies. H₂O₂ exposure resulted in cell shrinkage, chromatin condensation, and increased caspase-3 activity, hallmarks of apoptosis (Fig. 1B and see Fig. 5A). With the use of Trypan blue staining as a marker of necrotic cell death, less than 5% of cells were necrotic during our experimental period (data not shown).

H₂O₂-induced activation of ERK, p38, and Akt in a time-dependent manner. Our previous studies showed that RPTC exposure to H₂O₂ resulted in ERK1/2, p38, and Akt activation, but not JNK (44, 45). Because these kinases are involved in the regulation of apoptosis and cell survival, we examined their activation profiles over longer time points. ERK1/2 was constitutively phosphorylated under basal conditions, and phosphorylation increased within 10 min following H₂O₂ exposure and persisted for 60 min (Fig. 2). The level of ERK1/2 phosphorylation began to decrease at 120 min and returned to basal levels at 180 min. p38 phosphorylation also increased within 10 min following H₂O₂ exposure and then decreased gradually over 240 min (Fig. 2). Total expression level of all these proteins did not change (Fig. 2). These data reveal that the time courses for activation of these kinases are different following oxidant injury in RPTC and may be associated with different functions.

Role of MAPK activation in H₂O₂-induced apoptosis. Since both pro- and anti-apoptotic roles for ERK have been reported in renal epithelial cells following oxidant injury (6, 30), we further evaluated the role of ERK activation in H₂O₂-induced cell death using U0126 and PD98059, two specific inhibitors of the ERK upstream kinase MEK1/2 (3, 14). Treatment of RPTC with H₂O₂ for 5 h resulted in apoptotic nuclear changes in 49 ± 7% of the cells (Fig. 3A). Inhibition of ERK using U0126 or PD98059 decreased RPTC apoptotic nuclear changes to

F441
23 ± 8 and 28 ± 11%, respectively. The exposure of RPTC to U0126 or PD98059 alone for 5 h had no effect on apoptosis. Consistent with this observation, pretreatment of U0126 or PD98059 enhanced cell viability as measured by the MTT assay in H2O2-treated cells (see Fig. 8B). At the concentrations used, U0126 and PD98059 selectively inhibited the ERK1/2 phosphorylation as shown in our previous study (43).

We also examined the role of p38 and JNK pathways in H2O2-induced RPTC apoptosis. Preincubation of cells with SB203580, a p38 inhibitor, or SP600125, a JNK inhibitor, had no effect on apoptotic nuclear changes in H2O2-treated cells (Fig. 3B). These data reveal that ERK, but not p38 nor JNK, mediates apoptosis in RPTC following oxidant injury.

Effects of overexpression of constitutively active MEK1 and negative mutant of MEK1 on RPTC viability following H2O2 exposure. To confirm the role of ERK in H2O2-induced cell death, we evaluated the viability of RPTC overexpressing constitutive active MEK1 (Ad-MEK1ca) and negative mutant of MEK1 (Ad-MEK1dn) after H2O2 exposure. A further decrease in cell viability was observed in RPTC expressing Ad-MEK1ca and exposed to H2O2 (Fig. 4A). In contrast, overexpression of Ad-MEK1dn resulted in increased cell viability in RPTC exposed to H2O2 (Fig. 4C). The efficacy of both Ad-MEK1ca and Ad-MEK1dn was determined by immunoblot analysis. ERK1/2 phosphorylation levels increased in Ad-MEK1ca- and Ad-MEK1dn-
transfected cells, respectively (Fig. 4, B and D). These results confirm the participatory role of ERK1/2 activation in RPTC apoptosis.

Caspase-3-like activity mediates H2O2-induced apoptosis. Because many apoptotic stimuli induce apoptosis through activation of caspase-3 (8, 13, 22), ERK activation may induce cell death through a caspase-3-dependent pathway. To test this possibility, we examined whether H2O2 increased caspase-3 activity using DEVD-pNA as a substrate. RPTC exposure to H2O2 increased caspase-3 activity by 2.5-fold at 5 h (Fig. 5A). Caspase-3 activation was completely blocked by Z-VAD-FMK, a pan-caspase inhibitor, or Z-DEVD-FMK, a caspase-3, -6, and -7 inhibitor. Furthermore, Z-DEVD-FMK also blocked apoptotic nuclear changes (Fig. 5B). These data reveal that caspase-3 mediates apoptosis in H2O2-treated RPTC.

