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Mitochondria and Neuronal Death/Survival Signaling Pathways in Cerebral Ischemia*

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Apoptotic cell death pathways have been implicated in acute brain injuries, including cerebral ischemia, brain trauma, and spinal cord injury, and in chronic neurodegenerative diseases. Experimental ischemia and reperfusion models, such as transient focal/global ischemia in rodents, have been thoroughly studied and suggest the involvement of mitochondria and the cell survival/death signaling pathways in cell death/survival cascades. Recent studies have implicated mitochondria-dependent apoptosis involving pro- and anti-apoptotic protein binding, the release of cytochrome *c* and second mitochondria-derived activator of caspase, the activation of downstream caspases-9 and -3, and DNA fragmentation. Reactive oxygen species are known to be significantly generated in the mitochondrial electron transport chain in the dysfunctional mitochondria during reperfusion after ischemia, and are also implicated in the survival signaling pathway that involves phosphatidylinositol-3-kinase (PI3-K), Akt, and downstream signaling molecules, like Bad, 14-3-3, and the proline-rich Akt substrate (PRAS), and their bindings. Further studies of these survival pathways may provide novel therapeutic strategies for clinical stroke.

KEY WORDS: Mitochondria; oxidative stress; cerebral ischemia; neuronal death signaling; neuronal survival signaling; PI3-K/Akt.

ISCHEMIC APOPTOSIS SIGNALING PATHWAY INVOLVING MITOCHONDRIA

The cell death signaling pathway in mitochondria has recently been demonstrated in the ischemic brain with the release of mitochondrial cytochrome *c*, a water-soluble peripheral membrane protein of mitochondria and an essential component of the mitochondrial respiratory chain (Fig. 1). Cytochrome *c* is translocated from mitochondria to the cytosolic compartment after transient focal cerebral ischemia (tFCI) in rats (1), in brain slices that are subjected to hypoxia-ischemia (2,3), and in vulnerable hippocampal CA_1 neurons after transient global cerebral ischemia (4). Mitochondria are known to be involved in both the necrosis and apoptosis pathways, which depend on severity of the insult or the nature of the signaling pathways (5–8). In most instances, severe cerebral ischemia renders the mitochondria completely dysfunctional for adenosine triphosphate production, which ensures necrotic cell death. In contrast, various *in vitro* studies demonstrate that cellular or biochemical signaling pathways involve mitochondria in apoptosis by releasing cytochrome *c* to the cytoplasm. Cytochrome *c*

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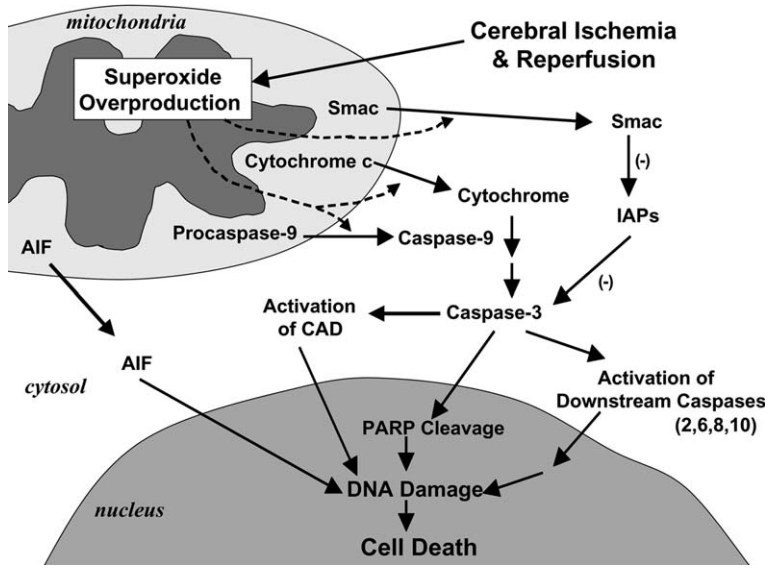

Fig. 1. Intrinsic mitochondria-dependent pathway of apoptosis in cerebral ischemia and reperfusion. AIF, apoptosis-inducing factor; CAD, caspase-activated DNase.

