JPET Fast Forward. Published on June 26, 2006 as DOI: 10.1124/jpet.106.107367

A Death-Promoting Role for Extracellular Signal-Regulated Kinase

Shougang Zhuang and Rick G. Schnellmann

Department of Pharmaceutical Sciences, Medical University of South Carolina,

Charleston, South Carolina 29425, USA.

Running title: ERK-mediated apoptosis

Corresponding author:

Rick G. Schnellmann, PhD

Department of Pharmaceutical Sciences

Medical University of South Carolina

280 Calhoun St., POB 250140, Charleston, SC 29425

Phone: 843-792-3754; Fax: 843-792-2620

E-mail: schnell@musc.edu

Number of pages:

Text page: 12

Figures: 1

Table: 1

Number of References:

95

Number of words:

Abstract: 184

Text body: 2883

Assignment section:

Review

Abbreviations: ERK; Extracellular signal-regulated protein kinases, RPTC, renal proximal tubular cells; PI3K, phosphoinositide-3-kinase; MAPK; mitogen-activated protein kinase

Abstract

Extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), which are members of the mitogen activated protein kinase (MAPK) superfamily, have been well characterized and are known to be involved in cell survival; however, recent evidence suggests that the activation of ERK1/2 also contributes to cell death in some cell types and organs under certain conditions. For example, ERK1/2 is activated in neuronal and renal epithelial cells upon exposure to oxidative stress and toxicants and deprivation of growth factors, and inhibition of the ERK pathway blocks apoptosis. ERK activation also occurs in animal models of ischemia- and trauma-induced brain injury and cisplatin-induced renal injury, and inactivation of ERK reduces the extent of tissue damage. In some studies, ERK has been implicated in apoptotic events upstream of mitochondrial cytochrome c release, whereas other studies have suggested the converse: ERK acts downstream of mitochondrial events and upstream of caspase-3 activation. ERK also can contribute to cell death through the suppression of the anti-apoptotic signaling molecule, Akt. Here we summarize the evidence and mechanism of ERK-induced apoptosis in both cell culture and in animal models.

General cascades of cell death

Cell death is divided into at least two categories, apoptosis and necrotic cell death. Apoptosis is a tightly orchestrated series of events that requires ATP and includes potassium efflux, cell shrinkage, mitochondria protein release and caspase activation (Yu et al., 2001; Boatright and Salvesen, 2003; Yu, 2003). In contrast, necrotic cell death is initiated by mitochondrial inhibition and the loss of ATP, and is associated with the breakdown of the cytoskeleton, cellular swelling, and progressive increases in plasma membrane permeability (Majno and Joris, 1995; Chen et al., 2001; Harriman et al., 2002; Liu et al., 2004). In general, apoptosis usually involves individual, noncontiguous cells whereas necrotic cell death usually involves multiple, contiguous cells (Columbano, 1995; Boatright and Salvesen, 2003). Interestingly, the extent of exposure to an insult determines the nature of cell death and both forms can occur simultaneously in tissue: apoptosis results from milder insults whereas necrosis follows more severe insults (Columbano, 1995) (Majno and Joris, 1995).

Extensive studies to elucidate the mechanisms by which apopotosis is induced and transduced have resulted in the generally accepted theory that intrinsic and extrinsic mechanisms are involved (Table 1) (Fadeel and Orrenius, 2005; Kim et al., 2006). Intrinsic pathways are activated by specific stress stimuli and lead to mithochondrial cytochrome c release, the activation of caspase-9 through Apaf-1, and the activation of executioner caspases (e.g. caspase-3) (Fadeel and Orrenius, 2005). The extrinsic apopototic pathway is initiated by the activation of a death receptor, such as the TNF-α receptor and Fas, and caspase-8 (Kim et al., 2006). Activated caspase-8 can directly activate executioner caspases and/or induce cleavage of Bid to truncated Bid (tBid). tBid

is translocated to the mitochrondia, prompting cytochrome c release and subsequent activation of caspase-3 (Li et al., 1998; Runden et al., 1998).

Concomitant with the activation of apoptotic pathways, survival signaling pathways are activated. Among them, the phosphatidylinositol 3-kinase (PI3K)/Akt pathway is activated by many apoptotic stimuli and plays a critical role in balancing apoptosis (Yang et al., 2004). Although the ERK pathway is attributed to survival for many cell types, ERK activation is now thought to contribute to apoptosis also.

ERK signaling pathway

ERK belongs to a family of MAPKs, which is comprised of ERKs, c-Jun N-terminal kinase/stress-activated protein kinases (JNK/SAPK), and p38 kinases. Within the ERK group there are eight isoforms (i.e. ERK1, 2, 3, 4, 5, 6, 7, and 8) (Bogoyevitch and Court, 2004; Yoon and Seger, 2006) of which ERK1/2 have been extensively studied. ERK1 and ERK2 are regulated by the dual-specificity kinases MEK1 and MEK2 through phosphorylation at both a threonine and an adjacent tyrosine residue within a dual-specificity motif (Thr-Glu-Tyr). MEK1 and MEK2 can be activated by multiple MAPK kinase kinases, although Raf kinase family members typically serve as dedicated MEK1/2 activators (Garrington and Johnson, 1999). Low molecular weight G-proteins (Ras, Rac, Rho, Cdc42, etc.), each associated with growth factor receptor activation and cellular adhesion can contribute to Raf activation either directly or indirectly. (Peyssonnaux and Eychene, 2001).

ERK1/2 also is activated in response to various stress stimuli through divergent mechanisms involving the Ras-Raf-MEK pathway (Strniskova et al., 2002; Kyosseva, 2004; Roux and Blenis, 2004; Baines and Molkentin, 2005; Rennefahrt et al., 2005).

Depending upon the cell type, the stimulus, and the duration of activation, a variety of biological responses (i.e. cell proliferation, migration, differentiation, and apoptosis) are associated with ERK activation (Strniskova et al., 2002; Kyosseva, 2004; Roux and Blenis, 2004; Baines and Molkentin, 2005; Rennefahrt et al., 2005). This pro-apoptotic perspective of ERK will comprise the remainder of this review.

