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Mitochondrial and apoptotic neuronal death signaling pathways in cerebral ischemia

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Abstract

Mitochondria play important roles as the powerhouse of the cell. After cerebral ischemia, mitochondria overproduce reactive oxygen species (ROS), which have been thoroughly studied with the use of superoxide dismutase transgenic or knockout animals. ROS directly damage lipids, proteins, and nucleic acids in the cell. Moreover, ROS activate various molecular signaling pathways. Apoptosis-related signals return to mitochondria, then mitochondria induce cell death through the release of pro-apoptotic proteins such as cytochrome *c* or apoptosis-inducing factor. Although the mechanisms of cell death after cerebral ischemia remain unclear, mitochondria obviously play a role by activating signaling pathways through ROS production and by regulating mitochondria-dependent apoptosis pathways.

Keywords

Mitochondria; Cerebral ischemia; SOD1; Reactive oxygen species; Neuronal death; PIDD

1. Introduction

Mitochondria are the powerhouse of the cell. Their primary physiological function is to generate adenosine triphosphate through oxidative phosphorylation via the electron transport chain, which contains five multi-subunit enzyme complexes, I to V. Reactive oxygen species (ROS) are generated in complex I and complex III during mitochondrial respiration [1]. Therefore, oxygen metabolism can be a potential threat to tissues and cells.

Numerous studies have shown the roles ROS play in the pathophysiology of neurological disorders, including ischemia, trauma, and degenerative diseases. ROS cause macromolecular damage such as lipid peroxidation, protein oxidation, and DNA oxidation, all of which can lead to cell injury and death [2,3]. In addition, ROS can act as intracellular messengers to transduce signals of various pathways, including cell death pathways [4,5], similar to the way in which reactive nitrogen species transduce signals in endothelial cells or neurons [6,7].

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Besides triggering molecular signals by overproduction of ROS, mitochondria regulate apoptotic pathways by sequestering Ca^{2+}, storing and releasing pro-apoptotic proteins such as cytochrome *c* and apoptosis-inducing factor (AIF), and probably by opening the permeability transition pore [8,9]. In this review, we discuss the roles of ROS generated in mitochondria and mitochondria-dependent apoptotic pathways in several in vivo models of cerebral ischemia.

2. The roles of ROS generated by mitochondria

2.1. Generation and clearance of ROS under normal physiological conditions

Because mitochondria generate superoxide anions (O_2-) and hydrogen peroxide (H_2O_2) during mitochondrial respiration under normal physiological conditions [1], oxygen metabolism poses a potential threat to cells. It is, nevertheless, essential for cell survival. Pro-oxidant enzymes, such as nitric oxide synthases (NOS), cyclooxygenases, xanthine dehydrogenase, xanthine oxidase, NADPH oxidase, myeloperoxidase, and monoamine oxidase, generate the ROS O_2-, H_2O_2, nitric oxide, and lipid peroxides.

To detoxify such ROS, cells develop ROS clearance systems. Superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase contribute to scavenging these ROS. SOD has three isoforms: copper/zinc SOD (SOD1), manganese SOD (SOD2), and extracellular SOD (SOD3) (Table 1). All three SOD isoforms dismutate O_2- to H_2O_2 and molecular oxygen. Then, GSHPx scavenges H_2O_2 to water at the expense of glutathione. Catalase also dismutates H_2O_2 to water [2]. Other small molecular non-enzymatic antioxidants such as vitamin E and vitamin C are also involved in the detoxification of free radicals [10].

Oxidative stress is defined as the pathogenic outcome of ROS overproduction beyond the capacity of ROS clearance in cells. After cerebral ischemia, the balance between ROS production and clearance shifts to the production side, resulting in induction of oxidative stress-induced signaling and cell injury.

2.2. Reperfusion injury and ROS

Reperfusion injury is brain damage caused by the return of blood flow, resulting in progression of vasogenic edema, hemorrhagic transformation, and an increase in stroke volume. ROS involvement in reperfusion injury has been described since the early 1980s [11,12]. Numerous subsequent reports have presented the relationship between reperfusion injury and ROS. In ischemic brain tissue, ROS generation is accelerated by cytosolic pro-oxidant enzymes and by mitochondria, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately replenish antioxidants [2]. These overproduced ROS cause macromolecular damage and activation of various pathways.