interacts with the CED-4 homolog, Apaf-1, and deoxyadenosine triphosphate, forming the apoptosome and leading to activation of caspase-9 (9–12). Caspase-9, which is an initiator of the cytochrome *c*-dependent caspase cascade, then activates caspase-3, followed by caspases-2, -6, -8 and -10 activation downstream (13). Caspase-3 also activates caspase-activated DNase and leads to DNA damage. In cerebral ischemia studies, caspases-3 and -9 have also been shown to play a key role in neuronal death after ischemia (14–16). Caspase-11 is also a critical initiator of caspases-1 and -3 activation, and caspase-11 knockout (KO) animals have shown reduced apoptosis after focal ischemia (17). Since caspase-11 is an upstream activator of caspase-1 in cytokine maturation, involvement of cytokines in apoptosis should also be considered after cerebral ischemia. The downstream caspases cleave many substrate proteins including poly(ADP-ribose) polymerase (PARP) (15,16,18). Substrate cleavage causes DNA injury and subsequently leads cells to apoptotic cell death, but excessive activation of PARP causes depletion of nicotinamide-adenine dinucleotide and adenosine triphosphate, which ultimately leads to cellular energy failure and death. Consistent with these notions, PARP KO mice showed decreased infarct after transient middle cerebral artery occlusion (MCAO) (19). A recent study has further demonstrated the role of PARP in the release of apoptosis-inducing factor from mitochondria and subsequent translocation to the nucleus for DNA damage and apoptosis (20).

Conversely, there are proteins that can prevent caspase activation in the cytosol. The inhibitor-of-apoptosis protein (IAP) family suppresses apoptosis by preventing activation of procaspases and also by inhibiting enzymatic activity of active caspases (21,22). The second mitochondria-derived activator of caspase (Smac) is also released by apoptotic stimuli and binds IAPs, thereby promoting activation of caspase-3 (23). A recent study showed that mitochondrial release of cytochrome *c* and Smac preceded caspase activation after global ischemia, suggesting the importance of IAP inhibition as well as caspase activation (14). It is important to point out that these cell death signaling pathways are regulated by reactive oxygen species and the redox state of the cell during cerebral ischemia and reperfusion. Overexpressed cytosolic copper/zinc-superoxide dismutase (SOD1) in mice or rats significantly reduces the cell death signaling pathways involving cytochrome *c* and Smac release, activation of caspase-9 and caspase-3, binding of Smac and IAP, PARP activation and DNA fragmentation; whereas a deficiency in either SOD1 or mitochondrial manganese-SOD significantly exacerbates these cell death signaling pathways (4,5,14,24–30). These data suggest that both oxidative stress and the redox state play a role as a molecular switch for cell death or survival in apoptosis during cerebral ischemia and reperfusion.

PRO- AND ANTI-APOPTOTIC PROTEINS THAT ARE ASSOCIATED WITH MITOCHONDRIAL-DEPENDENT APOPTOSIS/SURVIVAL

The Bcl-2 family proteins have one or more Bcl-2 homology domains and play a crucial role in intracellular apoptotic signal transduction by regulating permeability of the mitochondrial membrane (31). Although still controversial, many researchers believe that mitochondrial cytochrome *c* is released through the permeability transition pore (PTP), and that Bcl-2 family proteins directly regulate the PTP (32). Among these proteins, Bax, Bcl-X_S, Bak, Bid and Bad are pro-apoptotic. They eliminate the mitochondrial membrane potential by affecting the PTP and facilitating cytochrome *c* release (33). Conversely, Bcl-2 and Bcl-X_L function to conserve the membrane potential and block the release of cytochrome *c*. As expected, after focal cerebral ischemia, a decreased infarct was observed in transgenic mice that overexpress Bcl-2 (34) and in Bid KO animals (35), whereas, Bcl-2 KO mice showed an increased infarct (36). These findings, especially in the studies using pro-apoptotic/anti-apoptotic protein-transgenic/KO animals (Table I), suggest the importance of mitochondrial permeability regulation and Bcl-2 family proteins in ischemic cerebral injury.

Among the pro-apoptotic proteins, Bad, an important member of the Bax family, links the upstream cell survival signaling pathway and the downstream pathway to inactivate anti-apoptotic Bcl-2 family proteins (37). *In vitro* studies show that Bad resides in an inactive complex with the molecular chaperone 14-3-3 via the phosphorylation of four serine residues (Ser-112, -136, -155, -170) (38). With apoptotic stimuli, Bad is dephosphorylated, dissociated from 14-3-3, and translocated to the outer membrane of the mitochondria, where it subsequently dimerizes with Bcl-X_L and promotes mitochondrial cytochrome *c* release (38). Ser-155 residue is important for the direct interaction between Bad and Bcl-X_L and its phosphorylation is regulated by several upstream signaling pathways. After cerebral ischemia, dephosphorylation and translocation of Bad from the cytosol to the mitochondria are observed and dimerization of Bad progresses with Bcl-X_L in the early stages after MCAO (39). These results suggest the pivotal function of Bad in ischemic cell death.

