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Oxidative stress and mitochondrial dysfunction as determinants of ischemic neuronal death and survival

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Abstract

Mitochondria are the powerhouse of the cell. Their primary physiological function is to generate adenosine triphosphate through oxidative phosphorylation via the electron transport chain. Reactive oxygen species generated from mitochondria have been implicated in acute brain injuries such as stroke and neurodegeneration. Recent studies have shown that mitochondrially-formed oxidants are mediators of molecular signaling, which is implicated in the mitochondria-dependent apoptotic pathway that involves pro- and antiapoptotic protein binding, the release of cytochrome *c*, and transcription-independent p53 signaling, leading to neuronal death. Oxidative stress and the redox state of ischemic neurons are also implicated in the signaling pathway that involves phosphatidylinositol 3-kinase/Akt and downstream signaling, which lead to neuronal survival. Genetically modified mice or rats that overexpress or are deficient in superoxide dismutase have provided strong evidence in support of the role of mitochondrial dysfunction and oxidative stress as determinants of neuronal death/survival after stroke and neurodegeneration.

Keywords

apoptosis; ischemia; mitochondria; oxidative stress; p53; reactive oxygen species

Introduction

Mitochondria are central integrators for and transducers of apoptotic signals in neurons. Mitochondria physiologically generate adenosine triphosphate through oxidative phosphorylation via the electron transport chain. Reactive oxygen species (ROS) generated from mitochondria mediate molecular signaling, such as p53. In mitochondria-dependent apoptosis, molecular signaling returns to mitochondria, then triggers the release of critical apoptotic activators and effectors of cell death, such as cytochrome *c* or apoptosis-inducing factor, from the mitochondrial intermembrane space.

p53 is the master regulator of cell death by inducing apoptosis (Schmitt *et al*. 2002). p53 encodes a sequence-specific transcription factor that controls apoptosis-related gene expression. Bcl-2-associated X protein (Bax) (Miyashita and Reed 1995), BH3 interacting domain death agonist (Bid) (Wang *et al*. 1996; Sax *et al*. 2002), NADPH oxidase activator 1 (Noxa) (Oda *et al*. 2000b), p53 acetate-induced protein 1 (*p53AIP1*) (Oda *et al*. 2000a), and p53-upregulated modulator of apoptosis (PUMA) (Nakano and Vousden 2001; Yu *et al*. 2001), all of which are products of p53, act directly on mitochondria and induce apoptosis. p53-induced protein with a death domain (PIDD), which is also a product of p53, activates

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caspase-2, resulting in the activation of mitochondria-dependent apoptosis (Tinel and Tschopp 2004; Berube *et al*. 2005; Ren *et al*. 2005; Seth *et al*. 2005). Moreover, p53 mediates apoptosis in a transcription-independent manner (Chipuk *et al*. 2004) (Fig. 1).

Recent findings demonstrate that p53 is involved in neuronal death that occurs with stroke and neurodegeneration (Crumrine *et al*. 1994; Li *et al*. 1994; Tomasevic *et al*. 1999; Saito *et al*. 2005; Endo *et al*. 2006a). Overexpression of copper/zinc-superoxide dismutase (SOD1) downregulated PUMA (Niizuma *et al*. 2009), suggesting a functional relationship between oxidative stress and the p53 signaling pathway. Here, we discuss the role of mitochondrial dysfunction and oxidative stress as determinants of neuronal death after stroke and neurodegeneration, focusing on Bax, PUMA, PIDD, transcription-independent p53 translocation, and SOD1 overexpression.

Bax signaling pathway

Bax has an extensive amino acid homology with Bcl-2. Bax homodimerizes and forms heterodimers with Bcl-2 (Oltvai *et al*. 1993). Cell fractionation and confocal microscopy showed that Bax localized in the cytosol of most cells, although it has the C-terminal putative transmembrane domain, similar to that of Bcl-2 (Hsu *et al.* 1997). With apoptotic stimuli, Bax is post-transcriptionally activated, then it oligomerizes and translocates to mitochondria. Mitochondrial Bax triggers cytochrome *c* release from mitochondria (Gross *et al*. 1998; Fiskum *et al*. 1999).

Bax is known to have roles in neuronal death. Bax mRNA was upregulated after transient global cerebral ischemia (tGCI) (Honkaniemi *et al*. 1996). Bax protein levels increased after tGCI (Krajewski *et al*. 1995) and focal cerebral ischemia (FCI) (Gillardon *et al*. 1996). Recent studies indicate that Bax transcription was regulated by p53, and translocation was mediated by c-Jun N-terminal kinase (JNK) in focal ischemia and experimental Parkinson's disease (Okuno *et al*. 2004; Perier *et al*. 2007). Bax interacts with truncated Bid, Bim, or PUMA, which triggers cytochrome *c* release in neurons (Desagher *et al*. 1999; Okuno *et al*. 2004; Niizuma *et al*. 2009).