2.3. Detection and quantification of ROS

To detect and quantify various ROS in the ischemic brain, an indirect measurement method is required because of the short half-life of most ROS. One approach is to detect oxidative modification of biological targets of ROS such as lipid peroxidation, protein oxidation, or DNA oxidation. Another approach is to use reporter molecules, which are oxidized by ROS, resulting in the production of chromogenic, fluorescent, or luminescent molecules. Hydroethidine (HEt), one such reporter molecule, has been used to detect O_2- in cells and tissues [13,14]. “Ethidium fluorescence”, which is the red fluorescence arising from oxidation of HEt, has been attributed to O_2- trapping in cells [13,14]. However, a recent study revealed that ethidium could be generated by other ROS [15]. To specifically detect O_2-, 2-hydroxyethidium (2-HE), the two-electron oxidation product of HEt [16], is a more suitable diagnostic marker than HEt [15].

Although the fluorescence spectra from 2-HE and ethidium overlap and fluorescence from 2-HE cannot be separated under a fluorescent microscope, red fluorescence caused by HEt oxidation is still a powerful tool for detecting ROS, mainly O_2-. Upregulation of this red fluorescence suggests that O_2- affects signaling and injury after cerebral ischemia [17-20].

A disadvantage of HEt is that reliable quantification cannot be provided with a fluorescent microscope. For specific and quantitative detection of O_2-, a high-performance liquid chromatography/fluorescence assay [15], in addition to a fluorescent microscope study, may be required.

2.4. Transgenic and knockout studies of SOD

Although development of methodologies to detect and quantify ROS have enabled researchers to investigate their roles after cerebral ischemia, their causative roles in ischemic brain injury remain unclear. Advances in transgene and gene knockout (KO) technology have allowed us to investigate the contributions of ROS to molecular mechanisms of ischemic brain injury. Table 2 shows studies using cerebral ischemia models with transgenic (Tg) animals that carry human SOD genes or KO animals that are homozygously or heterozygously deficient in SOD genes.

SOD1 is neuroprotective. In heterozygous SOD1 Tg animals that carry the human SOD1 gene, SOD1 activity increased (a three-fold increase in SOD1 Tg mice [21] and an approximate four-fold increase in SOD1 Tg rats [22]) compared with wild-type (Wt) animals. In SOD1 Tg animals, a 35-50% decrease in infarct volume is usually observed after focal cerebral ischemia (FCI) [23,24]. After transient global cerebral ischemia (tGCI), delayed neuronal cell death decreases to about 50% in SOD1 Tg animals [25,26]. Regulation of various pathways contributes to neuroprotection, including activation of the phosphoinositide 3-kinase (PI3-K) pathway [27,28], and inhibition of the mitogen-activated protein kinase (MAPK) -related pathway [29,30], and the p53 signaling [18,31], nuclear factor-κB [19,20,32], and mitochondria-dependent apoptotic [22,29,33] pathways. Moreover, infarct volume and edema levels decrease after FCI in homozygous SOD1 KO mice [34-36], while cell death increases after tGCI [37].

SOD2 also has important neuroprotective roles. Heterozygous SOD2 Tg mice carrying the human SOD2 gene showed decreased injury [38] and reduced vascular endothelial cell death [39] after FCI. Moreover, infarct volume [40], brain edema [39], O_2- production [17], matrix metalloproteinase-9 activity [39], caspase-9 activation [41], and cytochrome *c* release [42] increase after FCI in SOD2 KO mice compared with Wt mice. Furthermore, hemorrhagic transformation after transient FCI (tFCI) significantly increases in SOD2 KO mice [39].

Although only a few studies have used SOD3 Tg or KO mice in cerebral ischemia models, they have shown that SOD3 has neuroprotective roles. Infarct volume after FCI decreases (−28%) in SOD3 Tg mice [43] that express a five-fold higher level of SOD3 in the brain compared with Wt mice [44]. Neuronal death after tGCI also decreases (−48%) in SOD3 Tg mice [45]. In contrast, infarct after FCI in homozygous SOD3 KO mice increases (+81%) [46]. In summary, studies using various SOD Tg and KO animals imply that ROS have important roles in activating various pathways and determining the outcome after cerebral ischemia.

2.5. Mitochondrial NOS

Three canonical isoforms of NOS are well known in mammals: neuronal NOS (nNOS), inducible NOS, and endothelial NOS. Recent findings reveal that mitochondria contain their own isoform of NOS, mitochondrial NOS (mtNOS), at their inner membrane [47,48]. Since

NOS isoforms are encoded not by mitochondrial DNA, but by nuclear DNA, mtNOS is thought to be synthesized in the cytosol and translocated to mitochondria [49], although the mechanism of this translocation remains unknown. mtNOS stays active because of mitochondrial Ca^{2+} content, in contrast to other nitric oxide sources. mtNOS continuously controls mitochondrial respiration [47,48] and is considered a key molecule of reperfusion injury [50].