PHOSPHATIDYLINOSITOL-3-KINASE/AKT AS SURVIVAL SIGNALING PATHWAYS

There are several pathways to the inhibition of the pro-apoptotic function of Bad. Ras is thought to play a central role in signaling for growth factor-mediated resistance to apoptosis (40). Recent studies have shown that pharmacological blockade of Ras results in inhibition of the protective effects of ischemic preconditioning in primary cultures, and conversely, overexpression of Ras by transfection provides protection for cultured ischemic neurons

Table I. Transgenic and Knockout Studies of Pro-Apoptotic and Anti-Apoptotic Proteins

Study	Insult	Findings	Reference
Bid −/−	Transient MCAO	Decreased infarct (−67%)	(35)
Bcl-2 Tg	Permanent MCAO	Decreased infarct (−50%)	(34)
Bcl-2 Tg	Global ischemia	Decreased injury	(54)
Bcl-2 Tg	Permanent MCAO	No protection	(55)
Bcl-2 Tg	Permanent MCAO	Decreased injury	(56)
Bcl-2 −/+, −/−	Transient MCAO	Increased infarct	(36)
Bcl-X_L Tg	Permanent MCAO	Decreased infarct (−21%)	(55)
Caspase-1 NM	Transient MCAO	Decreased infarct (−44%)	(57)
Caspase-1 NM	Permanent MCAO	Reduced injury	(58)
Caspase-1 −/−	Permanent MCAO	Reduced injury	(59)
Caspase-1 −/−	Transient MCAO	Decreased infarct	(60)
Caspase-11 −/−	Permanent MCAO	Reduced apoptosis	(17)
PARP −/−	Transient MCAO	Decreased infarct	(61)
PARP −/−	Transient MCAO	Decreased infarct in chronic stage	(62)
Fas NM	Transient MCAO	Decreased infarct	(63)
TNFR (p55 & p75) −/−	Transient MCAO	Increased injury	(64)
TNFR (p55 & p75) −/−	Transient MCAO	Increased injury	(65)
p53 −/+, −/−	Permanent MCAO	Decreased infarct (−27%, −15%)	(66)

Tg, transgenic; NM, negative mutant; TNFR, TNF-receptor-α.

(41). Ras can directly activate phosphatidylinositol-3-kinase (PI3-K), an upstream effector for activation of Akt. Akt is an initiator of the downstream pathways that inhibit the apoptotic pathways. Akt phosphorylates Bad and obviates its inhibitory effects on Bcl-X_L, ultimately inhibiting the release of cytochrome *c* by blocking channel formation by Bax on the mitochondrial membrane (40). Akt also inhibits proteolytic activity of caspase-9 by phosphorylating it on Ser-196 (42). In addition, Akt can translocate into the nuclei and inactivate a pro-apoptotic member of the Forkhead family of transcription factors by phosphorylation, thereby inhibiting activation of the Fas pathway of apoptosis (43). Mitogen-activated protein kinase (MAPK) family members play a critical role in the regulation of cell growth, differentiation and cellular response to cytokines and stress (44). One MAPK-family member, extracellular signal-regulated kinase (ERK), has two isoforms (ERK1/2), which are constitutively expressed in the normal brain (45) and are activated by MAPK/ERK kinase 1/2. In this pathway, Ras recruits the main effector, Raf-1, to activate MAPK/ERK kinase 1/2 (46). Active ERK1/2 inactivates Bad through phosphorylation of 90-kDa ribosomal S6 kinases (47). Transforming growth factor-β1 has been shown to suppress Bad activity by phosphorylation of Bad at the Ser-112 site via activation of the ERK pathway in both *in vivo* cerebral ischemia models and *in vitro* studies (48). Phosphorylation of ERK1/2 is involved in apoptosis and cell death after transient MCAO (49). Phosphorylation of the Ser-155 residue in Bad is regulated by protein kinase A (PKA) in studies *in vitro* (50). In rodent focal cerebral ischemia models, intraventricular injection of a PKA inhibitor, H89, effectively suppressed PKA activity (51) and dimerization of Bad/Bcl-X_L, and subsequently, apoptotic cell death (39). This cumulative evidence suggests that Akt, ERK1/2 and PKA pathways inhibit the function of Bad as a cell survival signaling pathway after cerebral ischemia.