In summary, apoptotic stimuli cause Bax to increase and translocate to mitochondria. Mitochondrial Bax interacts with other Bcl-2 family proteins, which triggers cytochrome *c* release (Fig. 1).

PUMA signaling pathway

PUMA was originally identified as a direct target of p53 with two putative p53 binding sites (Nakano and Vousden 2001; Yu *et al.* 2001). PUMA has been reported to be a strong inducer of apoptosis. In an *in vitro* study, PUMA expression caused rapid apoptosis (Yu *et al*. 2001) and PUMA inhibition by antisense oligonucleotide reduced apoptosis (Nakano and Vousden 2001). PUMA induced apoptosis through a mitochondria-dependent pathway (Nakano and Vousden 2001; Yu *et al*. 2001).

Recent reports have demonstrated that PUMA induces apoptosis by interacting with anti- or pro-apoptotic proteins (Nakano and Vousden 2001; Yu *et al*. 2001; Chen *et al*. 2005; Kim *et al*. 2006; Steckley *et al*. 2007). PUMA can interact with multiple Bcl-2 family members through the BH3 domain (Chen *et al.* 2005). It localized to mitochondria and interacted with both pro-apoptotic Bax (Kim *et al*. 2006; Steckley *et al*. 2007) and anti-apoptotic Bcl-2 or Bcl-X_L (Nakano and Vousden 2001; Yu *et al.* 2001) through a BH3 domain, followed by cytochrome *c* release and caspase activation.

PUMA is also known to have important roles in neuronal apoptosis. Its overexpression induced apoptosis in primary neurons (Cregan *et al*. 2004), and PUMA nullizygous neurons are resistant to araC-induced apoptosis (Wyttenbach and Tolkovsky 2006). PUMA mediated oxidative stress-induced neuronal apoptosis through cytochrome *c* release and caspase activation in a primary culture of mouse neurons (Steckley *et al*. 2007). It also mediated camptothecin-induced neuronal death in a primary mouse neuron culture (Uo *et al*. 2007). PUMA regulated neuronal death after tGCI (Reimertz *et al*. 2003; Niizuma *et al*. 2009) (Fig. 2).

In summary, PUMA is induced by p53, and interacts with pro-apoptotic and anti-apoptotic Bcl-2 family proteins, resulting in cytochrome *c* release (Fig. 1).

PIDD signaling pathway

PIDD was also identified as a target of p53 (Lin *et al*. 2000). Since PIDD overexpression in p53-deficient human cell lines induces cell-cycle arrest and apoptosis, PIDD is considered to act downstream of p53. Full length PIDD is constitutively cleaved into an N-terminal fragment and a C-terminal fragment (PIDD-C) by autoproteolysis. PIDD-C is further cleaved into PIDD-CC by autoproteolysis (Tinel and Tschopp 2004; Tinel *et al*. 2007).

Evidence for the role of PIDD-CC in the activation of caspase-2 has been accumulating (Tinel and Tschopp 2004; Berube *et al.* 2005; Ren *et al.* 2005; Seth *et al.* 2005). PIDD-CC, receptor-interacting protein-associated ICH-1/CED-3 homologous protein with a death domain (RAIDD), and procaspase-2 form a large protein complex, which is referred to the PIDDosome, similar to the caspase-9—activating apoptosome complex (Tinel and Tschopp 2004). PIDD interacts with RAIDD through the death domain, and RAIDD interacts with caspase-2 through the caspase recruitment domain, resulting in the crystal structure of the PIDDosome (Park *et al*. 2007). Procaspase-2 was schematically dimerized and activated by the PIDDosome (Park *et al*. 2007). Similar to the caspase-9—activating apoptosome complex, the PIDDosome regulates stress-induced apoptosis (Tinel and Tschopp 2004).

In contrast to PIDD-CC, PIDD-C is thought to have an anti-apoptotic role. In a recent study, PIDD-C formed a protein complex with a nuclear factor-κB essential modulator and receptor-interacting protein 1. This activated the transcription factor nuclear factor-κB pathway in response to genotoxic stress (Janssens *et al*. 2005).