The enzymatic activity of mtNOS was higher in hypoxic animals than in normoxic controls [51]. mtNOS is also considered a marker of brain aging. In aged mice, mtNOS activity was linearly correlated with neurological performance and survival [52]. Since mtNOS controls mitochondrial respiration and nitric oxide generation, it may correlate with apoptosis after stroke. Further studies may reveal the roles of mtNOS after stroke and may provide novel therapeutic strategies.

3. Ischemic neuronal apoptotic pathways (Fig. 1)

3.1. The intrinsic pathway

After mitochondria trigger various signaling pathways by overproduction of ROS, some, but not all, apoptotic signals return to mitochondria with the help of BH3-only proteins. Then, Bcl-2 family proteins (such as cytochrome *c*, AIF, endonuclease G [Endo G], and second mitochondria-derived activator of caspase [Smac]) interact with each other, resulting in the release of pro-apoptotic proteins stored in the mitochondrial intermembrane space, followed by neuronal apoptosis. This pathway is called the ‘intrinsic pathway’.

3.2. Bcl-2 family protein interactions

The Bcl-2 protein family, which is a principal regulator of mitochondrial membrane integrity and function, is classified into three subgroups according to structural homology: the anti-apoptotic proteins such as Bcl-2, Bcl-X_L, and Bcl-w, the pro-apoptotic proteins such as Bax and Bak, and the BH3-only proteins including Bad, Bid, Bim, Noxa, and p53-upregulated modulator of apoptosis (PUMA). Since neurons lack full-length Bak, Bax is the only pro-apoptotic protein in neurons. In response to apoptotic stimuli, specific BH3-only proteins are activated and transduce apoptotic signals to mitochondria. Studies have shown that after cerebral ischemia, BH3-only proteins were upregulated, meaning cerebral ischemia activates various apoptotic pathways.

Currently, two main ideas can explain Bcl-2 protein family interaction: the ‘direct model’ and the ‘hierarchy model’ (Fig. 2). In the direct model, anti-apoptotic proteins trap pro-apoptotic proteins. BH3-only proteins disrupt this interaction, resulting in liberation of pro-apoptotic proteins and subsequent apoptosis (Fig. 2A).

Recently, Kim et al. [53] advocated the ‘hierarchy model’. In this model, BH3-only proteins are subdivided into two groups: ‘activator’ and ‘inactivator’. Bim, PUMA, and truncated Bid (tBid) belong to the activator group and other BH3-only proteins belong to the inactivator group. Activator BH3-only proteins are trapped by anti-apoptotic proteins, whereas pro-apoptotic proteins are not. Inactivator BH3-only proteins disrupt this interaction, resulting in liberation of activator BH3-only proteins. Liberated activator BH3-only proteins interact with pro-apoptotic proteins, followed by apoptosis (Fig. 2B).

The Bcl-2 family plays various roles in cerebral ischemia. BH3-only proteins, including Bad [33,54,55], Bim [56,57], Noxa [57,58], PUMA [18,59], and tBid [18,60] contribute to cell death after cerebral ischemia, mainly through interactions with other Bcl-2 family members. Bax increases after tGCI [61] or FCI [62], and translocates from the cytosol to mitochondria, mediated by c-Jun N-terminal kinase with Bim_L [56]. Bim [56], tBid [63], and PUMA [18] have been reported to interact with Bax after cerebral ischemia, which may support the

hierarchy model. After interacting with other Bcl-2 family proteins, Bax is oligomerized and activated, which triggers release of apoptotic proteins stored in the mitochondrial intermembrane space, leading to neuronal apoptosis [8,56].

3.3. Bcl-2 family downstream interactions

Proteins in the mitochondrial intermembrane space, including cytochrome *c* [64,65], Smac [66], AIF [67], and Endo G [68], are released after cerebral ischemia, at which time they cause transduction of apoptotic signals. Release of these proteins leads to 'the point of no return'. Cytochrome *c* interacts with apoptosis activating factor-1, deoxyadenosine triphosphate, and procaspase-9, and forms the apoptosome, which activates procaspase-9 [69-71]. Caspase-9 activates procaspase-3, then caspase-3 cleaves inhibitor of caspase-activated DNase, which is an inhibitor and a chaperone of caspase-activated DNase. Liberated caspase-activated DNase damages DNA and induces apoptosis. Caspase-3 can also activate other effector caspases, which activate crucial substrates, including poly(ADP-ribose) polymerase (PARP), after cerebral ischemia [72,73]. Although PARP is involved in both apoptotic and non-apoptotic cell death, 89- and 21-kDa fragments are cleaved by caspases and are related to apoptosis after cerebral ischemia [73,74].