Besides Bad survival signaling, PI3-K/Akt is also involved in many other survival signaling pathways. One such pathway includes MDM2/p53 (52). Also, a novel proline-rich Akt substrate (PRAS) was recently detected and found to be involved in apoptosis. We have found that PRAS is phosphorylated by Akt in surviving cortical neurons and that phosphorylated PRAS (pPRAS) and the binding of pPRAS phosphorylated Akt (pPRAS/pAkt) to 14-3-3 (pPRAS/14-3-3) were altered, and their expression briefly decreased in mouse brains after tFCI. Liposome-mediated pPRAS cDNA transfection induced overexpression of pPRAS, promoted pPRAS/14-3-3, and inhibited apoptotic neuronal cell death after tFCI. The expression of pPRAS, pPRAS/pAkt and pPRAS/14-3-3 increased in nerve

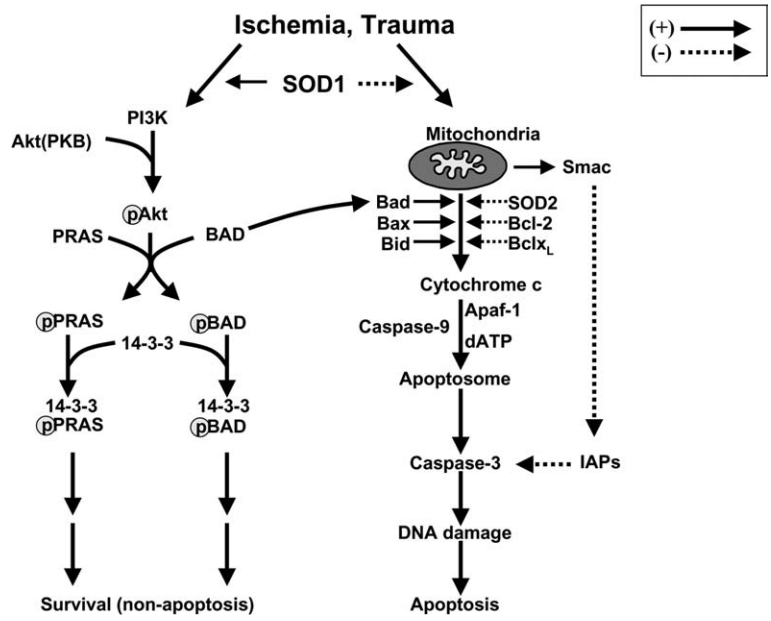

Fig. 2. Life and death signaling in ischemic neurons involving mitochondria and the PI3-K/Akt pathway. PKB, protein kinase B; SOD2, manganese-superoxide dismutase.

growth factor (NGF)-treated mice, but decreased with inhibition of PI3-K and the NGF trkA receptor after tFCI. These results suggest that PRAS phosphorylation and its interaction with pAkt and 14-3-3 might play an important role in neuroprotection mediated by NGF in anti-apoptotic neuronal cell death after tFCI. Further studies have also shown that oxidative stress is also involved in modulating the expression of pPRAS, pPRAS/pAkt, and pPRAS/14-3-3 binding (53), again suggesting that the PI3-K/Akt survival signaling pathway is upregulated by SOD1 overexpression (Fig. 2).

We now propose that mitochondria and the PI3-K/Akt signaling pathway are the determinants controlling pro-apoptosis and anti-apoptosis in ischemic neurons during stroke. Further studies of the survival signaling pathways may provide novel therapeutic strategies for clinical stroke.