In neuronal death, PIDD-CC increased after tGCI, followed by caspase-2 activation and Bid cleavage (Niizuma *et al.* 2008). Truncated Bid interacts with Bax, exposing the N-terminus of Bax and inducing its oligomerization followed by release of pro-apoptotic proteins from mitochondria (Desagher *et al*. 1999).

In summary, PIDD is transcriptionally induced by p53, then cleaved by autoproteolysis. PIDD-CC forms the PIDDosome, which activates caspase-2. Caspase-2 cleaved Bid, followed by the interaction of Bax and truncated Bid, resulting in cytochrome *c* release (Fig. 1).

Transcription-independent p53 translocation

Most of the effects of p53 are ascribed to its function as a transcription factor. However, reports have suggested that p53 can also induce apoptosis independently of its transcriptional activity (Caelles *et al*. 1994; Bennett *et al*. 1998; Mihara *et al*. 2003). In response to certain death stimuli, a fraction of stabilized p53 rapidly translocates to mitochondria in some cell types (Marchenko *et al*. 2000; Mihara *et al*. 2003; Erster *et al*. 2004). In p53 null cancer cells, exogenous p53 targeted to mitochondria induced apoptosis and suppressed colony formation in a transcription-independent manner (Mihara *et al*. 2003). Furthermore, endogenous mitochondrial p53 forms inhibitory complexes with anti-apoptotic Bcl-X_L and Bcl-2 proteins,

which cause cytochrome *c* release and caspase activation (Mihara *et al*. 2003). Mitochondrial translocation of p53 launches a rapid pro-apoptotic response in a transcription-independent manner that jump-starts and amplifies the slower transcription-dependent response (Erster *et al*. 2004). This translocation may be regulated by the Akt-Mdm2 pathway through monoubiquitylation of p53 (Marchenko *et al*. 2007).

In neuronal cell death, p53 translocated to mitochondria and interacted with anti-apoptotic Bcl-X_L, followed by cytochrome *c* release after tGCI (Endo *et al*. 2006a). Inhibition of p53 translocation caused by a specific dosage of the p53 inhibitor, pifithrin-α, resulted in neuroprotection of the hippocampal CA1 subregion against cerebral ischemia and reperfusion (Endo *et al*. 2006a).

In summary, p53 can induce apoptosis in a transcription-independent manner by interacting with Bcl-X_L after ischemia (Fig. 1).

SOD1 overexpression

Evidence is accumulating in support of the idea that activation of p53 signaling pathways, which precedes release of pro-apoptotic proteins from mitochondria, can cause apoptosis in ischemic neurons. However, the upstream events that lead to p53 signaling and neuronal death are unclear. ROS formation during reperfusion after cerebral ischemia in the mitochondria appears to be one such event. This is supported by the finding that p53 target genes upregulated in response to elevated oxidative stress in liver samples (Han *et al*. 2008). ROS cause DNA damage, which activates DNA-dependent kinase and ataxia telangiecta protein, resulting in phosphorylation of p53 at specific serine residues (Nakagawa *et al*. 1999; Shangary *et al*. 2000).

Specific scavengers of ROS, such as SOD1, may play a major role in modulating death signaling. SOD1 is an antioxidant isoenzyme mainly localized in the cytosol that dismutates superoxide anions to hydrogen peroxide (Fridovich 1975). SOD1 is constitutively present in all cells (Huang *et al*. 1999). In animals that overexpress SOD1, cytochrome *c* release and neuronal death were highly inhibited after FCI (Kinouchi *et al*. 1991; Chan 1996; Fujimura *et al*. 2000), tGCI (Murakami *et al*. 1997; Chan *et al*. 1998; Endo *et al*. 2006b), and subarachnoid hemorrhage (Endo *et al*. 2007). The phospho-Akt survival pathway was significantly upregulated in SOD1-overexpressing animals compared with wild-type animals (Noshita *et al*. 2003; Endo *et al*. 2006b, 2007). In contrast to the survival pathway, p53 upregulation was inhibited by SOD1 overexpression after FCI (Saito *et al*. 2005). Moreover, PUMA upregulation was inhibited in SOD1-overexpressing animals after tGCI (Niizuma *et al*. 2009), suggesting that oxidative stress may modulate pro-survival Akt signaling and pro-death p53 signaling that determine death or survival of ischemic neurons.

In contrast to SOD1 overexpression, SOD1 homozygous mutants (SOD1-/-) and heterozygous mutants (SOD1-/+) showed high mortality, increased infarct volume, and greater apoptotic neuronal cell death after FCI (Kondo *et al*. 1997). SOD1 deficiency also showed increased neuronal death after tGCI (Kawase *et al*. 1999).