Smac also contributes to activation of caspases. Smac released from mitochondria binds to and neutralizes the effect of the X chromosome-linked inhibitor-of-apoptosis protein, which prevents procaspase activation and inhibits activities of activated caspases [66,74] after cerebral ischemia.

Recent reports show the importance of the caspase-independent pathways. AIF translocates from mitochondria to the nucleus and induces apoptosis after tFCI [67]. In mutant mice that express low-level AIF, infarct volume decreased (−43%) after tFCI [67]. PARP helped nuclear translocation of AIF [75]. Endo G is also known to translocate to the nucleus, causing DNA fragmentation after tFCI [68].

3.4. Upstream of the intrinsic pathway

ROS activate a number of pathways, including PI3-K, MAPK, and p53 pathways. These pathways modulate the intrinsic pathway.

3.4.1. Kinase pathway—Akt is a key molecule for neuronal death and survival after cerebral ischemia [27]. Akt is a serine/threonine kinase and a major downstream target of PI3-K. Akt phosphorylates and inactivates Bad after cerebral ischemia [55]. Since phosphorylated Bad is unable to inhibit the pro-survival Bcl-2 family proteins, Bad phosphorylation results in inactivation of the apoptotic pathway. Akt also phosphorylates procaspase-9 and caspase-9 on serine-196; procaspase-9 phosphorylation inhibits activation of procaspase-9, and caspase-9 phosphorylation inhibits protease activity [76]. Akt modulates p53 degradation through MDM2 phosphorylation [31].

Other kinases also have regulative roles in the intrinsic pathway. Phosphorylated extracellular signal-regulated kinase, which also phosphorylates and inactivates Bad, is upregulated after tFCI [29]. Protein kinase A phosphorylates and inactivates Bad after cerebral ischemia [33].

3.4.2. p53 signaling pathway—Since a number of Bcl-2 family proteins such as Bax, Bid, Noxa, PUMA, and *p53AIP1* are the products of p53, p53 plays important roles in the intrinsic pathway. These Bcl-2 family proteins increase and regulate cell death after cerebral ischemia, as described in section 3.2. Recent findings suggest that p53 can activate the intrinsic pathway in a transcription-independent manner, as well as in a transcription-dependent manner [58]. p53 translocates to mitochondria and interacts with anti-apoptotic Bcl-X_L, which precedes

cytochrome *c* release after tGCI [58]. A p53 inhibitor, pifithrin-α, decreased the translocation of p53, and resulted in neuroprotection in the hippocampal CA1 subregion after tGCI [58]. In summary, p53 acts as a BH3-only protein in this transcription-independent manner in addition to transcription of apoptosis-related proteins such as Bcl-2 family proteins.

3.4.3. PIDD signaling pathway—p53 and caspase-2 are involved with stress-induced apoptosis. However, the key molecules connecting them have not been determined. Tinel and Tschopp [77] reported that p53-induced protein with a death domain (PIDD), which is a target of p53, formed a high-molecular weight protein complex with RAIDD and procaspase-2. This molecular complex is referred to the 'PIDDosome', in which caspase-2 is activated, similar to caspase-9 activation in the apoptosome [77]. After tGCI, the PIDDosome increased in the hippocampal CA1 subregion, followed by caspase-2 activation and Bid cleavage, which preceded neuronal death [78].

Recently, in vitro studies have presented new findings regarding this PIDD pathway. One finding is that caspase-2 can directly interact with mitochondria and activate the mitochondria-dependent apoptotic pathway [79,80]. Interestingly, this interaction occurs independently of its proteolytic activity. Another finding is that a cleaved fragment of PIDD (PIDD-C) forms protein complexes that differ from the PIDDosome. The protein complex containing PIDD-C and nuclear factor-κB has an anti-apoptotic role in response to genotoxic stress [81]. These interactions after cerebral ischemia are unknown and require further study.

3.4.4. Crosstalk between the intrinsic pathway and the extrinsic pathway—The extrinsic pathway is the death-receptor-mediated pathway that receives extracellular signals and transduces them to intracellular signals. Recent studies have shown that the death receptor pathway has various physiological functions as well as apoptotic roles.