ERK acts upstream of caspase-3 during H2O2-induced apoptosis. Involvement of both ERK and caspase-3 in H2O2-induced apoptosis suggests the possibility that they may act in the same apoptotic pathway. Inhibition of ERK with U0126 or PD98059 blocked H2O2-induced caspase-3 activation (Fig. 6A). In contrast, neither Z-VAD-FMK nor Z-DEVD-FMK treatment altered the phosphorylation level of ERK1/2 induced by this oxidant (Fig. 6B). These data clearly illustrate that ERK acts upstream of caspase-3 in the signaling pathway which leads to apoptosis in H2O2-treated RPTC.

Effects of U0126 on phosphorylation of histone H2B induced by H2O2. The above studies revealed that nuclear condensation is dependent on caspase-3 activation. Because phosphorylation of histone H2B at serine 14 is associated with nuclear condensation during apoptosis (9), we determined whether H2O2 induces histone H2B phosphorylation in RPTC and, if so, whether this is mediated by ERK and/or caspase-3. Treatment with H2O2 induced histone H2B phosphorylation at S14 at 3 h and was persistent until 5 h (Fig. 7A). In the presence of U0126, the phosphorylation of H2B was inhibited largely (Fig. 7B). Similarly, incubation of cells with Z-DEVD-FMK blocked H2B phosphorylation (Fig. 7C). We suggest that the proapoptotic role of ERK in H2O2-treated RPTC apoptosis is through caspase-3 activation and, subsequently, the phosphorylation of histone H2B.

Blockade of PI3K/Akt enhances H2O2-induced cell death and attenuates the protective effect resulting from ERK inhibition. It has been reported that activation of PI3K/Akt is required for cell survival in cells exposed to a variety of stress stimuli, including H2O2 (4, 22). Given the effects of ERK1/2 inhibition on RPTC survival following H2O2 exposure, it is possible that ERK inhibition further activates PI3K/Akt-mediated survival signaling. To test this hypothesis, we examined the effect of ERK inhibition on Akt phosphorylation in RPTC exposed to H2O2. H2O2 treatment increased Akt phosphorylation, which was enhanced in the presence of U0126 (Fig. 8A). However, U0126 alone did not affect the basal level of Akt phosphorylation. In the presence of LY294002, a specific PI3K inhibitor, basal levels and H2O2-induced Akt phosphorylation were blocked. LY-
294002 had no effect on basal nor H$_2$O$_2$-induced ERK1/2 phosphorylation (Fig. 8A).

The effect of LY294002 inhibition of PI3K/Akt on cell viability following H$_2$O$_2$ injury in the presence or absence of U0126 was examined. As discussed above, inhibition of ERK1/2 with U0126 or PD98059 blocked H$_2$O$_2$-induced apoptosis in RPTC (Fig. 3A). If PI3K/Akt plays an important role in cell survival, then inhibition of PI3K/Akt should potentiate H$_2$O$_2$-induced decreases in RPTC viability; indeed this was observed (Fig. 8B). If ERK inhibition elicited cell survival through enhanced Akt phosphorylation, then inhibition of the PI3K/Akt pathway in the presence of ERK1/2 inhibition should partly decrease the cytoprotective effect of ERK1/2 inhibition alone; indeed this was observed (Fig. 8B). These inhibitors alone or in combination did not significantly affect cell survival during the course of the 5-h incubation (Fig. 8B). We suggest that although both PI3/Akt and ERK pathways are activated following oxidant injury in RPTC, they play opposite roles. Furthermore, induction of apoptosis by H$_2$O$_2$ injury is partially mediated by the inactivation of the PI3K-Akt pathway.