ERK signaling mediates apoptosis in cultured cells

Bhat *et al*., first reported that inhibition of ERK using the MEK1 inhibitor PD98059 rescues oligodendrocytes from H_2O_2-induced cell death (Bhat and Zhang, 1999) and this observation was subsequently confirmed in Hela cells (Wang et al., 2000), cortical neurons (Lesuisse and Martin, 2002), and primary β-cells (Pavlovic et al., 2000) (Table 1). In renal cell lines (LLC-PK1; OK) and primary cultures of renal proximal tubular cells, inhibition of ERK improved cell survival by inhibiting apoptosis after cisplatin exposure (Ishikawa and Kitamura, 2000; Nowak, 2002; Kim et al., 2005). The pro-apoptotic role of ERK in renal epithelial cells is not limited to cisplatin exposure; ERK activation is also associated with cell death induced by reactive oxygen species (ROS) (Tikoo et al., 2001; Ramachandiran et al., 2002; Dong et al., 2004), *E. coli* toxins (Chen et al., 2004), zinc (Matsunaga et al., 2005), and cephaloridine (Kohda et al., 2003). ERK activation also has been implicated in cell death induced by deprivation of survival factors. For example, withdrawal of survival factors from mouse kidney proximal tubular epithelial cells led to a progressive increase in ERK1/2 activity, and inhibition of ERK1/2 maintained cell survival (Sinha et al., 2004). Finally, ERK1/2 activation is involved in doxorubicin-induced apoptosis in human hepatoma cell lines (HepG2 and Huh-7) (Alexia et al., 2004), amloid-induced neurotoxicity in primary hippocampal neurons (Medina et

al., 2005) and CD40-mediated apoptosis in cholangiocytes (Ahmed-Choudhury et al., 2006).

Most of the above-mentioned studies addressed the role of ERK in apoptosis using MEK inhibitors PD98059 or U0126 (Alessi et al., 1995; Favata et al., 1998). Recently, Kim *et al*. used molecular approaches to examine the role of the ERK pathway in cell death of renal epithelial cells (Kim et al., 2005). They found that transient transfection of constitutively active MEK1 increased H_2O_2-induced apoptosis whereas the dominant-negative mutant of MEK1 led to an inhibition of H_2O_2-mediated cell death. Similarly, Goillot *et al*. demonstrated that interference with the ERK pathway by expression of dominant-negative MEK1 resulted in inhibition of Fas-mediated apoptosis (Goillot et al., 1997). These *in vitro* studies support a significant role for ERK in mediating apoptosis.

ERK signaling mediates injury-induced tissue damage in vivo

ERK-mediated cell death also has been reported in numerous animal models. In a mouse model of stroke induced by transient occlusion of the middle cerebral artery (MCA) an increase in phosphorylated ERK in the nuclei of cortical cells was detected and pretreatment with PD98059 blocked ERK phosphorylation, resulting in a 55% decrease in focal infarct volume at 22 hr and a 36% decrease at 72 hr (Alessandrini et al., 1999). Using the same model, researchers confirmed these results using two additional MEK1 inhibitors, U0126 and SL327 (Namura et al., 2001; Wang et al., 2003; Wang et al., 2004b). In a gerbil model of forebrain ischemia induced by bilateral carotid artery occlusion, Namura *et al*. obtained similar results with MEK1 inhibitors (Namura et al., 2001). ERK also was rapidly activated in damaged tissue in a controlled cortical-impact

model of traumatic injury in mice, and ERK pathway inhibition with PD98059 prior to trauma resulted in a significant reduction of cortical lesion volumes 7 days after trauma (Mori et al., 2002; Clausen et al., 2004), Confocal immunohistochemistry showed that phospho-ERK co-localized with the neuronal nuclei marker Neu-N in the injured brain.

However, increased phospho-ERK1/2 staining was also detected in neurons that survived ischemic injury in a rat and mouse model of focal cerebral ischemia (Irving et al., 2000; Namura et al., 2001) suggesting that ERK1/2 activation may mediate cell survival in these areas. In support of an anti-apoptotic role of ERK, Gonzalez-Zulueta *et al*., reported that activation of the Ras/ERK cascade was critical for the development of ischemic tolerance in cortical neurons (Gonzalez-Zulueta et al., 2000). Finally, inhibition of ERK1/2 with PD98059 failed to protect gerbils against ischemic cell death in the hippocampal CA1 region (Sugino et al., 2000) and did not block cerebral endothelial cell death after hypoxia-reoxygenation (Lee and Lo, 2003). These differences in the outcome of ERK activation may be attributed to the differences in the experimental models.

In a mouse model of cisplatin-induced nephrotoxicity, Jo *et al,* observed that ERK1/2 phosphorylation increased mainly in distal tubules and collecting ducts 24 hr after cisplatin administration and persisted until 72 hr (Jo et al., 2005). Inhibition of ERK phosphorylation by U0126 pretreatment reduced tissue damage and improved renal function. A crucial role of ERK activation in mediating renal cell injury also was observed in rat renal cortical slices treated with cephaloridine, a cephalosporin antibiotic (Kohda et al., 2003). Further, serum thymic factor attenuated cisplatin nephrotoxicity by suppressing cisplatin-induced ERK activation (Kohda et al., 2005) . These studies, coupled with the *in vitro* studies, reveal that ERK is a critical mediator in nephrotoxicity.

Although ERK is activated after renal ischemia/reperfuson injury in animals (Park et al., 2001), the role of ERK in ischemia/reperfuson-induced renal injury remains to be established.