The Fas pathway (Fas is a death receptor) is involved in apoptosis after cerebral ischemia. mRNA and protein levels of both Fas and the Fas ligand are upregulated after cerebral ischemia [82,83]. Mutant mice that have a loss-of-function mutation for Fas show reduced infarct volume after FCI [82]. Fas, Fas-associated death domain, and procaspase-8 form a protein complex that is referred to as the death-inducing signaling complex (DISC). DISC activates procaspase-8, similar to procaspase-9 activation by the apoptosome. Caspase-8 activation is followed by activation of caspases −3 and −10 after cerebral ischemia [83].

There is crosstalk between the intrinsic pathway and the extrinsic pathway. The key molecule involved in this crosstalk is Bid, which is also a key molecule for the p53-caspase-2 pathway as described above. Bid is truncated by caspase-8, translocates to mitochondria, and interacts with other Bcl-2 family proteins, which causes cytochrome *c* release followed by apoptotic cell death [60].

4. Conclusions

Numerous reports show the involvement of ROS in cell death after cerebral ischemia. ROS contribute not only to injury of macromolecules, but also to transduction of apoptotic signals. Although it is well known that various factors, including necrosis, are involved in the mechanisms of cell death after cerebral ischemia, mitochondria contribute to cell death by activating signaling pathways through ROS production and by regulating intrinsic apoptosis pathways. Future studies of these cell death mechanisms after ischemia may provide unique information regarding molecular targets for therapeutic strategies in clinical stroke.

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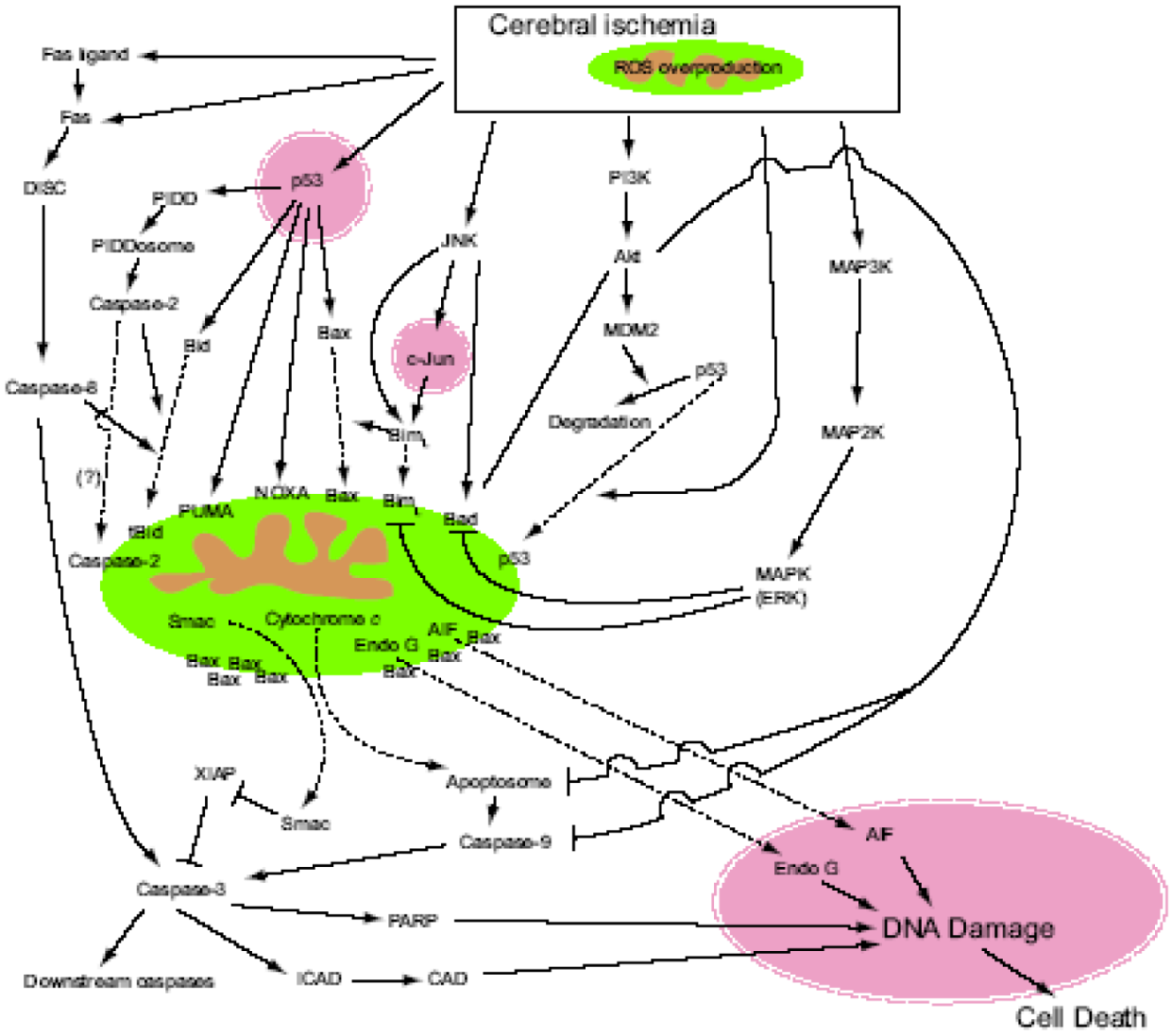