Fig. 5. Effect of Z-VAD-FMK and Z-DEVD-FMK on caspase-3 activity and apoptosis. RPTC were pretreated with 100 µM z-VAD-FMK (VAD), or 100 µM z-DEVD-FMK (DEVD) for 1 h, and then exposed to 1 mM H$_2$O$_2$ for 5 h (A) or 30 min (B). A and C: cell viability was assessed by MTT assay. Data are means ± SD of 3 independent experiments conducted in triplicate. Bars with different superscript letters are significantly different from each other ($P < 0.05$). B: cell lysates were analyzed by immunoblotting with antibodies to p-ERK1/2 or ERK. Representative blots are shown.

The effect of LY294002 inhibition of PI3K/Akt on cell viability following H$_2$O$_2$ injury in the presence or absence of U0126 was examined. As discussed above, inhibition of ERK1/2 with U0126 or PD98059 blocked H$_2$O$_2$-induced apoptosis in RPTC (Fig. 3A). If PI3K/Akt plays an important role in cell survival, then inhibition of PI3K/Akt should potentiate H$_2$O$_2$-induced decreases in RPTC viability; indeed this was observed (Fig. 8B). If ERK inhibition elicited cell survival through enhanced Akt phosphorylation, then inhibition of the PI3K/Akt pathway in the presence of ERK1/2 inhibition should partly decrease the cytoprotective effect of ERK1/2 inhibition alone; indeed this was observed (Fig. 8B). These inhibitors alone or in combination did not significantly affect cell survival during the course of the 5-h incubation (Fig. 8B). We suggest that although both PI3/Akt and ERK pathways are activated following oxidant injury in RPTC, they play opposite roles. Furthermore, induction of apoptosis by H$_2$O$_2$ injury is partially mediated by the inactivation of the PI3K-Akt pathway.
DISCUSSION

In this study, we investigated the role of the ERK1/2 pathway in apoptosis of RPTC in response to oxidant injury and showed that ERK1/2 is a critical mediator. This statement is based on the following observations. ERK1/2 are activated following oxidant injury, inhibition of the ERK signaling pathway either with PD98059 or U0126 blocked oxidant-induced loss of RPTC viability and apoptosis characterized by nuclear condensation, and overexpression of dominant negative mutant MEK1 blocked oxidant-induced loss of RPTC viability while expression of active MEK1 enhanced the loss of RPTC viability. Our results are in contrast with a report showing that ERK activation is associated with protective effect in mouse RPTC (6) and corroborate other studies indicating that ERK mediates cell death in renal epithelial cell lines (OK and LLC-PK1) (12, 25). The reason for the different results in the mouse RPTC is not clear but may be due to species or experimental protocol differences.

In addition to ERK1/2, p38 is also activated in RPTC following oxidant injury. However, activation of p38 kinase is not required for H2O2-induced apoptosis in RPTC as inhibition of p38 kinase by SB203580 did not affect the apoptotic nuclear condensation (Fig. 3B). Although JNK has also been reported to be associated with apoptosis in mouse RPTC following oxidant injury (6), the phosphorylation level of JNK did not change in H2O2-treated RPTC (45) and JNK inhibition, using SP600125, had no effect on apoptotic nuclear condensation (Fig. 3B). Of the MAPK pathways, we suggest that the ERK pathway uniquely mediates apoptosis in RPTC exposed to H2O2.

The mechanism by which ERK mediates apoptosis has not been well defined. Our studies revealed that H2O2-induced apoptosis is mainly mediated by caspase-3 and ERK inactivation blocked activation of caspase-3, suggesting that ERK induces apoptosis through activating the caspase-3-mediated apoptotic pathway. Because it was reported that ERK activation resulted in depolarization of mitochondrial potential and cytochrome c release (22), which are generally considered to be a prerequisite for activation of caspase-3, it is possible that ERK acts upstream of mitochondria in our model. Alternatively, ERK may act downstream of mitochondrial events and upstream of caspase-3. Consistent with this proposal, Nowak (26) reported that ERK inhibition blocked caspase-3 activation without affecting cytochrome c release from mitochondria. Further studies are needed to define the target(s) where ERK is coupled to the apoptotic pathway.