Mechanisms of ERK-induced apoptosis

ERK-mediated regulation of the intrinsic pathway

Because the intrinsic pathway is characterized by mitochondrial outer membrane permeabilization, cytochrome c release, and caspase activation, the association of ERK with these events has been investigated *in vivo* and *in vitro*. *In vivo* studies revealed that ERK1/2 acts upstream of caspase-3 in cisplatin-induced cell death in the kidney (Jo et al., 2005) and in the brain after ischemia/reperfusion (Wang et al., 2003). Using Hela cells, Wang *et al*. showed that ERK1/2 inhibition blocks cytochrome c release and subsequent activation of caspase-3 in cisplatin-induced apoptosis (Wang et al., 2000). In a renal cell line (OK cells), Kim *et al*. reported that ERK activation is required for mitochondrial membrane depolarization, cytochrome c release, and caspase-3 activation after cisplatin exposure (Kim et al., 2005). Nowak reported that phosphorylated ERK1/2 was detected in mitochondria and associated with loss of mitochondrial function in cisplatin-treated primary cultures of renal proximal tubular cells (Nowak, 2002). However, ERK inhibition blocked caspase-3 activation without affecting cytochrome c release from mitochondria (Nowak, 2002). Thus, ERK1/2 may act on mitochondria to cause cytochrome c release and/or may regulate activation of caspase-3 downstream of cytochrome c release.

ERK may act on mitochondria through Bax and/or p53. Bax is a member of the Bcl-2 family of proteins and acts as pro-apoptotic molecule. During apoptosis induced

by a variety of stimuli, Bax is translocated to the mitochondria, where it promotes the release of pro-apoptotic proteins from the intermembrane space (Degli Esposti and Dive, 2003). In renal epithelial and osteoblastic cells, Bax expression is increased after cisplatin or H_2O_2, and inhibition of the ERK pathway decreased Bax expression (Kim et al., 2005; Park et al., 2005). Similar to the role of ERK in the regulation of Bax expression, ERK activation is also associated with up-regulation of p53 expression in HeLa cells treated with shikonin (a red naphthoquinone pigment isolated from the ground rhizome of lithospermum erythrorhizon) (Wu et al., 2005) and in lens epithelial cells treated with calcimycin, a calcium mobilizer (Li et al., 2005). In the presence of apoptotic stimuli, p53 has been reported to translocate to the mitochondria to inhibit the action of the anti-apoptotic Bclx through the formation of a p53/Bclx complex (Petros et al., 2004) or to directly promote the pro-apoptotic activities of Bak (Mihara et al., 2003; Leu et al., 2004). Inhibition of Bclx and induction of Bax activity leads to cytochrome c release and caspase activation (Moll et al., 2005). In addition, ERK may regulate apoptosis via direct phosphorylation of p53. ERK can induce p53 phosphorylation at serine residue 15 (Persons et al., 2000) and inactivation of ERK resulted in p53 dephosphorylation and inhibited apoptosis (Brown and Benchimol, 2006). Together, these data suggest that Bax and p53 are important components in the ERK mediated apoptotic signaling pathways.

ERK-mediated regulation of the extrinsic pathway

ERK may induce apoptosis through regulation of the extrinsic apoptotic pathway. Jo *et al.* examined the effect of ERK inhibition on TNF–α expression and subsequent caspase-3 activation in cisplatin-induced acute renal failure in mice (Jo et al., 2005). They found that inhibiting ERK1/2 reduced TNF-α expression, caspase-3 activation, and

apoptosis in kidney tissue, suggesting that ERK1/2 pathway may also participate in apoptosis by increasing an upstream signal for TNF- α production. It is established that increases in interleukin IL-β are closely associated with brain injury after cerebral ischemia (Barone and Feuerstein, 1999; Emsley and Tyrrell, 2002). Recently, Wang *et al*. showed that cerebral ischemia in mice induces ERK activation and interleukin-1β (IL-1β) expression, and inhibition of ERK activation by U0126 prevented increases in IL-1β mRNA (Wang et al., 2004a). These studies implicate ERK-mediated expression of death ligands and pro-inflammatory cytokines as an important mechanism in exacerbating tissue injury.

The extrinsic apoptotic pathway relies on the activation of an initial caspase, caspase-8. Cagnol *et al*. examined the effect of ERK pathway activation on caspase-8 and apoptosis in HEK 293 cells that express an inducible form of Raf-1 (Raf-1:ER)(Cagnol et al., 2006). They showed that prolonged ERK stimulation activated caspase-8 and potentiated Fas signaling and apoptosis whereas expression of Bcl-X_L, an inhibitor of apoptosis, did not significantly alter the apoptotic rate (Cagnol et al., 2006). These data suggest that ERK can regulate the apoptotic pathway at the level of caspase-8.

ERK-mediated suppression of survival signaling

Promotion of cell death by ERK activation also may result from the suppression of survival signaling pathways. The phosphoinositide-3-kinase (PI3K)-Akt pathway plays a critical role in the regulation of cell survival and most growth and survival factors activate this pathway (Amaravadi and Thompson, 2005). Recently, it was reported that withdrawal of soluble survival factors from primary cultures of mouse renal proximal tubular cells led to ERK1/2 activation that was accompanied by a gradual decrease in Akt

activity and apoptosis (Sinha et al., 2004). Inhibition of ERK1/2 with U0126 or PD98059 in the cells deprived of survival factors not only prevented the decline in Akt activity, but also resulted in cell survival (Sinha et al., 2004). These results support the idea that ERK1/2 activation in response to survival factor deprivation contributed to cell death via the suppression of the Akt pathway. Although the precise mechanism by which ERK1/2 inhibits Akt remains unclear, ERK1/2 and Akt have been reported to coexist in a multimolecular complex containing at least ERK1/2, Akt, ribosomal S6 kinase 1 (RSK), and phosphoinositide-dependent kinase 1 (PDK1) (Sinha et al., 2004). Whether ERK suppresses Akt activity through regulation of RSK and PDK1 or through other signaling molecules requires further investigation.