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REFERENCES

1. Fujimura, M., Morita-Fujimura, Y., Murakami, K., Kawase, M., and Chan, P. H. 1998. Cytosolic redistribution of cytochrome *c* after transient focal cerebral ischemia in rats. J. Cereb. Blood Flow Metab. 18:1239–1247.
2. Pérez-Pinzón, M. A., Xu, G. P., Born, J., Lorenzo, J., Busto, R., Rosenthal, M., and Sick, T. J. 1999. Cytochrome *c* is released from mitochondria into the cytosol after cerebral anoxia or ischemia. J. Cereb. Blood Flow Metab. 19:39–43.
3. Hirai, K., Sugawara, T., Chan, P. H., Basus, V. J., James, T. L., and Litt, L. 2002. Cytochrome *c* associated apoptosis during ATP recovery after hypoxia in neonatal rat cerebrocortical slices. J. Neurochem. 83:309–319.
4. Sugawara, T., Fujimura, M., Morita-Fujimura, Y., Kawase, M., and Chan, P. H. 1999. Mitochondrial release of cytochrome *c* corresponds to the selective vulnerability of hippocampal CA1 neurons in rats after transient global cerebral ischemia. J. Neurosci. 19:RC39:1–6.
5. Fujimura, M., Morita-Fujimura, Y., Noshita, N., Sugawara, T., Kawase, M., and Chan, P. H. 2000. The cytosolic antioxidant copper/zinc-superoxide dismutase prevents the early release of mitochondrial cytochrome *c* in ischemic brain after transient focal cerebral ischemia in mice. J. Neurosci. 20:2817–2824.
6. Ankarcrona, M. 1998. Glutamate induced cell death: Apoptosis or necrosis? Prog. Brain Res. 116:265–272.
7. Bonfoco, E., Krainc, D., Ankarcrona, M., Nicotera, P., and Lipton, S. A. 1995. Apoptosis and necrosis: Two distinct events induced, respectively, by mild and intense insults with *N*-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. Proc. Natl. Acad. Sci. USA 92:7162–7166.
8. Green, D. R. and Reed, J. C. 1998. Mitochondria and apoptosis. Science 281:1309–1312.
9. Kuida, K., Haydar, T. F., Kuan, C. Y., Gu, Y., Taya, C., Karasuyama, H., Su, M. S., Rakic, P., and Flavell, R. A. 1998. Reduced apoptosis and cytochrome *c*-mediated caspase activation in mice lacking caspase 9. Cell 94:325–337.
10. Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S. M., Ahmad, M., Alnemri, E. S., and Wang, X. 1997. Cytochrome *c* and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91:479–489.
11. Yoshida, H., Kong, Y.-Y., Yoshida, R., Elia, A. J., Hakem, A., Hakem, R., Penninger, J. M., and Mak, T. W. 1998. Apaf1 is required for mitochondrial pathways of apoptosis and brain development. Cell 94:739–750.
12. Zou, H., Henzel, W. J., Liu, X., Lutschg, A., and Wang, X. 1997. Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome *c*-dependent activation of caspase-3. Cell 90:405–413.
13. Slee, E. A., Harte, M. T., Kluck, R. M., Wolf, B. B., Casiano, C. A., Newmeyer, D. D., Wang, H. -G., Reed, J. C., Nicholson, D. W., Alnemri, E. S., Green, D. R., and Martin, S. J. 1999. Ordering the cytochrome *c*-initiated caspase cascade: Hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. J. Cell Biol. 144:281–292.
14. Sugawara, T., Noshita, N., Lewén, A., Gasche, Y., Ferrand-Drake, M., Fujimura, M., Morita-Fujimura, Y., and Chan, P. H. 2002. Overexpression of copper/zinc superoxide dismutase in transgenic rats protects vulnerable neurons against ischemic damage by blocking the mitochondrial pathway of caspase activation. J. Neurosci. 22:209–217.
15. Chen, J., Nagayama, T., Jin, K., Stetler, R. A., Zhu, R. L., Graham, S. H., and Simon, R. P. 1998. Induction of caspase-3-like protease may mediate delayed neuronal death in the hippocampus after transient cerebral ischemia. J. Neurosci. 18:4914–4928.
16. Namura, S., Zhu, J., Fink, K., Endres, M., Srinivasan, A., Tomaselli, K. J., Yuan, J., and Moskowitz, M. A. 1998. Activation and cleavage of caspase-3 in apoptosis induced by experimental cerebral ischemia. J. Neurosci. 18:3659–3668.
17. Kang, S. -J., Wang, S., Hara, H., Peterson, E. P., Namura, S., Amin-Hanjani, S., Huang, Z., Srinivasan, A., Tomaselli, K. J., Thornberry, N. A., Moskowitz, M. A., and Yuan, J. 2000. Dual role of caspase-11 in mediating activation of caspase-1 and caspase-3 under pathological conditions. J. Cell Biol. 149:613–622.
18. Endres, M., Wang, Z. -Q., Namura, S., Waeber, C., and Moskowitz, M. A. 1997. Ischemic brain injury is mediated by the activation of poly(ADP-ribose)polymerase. J. Cereb. Blood Flow Metab. 17:1143–1151.
19. Eliasson, M. J. L., Huang, Z., Ferrante, R. J., Sasamata, M., Molliver, M. E., Snyder, S. H., and Moskowitz, M. A. 1999. Neuronal nitric oxide synthase activation and peroxynitrite formation in ischemic stroke linked to neural damage. J. Neurosci. 19:5910–5918.
20. Yu, S. -W., Wang, H., Poitras, M. F., Coombs, C., Bowers, W. J., Federoff, H. J., Poirier, G. G., Dawson, T. M., and Dawson, V. L. 2002. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science 297:259–263.
21. Deveraux, Q. L., and Reed, J. C. 1999. IAP family proteins–suppressors of apoptosis. Genes Dev. 13:239–252.
22. Miller, L. K. 1999. An exegesis of IAPs: Salvation and surprises from BIR motifs. Trends Cell Biol. 9:323–328.
23. Chai, J., Du, C., Wu, J. -W., Kyin, S., Wang, X., and Shi, Y. 2000. Structural and biochemical basis of apoptotic activation by Smac/DIABLO. Nature 406:855–862.