Fig. 1.

Mitochondria-dependent pathways of apoptosis in cerebral ischemia and reperfusion. After cerebral ischemia, various pathways, such as the death receptor pathway, p53 pathway, c-Jun N-terminal kinase (JNK) pathway, PI3-K pathway, and the MAPK pathway are activated. Most signaling pathways induce apoptosis with the help of pro-apoptotic proteins, such as cytochrome *c*, Endo G, AIF and Smac, which are stored in mitochondria.

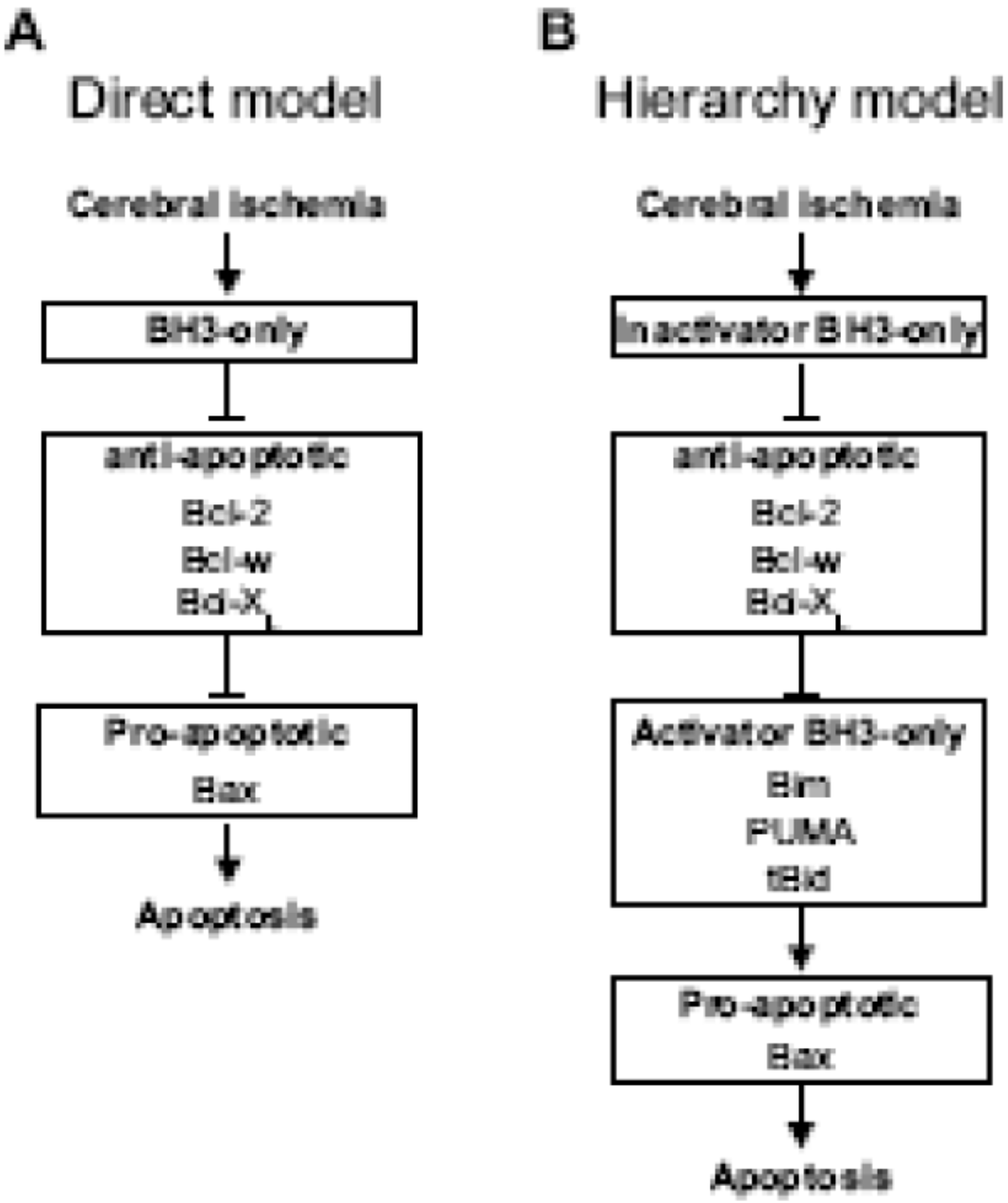

Fig. 2.