Upstream inducers of ERK in apoptosis

ERK activation can be induced by different stimuli via distinct mechanisms. For example, Arany *et al*. reported that the EGF receptor mediates ERK activation, and inhibition of the EGF receptor blocked cisplatin-induced ERK activation and apoptosis in mouse renal proximal tubules (Arany et al., 2004). Similarly, Lee *et al* showed that H_2O_2 triggers EGFR activation, and EGF receptor inactivation attenuated apoptosis in OK cells (Lee et al., 2005). Consequently, the death signal may initiate at a cellular membrane receptor and then propagate through an ERK-mediated signaling pathway.

Ras and Raf are downstream of growth factor receptors and mediate activation of MEK-ERK (Magnuson et al., 1994; Downward, 1998). Li *et al* demonstrated that calcimycin-induced apoptosis of lens epithelial cells is mediated by MEK and ERK through a Ras and Raf-dependent pathway (Li et al., 2005). Woessmann *et al*., showed that Ras-mediated activation of ERK by cisplatin induces cell death in osteosarcoma and

neuroblastoma cell lines (Woessmann et al., 2002). In addition, calcium can induce ERK and apoptosis through a Ras-dependent and Raf-independent mechanism (Vossler et al., 1997; Li et al., 2005). Src also can transduce EGF receptor activation to ERK in cisplatin-induced apoptosis of mouse proximal tubular cells (Arany et al., 2004). In contrast, Sinha *et al*. reported that growth factor depletion-mediated cell death is dependent on ERK but not Raf (Sinha et al., 2004). These data suggest that divergent intermediates are involved in transducing the death signal from the cellular membrane to ERK.

ROS play an important role in regulating cellular events leading to ERK activation and subsequent apoptosis. In addition to exogenous ROS activation of ERK and apoptosis, cellular production of ROS—due to a stimulus—can result in ERK activation. For example, cisplatin-induced cytotoxicity is closely related to increased generation of ROS (Sasada et al., 1996; Miyajima et al., 1997). ERK activation prompted by asbestos or the deprivation of growth factors can be blocked by the addition of ROS scavengers. Furthermore, H_2O_2 generation and ERK activation are involved in 2,3,5-tris(glutathion-S-yl)hydroquinone (TGHQ)-induced cell death in LLC-PK1 cells (Tikoo et al., 2001; Ramachandiran et al., 2002; Dong et al., 2004). These studies suggest that ROS produce a specific environment that results in ERK-induced cell death.

The role of ERK5 in apoptosis

Studies examining the role of ERK in cell death have primarily relied upon MEK1 inhibitors, U0126 and PD98059. Recently, it was reported that biological actions previously attributed to ERK1/2 may, in fact, be mediated by ERK5, a newer member of the MAPK family, because PD98059 and U0126 inhibit MEK1/2 as well as MEK5

(Kamakura et al., 1999; Cavanaugh et al., 2001). ERK5, also called big MAP kinase 1 (BMK1), contains a dual-phosphorylation motif (Thr-Glu-Tyr) similar to that in ERK1/2 and the N-terminal half of ERK5 shares sequence homology with other members of the MAPK family. However, a large C terminus and a unique loop-12 sequence distinguish it from ERK1/2. ERK5 is phosphorylated and activated by MEK5, but not by MEK1 or MEK2 (English et al., 1995; Zhou et al., 1995).

Similar to ERK1/2, activation of ERK5 plays a role in cell survival in response to a variety of stimuli, including oxidants and toxicants (Suzaki et al., 2002; Pi et al., 2004). Recently, ERK5 activation has been shown to promote apoptosis. For example, Sturla *et al*. reported that over-expression of ERK5 increased apoptosis in two medulloblastoma cell lines and primary cultures of patched heterozygous mouse medulloblastomas upon exposure to neurotrophin-3 whereas expression of small interfering RNA for the ERK 5 activator, MEK5, inhibited neuotrophin-3-induced apoptosis (Sturla et al., 2005). Furthermore, inhibition of myocyte enhancer factor (MEF2), a specific target of ERK5, by a dominant-negative mutant of MEF2 blocked MEK5/ERK5-induced cell death (Sturla et al., 2005). Although ERK5 has an apparent role in cell death, the mechanisms for this are unclear. Because many stimuli that induce ERK1/2 activation also activate ERK5, it is tempting to speculate that the ERK5 pathway may also be involved in the death of other cell types.

Conclusions and future directions

Although ERK has generally been considered a survival signaling pathway, clear evidence exists that the ERK pathway mediates apoptosis induced by different stimuli in different tissues. The mechanisms by which ERK mediates apoptosis remain poorly

understood, and may occur at different levels. ERK1/2 may act upstream of mitochondrial cytochrome c release and caspase-3 activation through up-regulation of Bax and p53 and through suppression of Akt-mediated survival signaling. Further studies are necessary to elucidate the activated signal transduction upstream and downstream of the cascades and to define the crosstalk among the cascades and other signaling pathways.

The rationale for a signaling pathway being responsible for cell survival and apoptosis is not clear. At present, the signaling of survival or apoptosis by ERK appears to be dependent on the model system and injury paradigm. Furthermore, the kinetics and duration of ERK activation may play an important role in influencing its effect on cell fate. It has been reported that prolonged ERK activation is accompanied by the pro-apoptotic effect of ERK (di Mari et al., 1999) whereas a transient activation of ERK protects cells from death (Arany et al., 2004). Nevertheless, the protective effect of ERK inhibition has been reported in animal models of ischemia/reperfusion, and ERK activation was reported in humans who had experienced a stroke (Slevin et al., 2000), so the protection strategies which target these mechanisms merit further exploration.

References

Ahmed-Choudhury J, Williams KT, Young LS, Adams DH and Afford SC (2006) CD40 mediated human cholangiocyte apoptosis requires JAK2 dependent activation of STAT3 in addition to activation of JNK1/2 and ERK1/2. *Cell Signal* **18**:456-468.

Alessandrini A, Namura S, Moskowitz MA and Bonventre JV (1999) MEK1 protein kinase inhibition protects against damage resulting from focal cerebral ischemia. *Proc Natl Acad Sci U S A* **96**:12866-12869.