Fig. 6. Effect of ERK inhibitors on caspase-3 activity and caspase inhibitors on ERK activation. RPTC were pretreated with 10 μM U0126, 50 μM PD98059, 100 μM VAD, or 100 μM DEVD for 1 h, and then exposed to 1 mM H2O2 for 5 h (A) or 30 min (B). Cell lysates were harvested for measuring caspase-3 activity (A) or analyzed by immunoblotting with antibodies to p-ERK1/2 or ERK (B). Representative blots are shown.

Fig. 7. H2O2 induces H2B phosphorylation and the effects of caspase and ERK inhibition. RPTC were incubated for the time indicated (A) or pretreated with 10 μM U0126 (B), or 100 μM DEVD (C) for 1 h, and then exposed to 1 mM H2O2 for 4 h. Cell lysates were harvested and analyzed by immunoblotting with antibodies to p-H2B or H2B. Representative blots are shown.
ERK-mediated apoptosis also occurs through suppression of the PI3K-Akt pathway. PI3K/Akt elicits a survival signaling following stress, including oxidant injury (42). In line with this general view, exposure of RPTC to H2O2 induced Akt phosphorylation and pretreatment of cells with LY294002 reduced the viability of RPTC. However, unlike most observations in which Akt and ERK represent two separate survival pathways, inactivation of ERK resulted in elevated Akt phosphorylation and inhibition of the PI3K/Akt pathway partially reversed anti-apoptotic ERK inhibition. Thus, ERK induces apoptosis, in part, by suppressing the PI3K/Akt pathway. This pathway may be restricted to stress conditions because we observed that ERK inhibition only potentiated Akt phosphorylation in cells exposed to H2O2, not cells under basal conditions. Consistent with our observations, Sinha et al. (34) recently reported that inhibition of ERK1/2 not only prevented the decline in Akt activity but resulted in cell survival in kidney proximal tubular cells deprived of soluble survival factors. Interestingly, activation of ERK1/2 appears to be dependent on oxidant stress under this condition as addition of several different antioxidants and ROS scavengers prevented activation of ERK1/2 (34). The mediator(s) responsible for ERK-mediated suppression of Akt activation remains unclear but may be associated with activation of ribosomal S6 kinase 1 (rsk) and PDK1. In kidney epithelial cells subjected to growth factor withdrawal, rsk and PDK1 are found to coexist with ERK1/2 and Akt in a multimolecular complex (34). Additional studies are needed to determine whether these molecules are involved in apoptosis following oxidant injury.

It has been suggested that different durations of ERK phosphorylation are associated with different actions of ERK. For example, in situations where ERK enhances survival, the activation occurs rapidly and is more transient (2, 17) and in situations where ERK induces cell death the activation tends to be delayed and sustained (20, 38). However, this scenario was not observed in our study. As shown in Fig. 2, the phosphorylated ERK was elevated within 5 min following H2O2 exposure and only persisted for 2 h while loss of cell viability occurred after 3 h (Fig. 1A) when ERK phosphorylation level had returned to the basal level. These data suggest that prolonged activation of ERK may not be only factor in driving cells to undergo apoptosis. In support of this idea, acute and transient increases in ERK phosphorylation also occur immediately after reperfusion and mediate brain injury in a stroke model induced by transient occlusion of the middle cerebral artery (39). Therefore, once ERK is activated, the death signal may propagate later without needing additional ERK activation.

In summary, we suggest that ERK1/2 activation signals apoptosis in RPTC through at least two distinct pathways: activation of caspase-3 and inhibition of PI3K/Akt. Furthermore, ERK activation is coupled to phosphorylation of H2B at serine 14, a critical event for inducing nuclear condensation. Because ROS, including H2O2, is an important mediator in ischemia and toxicant-induced death of renal epithelial cell, it will be intriguing to further investigate the role of ERK1/2 in animal models of acute renal failure.