24. Chan, P. H. 2001. Reactive oxygen radicals in signaling and damage in the ischemic brain. J. Cereb. Blood Flow Metab. 21:2–14.
25. Sugawara, T., Lewén, A., Gasche, Y., Yu, F., and Chan, P. H. 2002. Overexpression of SOD1 protects vulnerable motor neurons after spinal cord injury by attenuating mitochondrial cytochrome *c* release. FASEB J. express article 10.1096/fj.02-0251fje; Summary: FASEB J 16:1997–1999.
26. Chan, P. H., Kawase, M., Murakami, K., Chen, S. F., Li, Y., Calagui, B., Reola, L., Carlson, E., and Epstein, C. J. 1998. Overexpression of SOD1 in transgenic rats protects vulnerable neurons against ischemic damage after global cerebral ischemia and reperfusion. J. Neurosci. 18:8292–8299.
27. Kondo, T., Reaume, A. G., Huang, T. -T., Murakami, K., Carlson, E., Chen, S., Scott, R. W., Epstein, C. J., and Chan, P. H. 1997. Edema formation exacerbates neurological and histological outcomes after focal cerebral ischemia in CuZn-superoxide dismutase gene knockout mutant mice. Acta Neurochir. Suppl. 70:62–64.
28. Murakami, K., Kondo, T., Kawase, M., Li, Y., Sato, S., Chen, S. F., and Chan, P. H. 1998. Mitochondrial susceptibility to oxidative stress exacerbates cerebral infarction that follows permanent focal cerebral ischemia in mutant mice with manganese superoxide dismutase deficiency. J. Neurosci. 18:205–213.
29. Fujimura, M., Morita-Fujimura, Y., Kawase, M., Copin, J.-C., Calagui, B., Epstein, C. J., and Chan, P. H. 1999. Manganese superoxide dismutase mediates the early release of mitochondrial cytochrome *c* and subsequent DNA fragmentation after permanent focal cerebral ischemia in mice. J. Neurosci. 19:3414–3422.
30. Noshita, N., Sugawara, T., Fujimura, M., Morita-Fujimura, Y., and Chan, P. H. 2001. Manganese superoxide dismutase affects cytochrome *c* release and caspase-9 activation after transient focal cerebral ischemia in mice. J. Cereb. Blood Flow Metab. 21:557–567.
31. Yuan, J. and Yankner, B. A. 2000. Apoptosis in the nervous system. Nature 407:802–809.
32. Shi, Y. 2001. A structural view of mitochondria-mediated apoptosis. Nat. Struct. Biol. 8:394–401.
33. Merry, D. E. and Korsmeyer, S. J. 1997. Bcl-2 gene family in the nervous system. Annu. Rev. Neurosci. 20:245–267.
34. Martinou, J. -C., Dubois-Dauphin, M., Staple, J. K., Rodriguez, I., Frankowski, H., Missotten, M., Albertini, P., Talabot, D., Catsicas, S., Pietra, C., and Huarte, J. 1994. Overexpression of BCL-2 in transgenic mice protects neurons from naturally occurring cell death and experimental ischemia. Neuron 13:1017–1030.
35. Plesnila, N., Zinkel, S., Le, D. A., Amin-Hanjani, S., Wu, Y., Qiu, J., Chiarugi, A., Thomas, S. S., Kohane, D. S., Korsmeyer, S. J., and Moskowitz, M. A. 2001. BID mediates neuronal cell death after oxygen/glucose deprivation and focal cerebral ischemia. Proc. Natl. Acad. Sci. USA 98:15318–15323.
36. Hata, R., Gillardon, F., Michaelidis, T. M., and Hossmann, K. A. 1999. Targeted disruption of the bcl-2 gene in mice exacerbates focal ischemic brain injury. Metab. Brain Dis. 14:117–124.
37. Yang, E., Zha, J., Jockel, J., Boise, L. H., Thompson, C. B., and Korsmeyer, S. J. 1995. Bad, a heterodimeric partner for Bcl-XL and Bcl-2, displaces Bax and promotes cell death. Cell 80:285–291.
38. Zha, J., Harada, H., Yang, E., Jockel, J., and Korsmeyer, S. J. 1996. Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X(L). Cell 87:619–628.