Alessi DR, Cuenda A, Cohen P, Dudley DT and Saltiel AR (1995) PD 098059 is a specific inhibitor of the activation of mitogen-activated protein kinase kinase in vitro and in vivo. *J Biol Chem* **270**:27489-27494.

Alexia C, Fallot G, Lasfer M, Schweizer-Groyer G and Groyer A (2004) An evaluation of the role of insulin-like growth factors (IGF) and of type-I IGF receptor signalling in hepatocarcinogenesis and in the resistance of hepatocarcinoma cells against drug-induced apoptosis. *Biochem Pharmacol* **68**:1003-1015.

Amaravadi R and Thompson CB (2005) The survival kinases Akt and Pim as potential pharmacological targets. *J Clin Invest* **115**:2618-2624.

Arany I, Megyesi JK, Kaneto H, Price PM and Safirstein RL (2004) Cisplatin-induced cell death is EGFR/src/ERK signaling dependent in mouse proximal tubule cells. *Am J Physiol Renal Physiol* **287**:F543-549.

Baines CP and Molkentin JD (2005) STRESS signaling pathways that modulate cardiac myocyte apoptosis. *J Mol Cell Cardiol* **38**:47-62.

Barone FC and Feuerstein GZ (1999) Inflammatory mediators and stroke: new opportunities for novel therapeutics. *J Cereb Blood Flow Metab* **19**:819-834.

Bhat NR and Zhang P (1999) Hydrogen peroxide activation of multiple mitogen-activated protein kinases in an oligodendrocyte cell line: role of extracellular signal-regulated kinase in hydrogen peroxide-induced cell death. *J Neurochem* **72**:112-119.

Boatright KM and Salvesen GS (2003) Mechanisms of caspase activation. *Curr Opin Cell Biol* **15**:725-731.

Bogoyevitch MA and Court NW (2004) Counting on mitogen-activated protein kinases--ERKs 3, 4, 5, 6, 7 and 8. *Cell Signal* **16**:1345-1354.

Brown L and Benchimol S (2006) The involvement of MAPK signaling pathways in determining the cellular response to p53 activation: cell cycle arrest or apoptosis. *J Biol Chem* **281**:3832-3840.

Cagnol S, Van Obberghen-Schilling E and Chambard JC (2006) Prolonged activation of ERK1,2 induces FADD-independent caspase 8 activation and cell death. *Apoptosis*.

Cavanaugh JE, Ham J, Hetman M, Poser S, Yan C and Xia Z (2001) Differential regulation of mitogen-activated protein kinases ERK1/2 and ERK5 by neurotrophins, neuronal activity, and cAMP in neurons. *J Neurosci* **21**:434-443.

Chen J, Liu X, Mandel LJ and Schnellmann RG (2001) Progressive disruption of the plasma membrane during renal proximal tubule cellular injury. *Toxicol Appl Pharmacol* **171**:1-11.

Chen M, Bao W, Aizman R, Huang P, Aspevall O, Gustafsson LE, Ceccatelli S and Celsi G (2004) Activation of extracellular signal-regulated kinase mediates apoptosis

induced by uropathogenic Escherichia coli toxins via nitric oxide synthase: protective role of heme oxygenase-1. *J Infect Dis* **190**:127-135.

Clausen F, Lundqvist H, Ekmark S, Lewen A, Ebendal T and Hillered L (2004) Oxygen free radical-dependent activation of extracellular signal-regulated kinase mediates apoptosis-like cell death after traumatic brain injury. *J Neurotrauma* **21**:1168-1182.

Columbano A (1995) Cell death: current difficulties in discriminating apoptosis from necrosis in the context of pathological processes in vivo. *J Cell Biochem* **58**:181-190.

de Bernardo S, Canals S, Casarejos MJ, Solano RM, Menendez J and Mena MA (2004) Role of extracellular signal-regulated protein kinase in neuronal cell death induced by glutathione depletion in neuron/glia mesencephalic cultures. *J Neurochem* **91**:667-682.

Degli Esposti M and Dive C (2003) Mitochondrial membrane permeabilisation by Bax/Bak. *Biochem Biophys Res Commun* **304**:455-461.

di Mari JF, Davis R and Safirstein RL (1999) MAPK activation determines renal epithelial cell survival during oxidative injury. *Am J Physiol* **277**:F195-203.

Dong J, Ramachandiran S, Tikoo K, Jia Z, Lau SS and Monks TJ (2004) EGFR-independent activation of p38 MAPK and EGFR-dependent activation of ERK1/2 are required for ROS-induced renal cell death. *Am J Physiol Renal Physiol* **287**:F1049-1058.

Downward J (1998) Ras signalling and apoptosis. *Curr Opin Genet Dev* **8**:49-54.

Emsley HC and Tyrrell PJ (2002) Inflammation and infection in clinical stroke. *J Cereb Blood Flow Metab* **22**:1399-1419.

English JM, Vanderbilt CA, Xu S, Marcus S and Cobb MH (1995) Isolation of MEK5 and differential expression of alternatively spliced forms. *J Biol Chem* **270**:28897-28902.

Fadeel B and Orrenius S (2005) Apoptosis: a basic biological phenomenon with wide-ranging implications in human disease. *J Intern Med* **258**:479-517.

Favata MF, Horiuchi KY, Manos EJ, Daulerio AJ, Stradley DA, Feeser WS, Van Dyk DE, Pitts WJ, Earl RA, Hobbs F, Copeland RA, Magolda RL, Scherle PA and Trzaskos JM (1998) Identification of a novel inhibitor of mitogen-activated protein kinase kinase. *J Biol Chem* **273**:18623-18632.

Garrington TP and Johnson GL (1999) Organization and regulation of mitogen-activated protein kinase signaling pathways. *Curr Opin Cell Biol* **11**:211-218.

Goillot E, Raingeaud J, Ranger A, Tepper RI, Davis RJ, Harlow E and Sanchez I (1997) Mitogen-activated protein kinase-mediated Fas apoptotic signaling pathway. *Proc Natl Acad Sci U S A* **94**:3302-3307.