These results cumulatively suggest that oxidative stress, known to be generated during reperfusion following an ischemic event, is associated with cell survival signaling such as Akt, cell death pathways such as p53, and the determination of subsequent neuronal survival or death.

Conclusion

Oxidative stress may regulate p53-dependent transcription, p53 translocation, and pro-survival Akt signaling through phosphorylation, at least in part. Decreasing oxidative stress by SOD1 overexpression results in neuroprotection. Mitochondrial dysfunction and oxidative stress may determine neuronal death/survival after stroke and neurodegeneration.

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Abbreviations used

ROS, reactive oxygen species
Bax, Bcl-2-associated X protein
Bid, BH3 interacting domain death agonist
Noxa, NADPH oxidase activator 1
p53AIP1, p53 acetate-induced protein 1
PUMA, p53-upregulated modulator of apoptosis
PIDD, p53-induced protein with a death domain
SOD1, copper/zinc-superoxide dismutase
tGCI, transient global cerebral ischemia
FCI, focal cerebral ischemia
JNK, c-Jun N-terminal kinase
RAIDD, receptor-interacting protein-associated ICH-1/CED-3 homologous protein with a death domain

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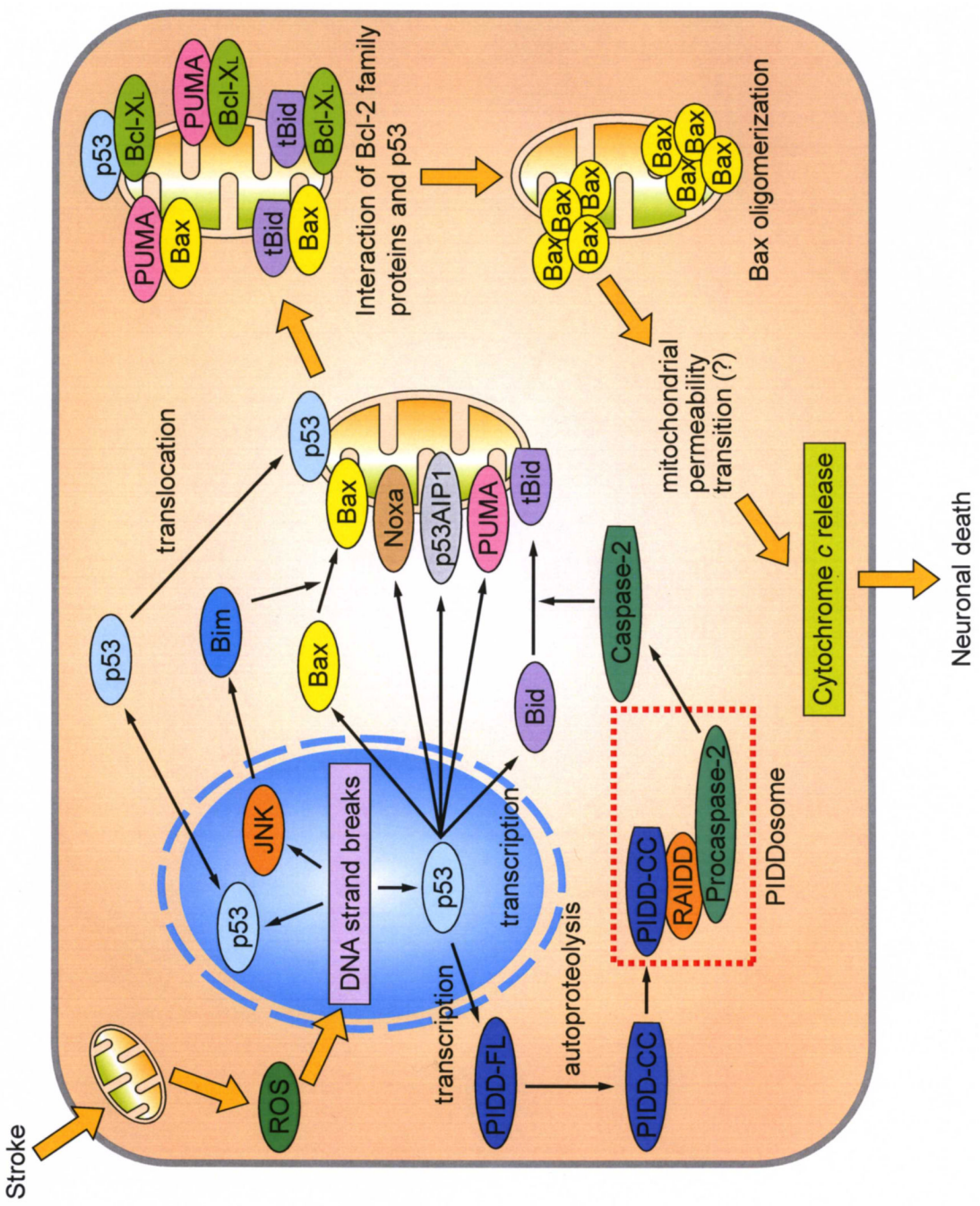

Fig. 1.