39. Saito, A., Hayashi, T., Okuno, S., Ferrand-Drake, M., and Chan, P. H. 2003. Overexpression of copper/zinc superoxide dismutase in transgenic mice protects against neuronal cell death after transient focal ischemia by blocking activation of the Bad cell death signaling pathway. J. Neurosci. 23:1710–1718.
40. White, B. C., Sullivan, J. M., DeGracia, D. J., O'Neil, B. J., Neumar, R. W., Grossman, L. I., Rafols, J. A., and Krause, G. S. 2000. Brain ischemia and reperfusion: Molecular mechanisms of neuronal injury. J. Neurol. Sci. 179:1–33.
41. Gonzalez-Zulueta, M., Feldman, A. B., Klesse, L. J., Kalb, R. G., Dillman, J. F., Parada, L. F., Dawson, T. M., and Dawson, V. L. 2000. Requirement for nitric oxide activation of $p21^{ras}$/extracellular regulated kinase in neuronal ischemic preconditioning. Proc. Natl. Acad. Sci. USA 97:436–441.
42. Cardone, M. H., Roy, N., Stennicke, H. R., Salvesen, G. S., Franke, T. F., Stanbridge, E., Frisch, S., and Reed, J. C. 1998. Regulation of cell death protease caspase-9 by phosphorylation. Science 282:1318–1321.
43. Brunet, A., Bonni, A., Zigmond, M. J., Lin, M. Z., Juo, P., Hu, L. S., Anderson, M. J., Arden, K. C., Blenis, J., and Greenberg, M. E. 1999. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 96:857–868.
44. Raingeaud, J., Gupta, S., Rogers, J. S., Dickens, M., Han, J., Ulevitch, R. J., and Davis, R. J. 1995. Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. J. Biol. Chem. 270:7420–7426.
45. Boulton, T. G., Nye, S. H., Robbins, D. J., Ip, N. Y., Radziejewska, E., Morgenbesser, S. D., DePinho, R. A., Panayotatos, N., Cobb, M. H., and Yancopoulos, G. D. 1991. ERKs: A family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. Cell 65:663–675.
46. Avruch, J., Zhang, X. F., and Kyriakis, J. M. 1994. Raf meets Ras: Completing the framework of a signal transduction pathway. Trends Biochem. Sci. 19:279–283.
47. Frodin, M. and Gammeltoft, S. 1999. Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction. Mol. Cell. Endocrinol. 151:65–77.
48. Zhu, Y., Yang, G. -Y., Ahlemeyer, B., Pang, L., Che, X.-M., Culmsee, C., Klumpp, S., and Krieglstein, J. 2002. Transforming growth factor-β1 increases Bad phosphorylation and protects neurons against damage. J. Neurosci. 22:3898–3909.
49. Noshita, N., Sugawara, T., Hayashi, T., Lewén, A., Omar, G., and Chan, P. H. 2002. Copper/zinc superoxide dismutase attenuates neuronal cell death by preventing extracellular signal-regulated kinase activation after transient focal cerebral ischemia in mice. J. Neurosci. 22:7923–7930.
50. Lizcano, J. M., Morrice, N., and Cohen, P. 2000. Regulation of BAD by cAMP-dependent protein kinase is mediated via phosphorylation of a novel site, Ser^{155}. Biochem. J. 349:547–557.
51. Kimura, S., Uchiyama, S., Takahashi, H. E., and Shibuki, K. 1998. cAMP-dependent long-term potentiation of nitric oxide release from cerebellar parallel fibers in rats. J. Neurosci. 18:8551–8558.
52. Saito, A., Ferrand-Drake, M., Hayashi, T., and Chan, P. H. 2004. Evidence of MDM2-mediated p53 ubiquitination in mouse brains after transient focal cerebral ischemia. Abstract No. 485. 29th International Stroke Conference, February 5, 2004, San Diego.
53. Saito, A., Narasimhan, P., Hayashi, T., Okuno, S., Ferrand-Drake, M., and Chan, P. H. 2004. Neuroprotective role of a proline-rich Akt substrate in apoptotic neuronal cell death after stroke: Relationships with nerve growth factor. J. Neurosci. 24:1584–1593.
54. Kitagawa, K., Matsumoto, M., Tsujimoto, Y., Ohtsuki, T., Kuwabara, K., Matsushita, K., Yang, G., Tanabe, H., Marti-