Gomez-Santos C, Ferrer I, Reiriz J, Vinals F, Barrachina M and Ambrosio S (2002) MPP+ increases alpha-synuclein expression and ERK/MAP-kinase phosphorylation in human neuroblastoma SH-SY5Y cells. *Brain Res* **935**:32-39.

Gonzalez-Zulueta M, Feldman AB, Klesse LJ, Kalb RG, Dillman JF, Parada LF, Dawson TM and Dawson VL (2000) Requirement for nitric oxide activation of p21(ras)/extracellular regulated kinase in neuronal ischemic preconditioning. *Proc Natl Acad Sci U S A* **97**:436-441.

Harriman JF, Liu XL, Aleo MD, Machaca K and Schnellmann RG (2002) Endoplasmic reticulum Ca(2+) signaling and calpains mediate renal cell death. *Cell Death Differ* **9**:734-741.

Irving EA, Barone FC, Reith AD, Hadingham SJ and Parsons AA (2000) Differential activation of MAPK/ERK and p38/SAPK in neurones and glia following focal cerebral ischaemia in the rat. *Brain Res Mol Brain Res* **77**:65-75.

Ishikawa Y and Kitamura M (2000) Anti-apoptotic effect of quercetin: intervention in the JNK- and ERK-mediated apoptotic pathways. *Kidney Int* **58**:1078-1087.

Jo SK, Cho WY, Sung SA, Kim HK and Won NH (2005) MEK inhibitor, U0126, attenuates cisplatin-induced renal injury by decreasing inflammation and apoptosis. *Kidney Int* **67**:458-466.

Kamakura S, Moriguchi T and Nishida E (1999) Activation of the protein kinase ERK5/BMK1 by receptor tyrosine kinases. Identification and characterization of a signaling pathway to the nucleus. *J Biol Chem* **274**:26563-26571.

Kim GS, Hong JS, Kim SW, Koh JM, An CS, Choi JY and Cheng SL (2003) Leptin induces apoptosis via ERK/cPLA2/cytochrome c pathway in human bone marrow stromal cells. *J Biol Chem* **278**:21920-21929.

Kim R, Emi M, Tanabe K, Murakami S, Uchida Y and Arihiro K (2006) Regulation and interplay of apoptotic and non-apoptotic cell death. *J Pathol* **208**:319-326.

Kim YK, Kim HJ, Kwon CH, Kim JH, Woo JS, Jung JS and Kim JM (2005) Role of ERK activation in cisplatin-induced apoptosis in OK renal epithelial cells. *J Appl Toxicol* **25**:374-382.

Kohda Y, Hiramatsu J and Gemba M (2003) Involvement of MEK/ERK pathway in cephaloridine-induced injury in rat renal cortical slices. *Toxicol Lett* **143**:185-194.

Kohda Y, Kawai Y, Iwamoto N, Matsunaga Y, Aiga H, Awaya A and Gemba M (2005) Serum thymic factor, FTS, attenuates cisplatin nephrotoxicity by suppressing cisplatin-induced ERK activation. *Biochem Pharmacol* **70**:1408-1416.

Kulich SM and Chu CT (2001) Sustained extracellular signal-regulated kinase activation by 6-hydroxydopamine: implications for Parkinson's disease. *J Neurochem* **77**:1058-1066.

Kyosseva SV (2004) Mitogen-activated protein kinase signaling. *Int Rev Neurobiol* **59**:201-220.

Lee JS, Kim SY, Kwon CH and Kim YK (2005) EGFR-dependent ERK activation triggers hydrogen peroxide-induced apoptosis in OK renal epithelial cells. *Arch Toxicol*:1-10.

Lee SR and Lo EH (2003) Interactions between p38 mitogen-activated protein kinase and caspase-3 in cerebral endothelial cell death after hypoxia-reoxygenation. *Stroke* **34**:2704-2709.

Lesuisse C and Martin LJ (2002) Immature and mature cortical neurons engage different apoptotic mechanisms involving caspase-3 and the mitogen-activated protein kinase pathway. *J Cereb Blood Flow Metab* **22**:935-950.

Leu JI, Dumont P, Hafey M, Murphy ME and George DL (2004) Mitochondrial p53 activates Bak and causes disruption of a Bak-Mcl1 complex. *Nat Cell Biol* **6**:443-450.

Li DW, Liu JP, Mao YW, Xiang H, Wang J, Ma WY, Dong Z, Pike HM, Brown RE and Reed JC (2005) Calcium-activated RAF/MEK/ERK signaling pathway mediates p53-dependent apoptosis and is abrogated by alpha B-crystallin through inhibition of RAS activation. *Mol Biol Cell* **16**:4437-4453.

Li H, Zhu H, Xu CJ and Yuan J (1998) Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* **94**:491-501.

Liu X, Van Vleet T and Schnellmann RG (2004) The role of calpain in oncotic cell death. *Annu Rev Pharmacol Toxicol* **44**:349-370.

Magnuson NS, Beck T, Vahidi H, Hahn H, Smola U and Rapp UR (1994) The Raf-1 serine/threonine protein kinase. *Semin Cancer Biol* **5**:247-253.

Majno G and Joris I (1995) Apoptosis, oncosis, and necrosis. An overview of cell death. *Am J Pathol* **146**:3-15.

Matsunaga Y, Kawai Y, Kohda Y and Gemba M (2005) Involvement of activation of NADPH oxidase and extracellular signal-regulated kinase (ERK) in renal cell injury induced by zinc. *J Toxicol Sci* **30**:135-144.

Medina MG, Ledesma MD, Dominguez JE, Medina M, Zafra D, Alameda F, Dotti CG and Navarro P (2005) Tissue plasminogen activator mediates amyloid-induced neurotoxicity via Erk1/2 activation. *Embo J* **24**:1706-1716.

Mihara M, Erster S, Zaika A, Petrenko O, Chittenden T, Pancoska P and Moll UM (2003) p53 has a direct apoptogenic role at the mitochondria. *Mol Cell* **11**:577-590.