Involvement of p53 signaling after ROS generation. After ROS generation from mitochondria, p53 transcriptionally generates pro-apoptotic proteins such as Bax, Noxa, *p53AIP1*, PUMA, and Bid. These products act directly on mitochondria. Mitochondrial translocation of Bax is promoted by JNK through transcriptional activation of Bim. Full-length PIDD (PIDD-FL) is also transcriptionally upregulated by p53. PIDD-CC, a fragment of PIDD-FL cleaved by autoproteolysis, activates caspase-2 through the formation of the PIDDosome, which precedes Bid truncation and translocation to mitochondria. Moreover, p53 translocates to the mitochondrial membrane and activates the mitochondria-dependent apoptotic pathway in a transcription-independent manner. BH3-only proteins and p53 interact with both pro-apoptotic

Bax and anti-apoptotic Bcl-X_L on the mitochondrial membrane. This interaction causes Bax oligomerization and activation, which triggers cytochrome c release, leading to neuronal death. tBid, truncated Bid.

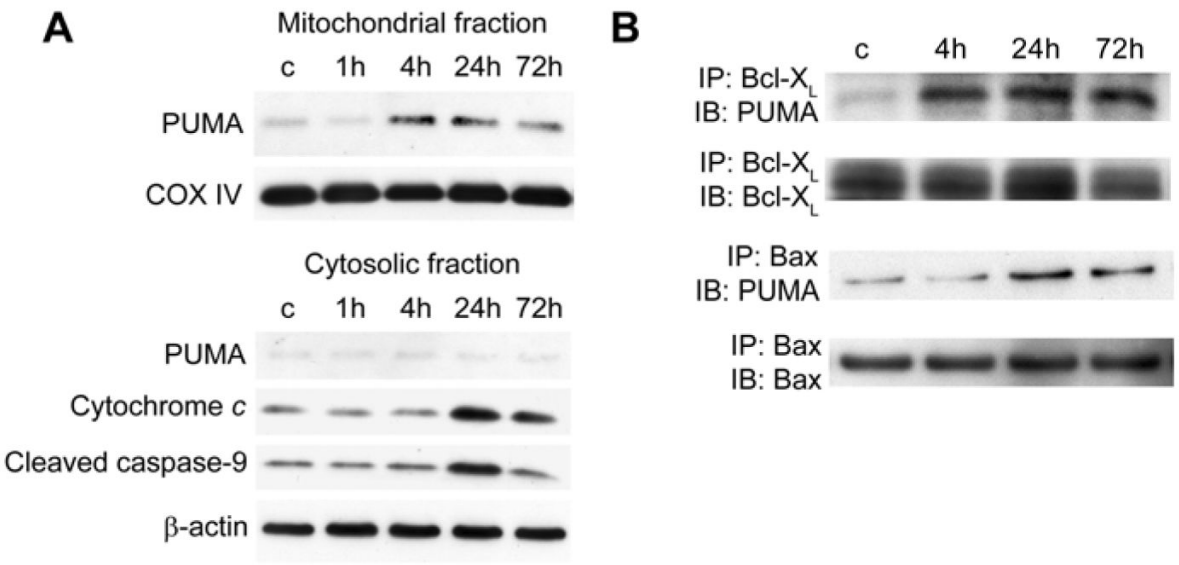

Fig. 2.

Mitochondrial PUMA upregulation after tGCI. (A) Western blot analysis shows that mitochondrial PUMA increased 4 and 24 h after tGCI, followed by cytosolic upregulation of cleaved caspase-9 and cytochrome *c* release. β-actin and cytochrome oxidase subunit IV (COX IV) analyses are shown as internal controls. c, control. (B) Coimmunoprecipitation analyses show that PUMA immunoreactivity precipitated by Bcl-X_L or Bax increased after tGCI. Bcl-X_L precipitated by Bcl-X_L, and Bax precipitated by Bax were used to show equal precipitation. IP, immunoprecipitation; IB, immunoblotting. (Data modified from Niizuma *et al*. 2009.)