Two models of Bcl-2 protein family interaction. (A) The direct model for Bax activation. After apoptotic stimuli, specific BH3-only proteins are activated and inhibit anti-apoptotic Bcl-2 family proteins. Liberated Bax oligomerizes and triggers the release of pro-apoptotic proteins stored in the mitochondrial intermembrane space. (B) The hierarchy model for Bax activation. After apoptotic stimuli, specific inactivator BH3-only proteins are activated and inhibit anti-apoptotic Bcl-2 family proteins. Then, liberated activator BH3-only proteins interact with Bax, resulting in the release of pro-apoptotic proteins stored in the mitochondrial intermembrane space.

Table 1

Mammalian superoxide dismutases

□	**SOD1 (CuZnSOD)**	**SOD2 (MnSOD)**	**SOD3 (ECSOD)**
Location	Cytosol	Mitochondria	Extracellular space
Molecular weight	32,000	88,000	120,000
Structure	Dimer	Tetramer	Tetramer
Metals, g-atoms/subunit	Cu 1, Zn 1	Mn 1	Cu 1, Zn 1
Phenotype of transgenic mouse (+/+)	Normal	Normal	Normal
Phenotype of knockout mutant (−/−)	Normal	Neonatal lethality	Normal
	21 (human)	6 (human)	4 (human)
	16 (mouse)	17 (mouse)	5 (mouse)
Chromosome	11 (rat)	1 (rat)	14 (rat)

CuZn, copper, zinc; Mn, manganese; EC, extracellular.

Table 2

Transgenic and knockout studies of superoxide dismutases using in vivo cerebral ischemia models