Miyajima A, Nakashima J, Yoshioka K, Tachibana M, Tazaki H and Murai M (1997) Role of reactive oxygen species in cis-dichlorodiammineplatinum-induced cytotoxicity on bladder cancer cells. *Br J Cancer* **76**:206-210.

Moll UM, Wolff S, Speidel D and Deppert W (2005) Transcription-independent pro-apoptotic functions of p53. *Curr Opin Cell Biol* **17**:631-636.

Mori T, Wang X, Jung JC, Sumii T, Singhal AB, Fini ME, Dixon CE, Alessandrini A and Lo EH (2002) Mitogen-activated protein kinase inhibition in traumatic brain injury: in vitro and in vivo effects. *J Cereb Blood Flow Metab* **22**:444-452.

Namura S, Iihara K, Takami S, Nagata I, Kikuchi H, Matsushita K, Moskowitz MA, Bonventre JV and Alessandrini A (2001) Intravenous administration of MEK inhibitor U0126 affords brain protection against forebrain ischemia and focal cerebral ischemia. *Proc Natl Acad Sci U S A* **98**:11569-11574.

Nowak G (2002) Protein kinase C-alpha and ERK1/2 mediate mitochondrial dysfunction, decreases in active Na+ transport, and cisplatin-induced apoptosis in renal cells. *J Biol Chem* **277**:43377-43388.

Park BG, Yoo CI, Kim HT, Kwon CH and Kim YK (2005) Role of mitogen-activated protein kinases in hydrogen peroxide-induced cell death in osteoblastic cells. *Toxicology* **215**:115-125.

Park KM, Chen A and Bonventre JV (2001) Prevention of kidney ischemia/reperfusion-induced functional injury and JNK, p38, and MAPK kinase activation by remote ischemic pretreatment. *J Biol Chem* **276**:11870-11876.

Pavlovic D, Andersen NA, Mandrup-Poulsen T and Eizirik DL (2000) Activation of extracellular signal-regulated kinase (ERK)1/2 contributes to cytokine-induced apoptosis in purified rat pancreatic beta-cells. *Eur Cytokine Netw* **11**:267-274.

Persons DL, Yazlovitskaya EM and Pelling JC (2000) Effect of extracellular signal-regulated kinase on p53 accumulation in response to cisplatin. *J Biol Chem* **275**:35778-35785.

Petros AM, Gunasekera A, Xu N, Olejniczak ET and Fesik SW (2004) Defining the p53 DNA-binding domain/Bcl-x(L)-binding interface using NMR. *FEBS Lett* **559**:171-174.

Peyssonnaux C and Eychene A (2001) The Raf/MEK/ERK pathway: new concepts of activation. *Biol Cell* **93**:53-62.

Pi X, Yan C and Berk BC (2004) Big mitogen-activated protein kinase (BMK1)/ERK5 protects endothelial cells from apoptosis. *Circ Res* **94**:362-369.

Ramachandiran S, Huang Q, Dong J, Lau SS and Monks TJ (2002) Mitogen-activated protein kinases contribute to reactive oxygen species-induced cell death in renal proximal tubule epithelial cells. *Chem Res Toxicol* **15**:1635-1642.

Rennefahrt U, Janakiraman M, Ollinger R and Troppmair J (2005) Stress kinase signaling in cancer: fact or fiction? *Cancer Lett* **217**:1-9.

Roux PP and Blenis J (2004) ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. *Microbiol Mol Biol Rev* **68**:320-344.

Runden E, Seglen PO, Haug FM, Ottersen OP, Wieloch T, Shamloo M and Laake JH (1998) Regional selective neuronal degeneration after protein phosphatase inhibition in hippocampal slice cultures: evidence for a MAP kinase-dependent mechanism. *J Neurosci* **18**:7296-7305.

Sasada T, Iwata S, Sato N, Kitaoka Y, Hirota K, Nakamura K, Nishiyama A, Taniguchi Y, Takabayashi A and Yodoi J (1996) Redox control of resistance to cis-diamminedichloroplatinum (II) (CDDP): protective effect of human thioredoxin against CDDP-induced cytotoxicity. *J Clin Invest* **97**:2268-2276.

Sinha D, Bannergee S, Schwartz JH, Lieberthal W and Levine JS (2004) Inhibition of ligand-independent ERK1/2 activity in kidney proximal tubular cells deprived of soluble survival factors up-regulates Akt and prevents apoptosis. *J Biol Chem* **279**:10962-10972.

Slevin M, Krupinski J, Slowik A, Rubio F, Szczudlik A and Gaffney J (2000) Activation of MAP kinase (ERK-1/ERK-2), tyrosine kinase and VEGF in the human brain following acute ischaemic stroke. *Neuroreport* **11**:2759-2764.

Strniskova M, Barancik M and Ravingerova T (2002) Mitogen-activated protein kinases and their role in regulation of cellular processes. *Gen Physiol Biophys* **21**:231-255.

Sturla LM, Cowan CW, Guenther L, Castellino RC, Kim JY and Pomeroy SL (2005) A novel role for extracellular signal-regulated kinase 5 and myocyte enhancer factor 2 in medulloblastoma cell death. *Cancer Res* **65**:5683-5689.

Subramaniam S, Strelau J and Unsicker K (2003) Growth differentiation factor-15 prevents low potassium-induced cell death of cerebellar granule neurons by differential regulation of Akt and ERK pathways. *J Biol Chem* **278**:8904-8912.

Subramaniam S, Zirrgiebel U, von Bohlen Und Halbach O, Strelau J, Laliberte C, Kaplan DR and Unsicker K (2004) ERK activation promotes neuronal degeneration

predominantly through plasma membrane damage and independently of caspase-3. *J Cell Biol* **165**:357-369.

Sugino T, Nozaki K, Takagi Y, Hattori I, Hashimoto N, Moriguchi T and Nishida E (2000) Activation of mitogen-activated protein kinases after transient forebrain ischemia in gerbil hippocampus. *J Neurosci* **20**:4506-4514.