nou, J. -C., Hori, M., and Yanagihara, T. 1998. Amelioration of hippocampal neuronal damage after global ischemia by neuronal overexpression of BCL-2 in transgenic mice. Stroke 29:2616–2621.
55. Wiessner, C., Allegrini, P. R., Rupalla, K., Sauer, D., Oltersdorf, T., McGregor, A. L., Bischoff, S., Böttiger, B. W., and van der Putten, H. 1999. Neuron-specific transgene expression of *Bcl-X_L* but not *Bcl-2* genes reduced lesion size after permanent middle cerebral artery occlusion in mice. Neurosci. Lett. 268:119–122.
56. de Bilbao, F., Guarin, E., Nef, P., Vallet, P., Giannakopoulos, P., and Dubois-Dauphin, M. 2000. Cell death is prevented in thalamic fields but not in injured neocortical areas after permanent focal ischaemia in mice overexpressing the anti-apoptotic protein Bcl-2. Eur. J. Neurosci. 12:921–934.
57. Hara, H., Fink, K., Endres, M., Friedlander, R. M., Gagliardini, V., Yuan, J., and Moskowitz, M. A. 1997. Attenuation of transient focal cerebral ischemic injury in transgenic mice expressing a mutant ICE inhibitory protein. J. Cereb. Blood Flow Metab. 17:370–375.
58. Friedlander, R. M., Gagliardini, V., Hara, H., Fink, K. B., Li, W., MacDonald, G., Fishman, M. C., Greenberg, A. H., Moskowitz, M. A., and Yuan, J. 1997. Expression of a dominant negative mutant of interleukin-1β converting enzyme in transgenic mice prevents neuronal cell death induced by trophic factor withdrawal and ischemic brain injury. J. Exp. Med. 185:933–940.
59. Schielke, G. P., Yang, G. -Y., Shivers, B. D., and Betz, A. L. 1998. Reduced ischemic brain injury in interleukin-1β converting enzyme-deficient mice. J. Cereb. Blood Flow Metab. 18:180–185.
60. Liu, X. -H., Kwon, D., Schielke, G. P., Yang, G. -Y., Silverstein, F. S., and Barks, J. D. E. 1999. Mice deficient in interleukin-1 converting enzyme are resistant to neonatal hypoxic-ischemic brain damage. J. Cereb. Blood Flow Metab. 19:1099–1108.
61. Eliasson, M. J., Sampei, K., Mandir, A. S., Hurn, P. D., Traystman, R. J., Bao, J., Pieper, A., Wang, Z. Q., Dawson, T. M., Snyder, S. H., and Dawson, V. L. 1997. Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. Nat. Med. 3:1089–1095.
62. Goto, S., Xue, R., Sugo, N., Sawada, M., Blizzard, K. K., Poitras, M. F., Johns, D. C., Dawson, T. M., Dawson, V. L., Crain, B. J., Traystman, R. J., Mori, S., and Hurn, P. D. 2002. Poly(ADP-ribose) polymerase impairs early and long-term experimental stroke recovery. Stroke 33:1101–1106.
63. Rosenbaum, D. M., Gupta, G., D'Amore, J., Singh, M., Weidenheim, K., Zhang, H., and Kessler, J. A. 2000. Fas (CD95/APO-1) plays a role in the pathophysiology of focal cerebral ischemia. J. Neurosci. Res. 61:686–692.
64. Bruce, A. J., Boling, W., Kindy, M. S., Peschon, J., Kraemer, P. J., Carpenter, M. K., Holtsberg, F. W., and Mattson, M. P. 1996. Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors. Nat. Med. 2:788–794.
65. Gary, D. S., Bruce-Keller, A. J., Kindy, M. S., and Mattson, M. P. 1998. Ischemic and excitotoxic brain injury is enhanced in mice lacking the p55 tumor necrosis factor receptor. J. Cereb. Blood Flow Metab. 18:1283–1287.
66. Crumrine, R. C., Thomas, A. L., and Morgan, P. F. 1994. Attenuation of p53 expression protects against focal ischemic damage in transgenic mice. J. Cereb. Blood Flow Metab. 14:887–891.