Study	Animal	Model	Main findings	References
SOD1 +/−	Mouse	pFCI	Decreased cortical infarct (−35%)	[23]
SOD1 +/−	Mouse	pFCI	No protection	[84]
SOD1 +/−	Mouse	tFCI	Decreased infarct	[85]
SOD1 +/−	Mouse	tFCI	Sustained hsp70 mRNA expression	[86]
SOD1 +/−	Mouse	tFCI	Sustained *c-fos* mRNA expression	[87]
SOD1 +/−	Mouse	tGCI	Induction of hsp 70	[88]
SOD1 +/−	Mouse	tFCI	Decreased injury (−50%)	[24]
SOD1 +/−	Rat	tGCI	Decreased injury (−50%)	[25]
SOD1 +/−	Mouse	tGCI	Decreased injury (−50%)	[26]
SOD1 +/−	Mouse	tFCI	Decreased DNA fragmentation	[89]
SOD1 +/−	Mouse	tFCI	Decreased cytochrome *c* release	[90]
SOD1 +/−	Mouse	tFCI	Decreased NF-κB expression	[32]
SOD1 +/−	Mouse	tFCI	Decreased activation of activator protein-1	[91]
SOD1 +/−	Mouse	pFCI	No difference in infarct volume	[92]
SOD1 +/−	Rat	tGCI	Decreased active caspase-3, -9	[22]
SOD1 +/−	Mouse	tFCI	Decreased ERK activation	[29]
SOD1 +/−	Mouse	tFCI	Decreased Bad activation	[33]
SOD1 +/−	Mouse	tFCI	Increased pAkt expression; decreased DNA fragmentation	[27]
SOD1 +/−	Mouse	tFCI	Decreased PARP activation	[93]
SOD1 +/−	Mouse	tFCI	Decreased lesion size and edema; decreased MMP-2, -9 expression	[36]
SOD1 +/−	Rat	tGCI	Decreased injury, PERK phosphorylation and GRP78 release	[94]
SOD1 +/−	Mouse	tFCI	Decreased injury, PERK phosphorylation and GRP78 release	[95]
SOD1 +/−	Mouse	tFCI	Decreased binding of XIAP/DNP, Smac/DNP and caspase-9/DNP	[96]
SOD1 +/−	Mouse	tFCI	Decreased Omi/HtrA2 activation	[96]
SOD1 +/−	Mouse	tFCI	Increased ILK expression and ILK/Akt complex	[97]
SOD1 +/−	Rat	tGCI	Inhibited ATF-4 induction and CHOP expression; decreased endoplasmic reticulum damage	[98]
SOD1 +/−	Mouse	tFCI	Inhibited ATF-4 induction and CHOP expression	[98]
SOD1 +/−	Mouse	tFCI	Increased proteasome activity and MDM2 activation; decreased nuclear p53	[31]
SOD1 +/−	Rat	tGCI	Inhibited APE/Ref-1 decrease; decreased injury	[99]
SOD1 +/−	Mouse	tFCI	Decreased MCP-1 and MIP-1α expression	[100]
SOD1 +/−	Mouse	tFCI	Decreased level of O_2-; decreased NF-κB activation and phosphorylation	[20]
SOD1 +/−	Mouse	tFCI	Increased pPRAS, pPRAS/pAkt binding and pPRAS/14-3-3 protein binding	[101]
SOD1 +/−	Rat	tGCI	Increased pAkt and pGSK-3β expression	[28]
SOD1 +/−	Rat	tGCI	Decreased p53 translocation to mitochondria	[58]
SOD1 +/−	Mouse	tFCI	Decreased level of O_2-; inhibited persistent upregulation of NF-κB	[19]
SOD1 +/−	Rat	tFCI with hyperglycemia	Decreased MMP activity and Evans blue leakage	[102]
SOD1 +/−	Rat	tFCI	Decreased activity of p38, phospho-p38, Evans blue leakage, edema and infarct	[30]
SOD1 +/−	Rat	tGCI	Decreased PUMA activation and injury; decreased level of O_2-	[18]
SOD1 −/−	Mouse	tFCI	Increased infarct (+40%)	[34]
SOD1 −/−	Mouse	tFCI	Increased lesion size and edema	[35]
SOD1 −/−	Mouse	tGCI	Increased cell death	[37]
SOD1 −/−	Mouse	pFCI	No difference in infarct volume	[92]
SOD1 −/−	Mouse	tFCI	Increased edema	[36]
SOD2 +/−	Mouse	tFCI	Decreased injury (−50%)	[38]
SOD2 +/−	Mouse	tFCI	Decreased vascular endothelial cell death	[39]
SOD2 −/+	Mouse	pFCI	Increased infarct (+66%)	[40]
SOD2 −/+	Mouse	pFCI	Increased active caspase-9	[41]
SOD2 −/+	Mouse	tFCI	Increased cytochrome *c* release	[42]
SOD2 −/+	Mouse	pFCI	Increased O_2- production	[17]
SOD2 −/+	Mouse	tFCI	Increased MMP-9 expression	[103]
SOD2 −/+	Mouse	tFCI	Increased MMP activity, edema, inflammation and hemorrhagic transformation	[39]
SOD3 +/−	Mouse	tFCI	Decreased infarct (−28%)	[43]

Study	Animal	Model	Main findings	References
SOD3 +/−	Mouse	tGCI	Decreased injury (−48%)	[45]
SOD3 −/−	Mouse	tFCI	Increased infarct (+81%)	[46]

+/−, heterozygous transgenic animals carrying human SOD genes; −/+ heterozygous knockout mutant of SOD genes; −/− homozygous knockout mutant of SOD genes.

Abbreviations used are: pFCI, permanent focal cerebral ischemia; tFCI, transient focal cerebral ischemia; tGCI, transient global cerebral ischemia; APE, apurinic/apyrimidinic endonuclease; ATF-4, activating transcription factor-4; CHOP, C/EBP homologous protein; DNP, 2,4-dinitrophenylhydrazone; GRP78, glucose-regulated protein 78; ILK, integrin-linked kinase; MCP-1, monocyte chemoattractant protein 1; MIP-1α, macrophage inflammatory protein-1α; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; pAkt, phosphorylated Akt; PERK, phosphorylation of RNA-dependent protein kinase-like endoplasmic reticulum eukaryotic initiation factor 2α kinase; PARP, poly(ADP-ribose) polymerase; pGSK-3β, phosphorylated glycogen synthase kinase-3β; pPRAS, phosphorylated proline-rich Akt substrate; PUMA, p53-upregulated modulator of apoptosis; Ref-1, redox factor-1; Smac, second mitochondria-derived activator of caspases; XIAP, X chromosome-linked inhibitor of apoptosis protein.