Suzaki Y, Yoshizumi M, Kagami S, Koyama AH, Taketani Y, Houchi H, Tsuchiya K, Takeda E and Tamaki T (2002) Hydrogen peroxide stimulates c-Src-mediated big mitogen-activated protein kinase 1 (BMK1) and the MEF2C signaling pathway in PC12 cells: potential role in cell survival following oxidative insults. *J Biol Chem* **277**:9614-9621.

Tikoo K, Lau SS and Monks TJ (2001) Histone H3 phosphorylation is coupled to poly-(ADP-ribosylation) during reactive oxygen species-induced cell death in renal proximal tubular epithelial cells. *Mol Pharmacol* **60**:394-402.

Ulisse S, Cinque B, Silvano G, Rucci N, Biordi L, Cifone MG and D'Armiento M (2000) Erk-dependent cytosolic phospholipase A2 activity is induced by CD95 ligand cross-linking in the mouse derived Sertoli cell line TM4 and is required to trigger apoptosis in CD95 bearing cells. *Cell Death Differ* **7**:916-924.

Vossler MR, Yao H, York RD, Pan MG, Rim CS and Stork PJ (1997) cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. *Cell* **89**:73-82.

Wang X, Martindale JL and Holbrook NJ (2000) Requirement for ERK activation in cisplatin-induced apoptosis. *J Biol Chem* **275**:39435-39443.

Wang X, Wang H, Xu L, Rozanski DJ, Sugawara T, Chan PH, Trzaskos JM and Feuerstein GZ (2003) Significant neuroprotection against ischemic brain injury by inhibition of the MEK1 protein kinase in mice: exploration of potential mechanism associated with apoptosis. *J Pharmacol Exp Ther* **304**:172-178.

Wang ZQ, Chen XC, Yang GY and Zhou LF (2004a) U0126 prevents ERK pathway phosphorylation and interleukin-1beta mRNA production after cerebral ischemia. *Chin Med Sci J* **19**:270-275.

Wang ZQ, Wu DC, Huang FP and Yang GY (2004b) Inhibition of MEK/ERK 1/2 pathway reduces pro-inflammatory cytokine interleukin-1 expression in focal cerebral ischemia. *Brain Res* **996**:55-66.

Woessmann W, Chen X and Borkhardt A (2002) Ras-mediated activation of ERK by cisplatin induces cell death independently of p53 in osteosarcoma and neuroblastoma cell lines. *Cancer Chemother Pharmacol* **50**:397-404.

Wong JK, Le HH, Zsarnovszky A and Belcher SM (2003) Estrogens and ICI182,780 (Faslodex) modulate mitosis and cell death in immature cerebellar neurons via rapid activation of p44/p42 mitogen-activated protein kinase. *J Neurosci* **23**:4984-4995.

Wu Z, Wu LJ, Tashiro S, Onodera S and Ikejima T (2005) Phosphorylated extracellular signal-regulated kinase up-regulated p53 expression in shikonin-induced HeLa cell apoptosis. *Chin Med J (Engl)* **118**:671-677.

Yang JY, Michod D, Walicki J and Widmann C (2004) Surviving the kiss of death. *Biochem Pharmacol* **68**:1027-1031.

Yoon S and Seger R (2006) The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. *Growth Factors* **24**:21-44.

Yu SP (2003) Regulation and critical role of potassium homeostasis in apoptosis. *Prog Neurobiol* **70**:363-386.

Yu SP, Canzoniero LM and Choi DW (2001) Ion homeostasis and apoptosis. *Curr Opin Cell Biol* **13**:405-411.

Zhou G, Bao ZQ and Dixon JE (1995) Components of a new human protein kinase signal transduction pathway. *J Biol Chem* **270**:12665-12669.

Figure Legends

Figure 1. Mechanisms of ERK-mediated apoptosis. ERK may regulate apoptosis and cell survival at multiple points that include increasing p53 and BAX action, increasing caspase-3 and caspase-8 activities, decreasing Akt activity, and increasing TNF-α production.

JPET Fast Forward. Published on June 26, 2006 as DOI: 10.1124/jpet.106.107367
This article has not been copyedited and formatted. The final version may differ from this version.

Table 1. Examples of ERK mediated apoptosis in different cell types and stimuli

Cell types	Inducers	References
Oligodendrocyte	H_2O_2	(Bhat and Zhang, 1999)
Hippocampal neurons	Amloid	(Medina et al., 2005)
Glial cells	gluthathione depetion	(de Bernardo et al., 2004)
CGN (cerebellar granule neurons)	low potassium	(Subramaniam et al., 2003; Subramaniam et al., 2004)
	steroid hormone	(Wong et al., 2003)
Pyramidal cells	Okadaic acid	(Runden et al., 1998)
Renal proximal tubular cells	Cisplatin	(Nowak, 2002; Arany et al., 2004; Kim et al., 2005)
Hepatoma cell line (HepG2)	doxorubicin	(Alexia et al., 2004)
HeLa (cervical carcinoma cells)	Cisplatin	(Wang et al., 2000)
Renal epithelial cells	Growth factors depletion	(Sinha et al., 2004)
Renal epithelial cells	Escherichia coli toxin	(Chen et al., 2004)
Renal epithelial cells	ROS	(Tikoo et al., 2001; Ramachandiran et al., 2002; Dong et al., 2004)
Osteoblastic cells	H_2O_2	(Park et al., 2005)
Pancreatic beta-cells	interleukin-1 beta	(Pavlovic et al., 2000)
TM4 cells (Sertoli cell line)	CD95L	(Ulisse et al., 2000)
Lens epithelial cells	Calcium	(Li et al., 2005)
Bone marrow stromal cells	Leptin	(Kim et al., 2003)
B65 cell line (dompamine neuron)	6-hydroxydopamine	(Kulich and Chu, 2001)
SH-SY5Y (neuroblastoma cell line)	1-Methyl-4-phenylpyridinium	(Gomez-Santos et al., 2002)

Figure 1