Mol Neurobiol (2008) 37:7–38
DOI 10.1007/s12035-007-8013-9

Molecular Mechanisms of Apoptosis in Cerebral Ischemia: Multiple Neuroprotective Opportunities

Venkata Prasuja Nakka · Anchal Gusain · Suresh L. Mehta · Ram Raghubir

Received: 1 May 2007 / Accepted: 5 November 2007 / Published online: 8 December 2007
© Humana Press Inc. 2007

Abstract Cerebral ischemia/reperfusion (I/R) injury triggers multiple and distinct but overlapping cell signaling pathways, which may lead to cell survival or cell damage. There is overwhelming evidence to suggest that besides necrosis, apoptosis do contributes significantly to the cell death subsequent to I/R injury. Both extrinsic and intrinsic apoptotic pathways play a vital role, and upon initiation, these pathways recruit downstream apoptotic molecules to execute cell death. Caspases and Bcl-2 family members appear to be crucial in regulating multiple apoptotic cell death pathways initiated during I/R. Similarly, inhibitor of apoptosis family of proteins (IAPs), mitogen-activated protein kinases, and newly identified apoptogenic molecules, like second mitochondrial-activated factor/direct IAP-binding protein with low p*I* (Smac/Diablo), omi/high-temperature requirement serine protease A2 (Omi/HtrA2), X-linked mammalian inhibitor of apoptosis protein-associated factor 1, and apoptosis-inducing factor, have emerged as potent regulators of cellular apoptotic/antiapoptotic machinery. All instances of cell survival/death mechanisms triggered during I/R are multifaceted and interlinked, which ultimately decide the fate of brain cells. Moreover, apoptotic cross-talk between major subcellular organelles suggests that therapeutic strategies should be optimally directed at multiple targets/mechanisms for better therapeutic outcome. Based on the current knowledge, this review briefly focuses I/R injury-induced multiple mechanisms of apoptosis, involving key apoptotic regulators and their emerging roles in orchestrating cell death programme. In addition, we have also highlighted the role of autophagy in modulating cell survival/death during cerebral ischemia. Furthermore, an attempt has been made to provide an encouraging outlook on emerging therapeutic approaches for cerebral ischemia.

Keywords Cerebral ischemia · Oxidative stress · Apoptosis · Caspases · Bcl-2 family · IAPs · AIF · Mitochondria · Endoplasmic reticulum

Introduction

Cerebral stroke is the major leading cause of death and disability world over. It is estimated that stroke is responsible for nearly half of all the patients hospitalized for acute neurological disorders. In practice, stroke usually referred to a condition caused by the occlusion or hemorrhage of blood vessels supplying the brain [1]. Focal ischemia is represented by a reduction in blood flow to a very specific brain region as in embolic occlusion of the middle cerebral artery, whereas global ischemia occurs when cerebral blood flow (CBF) is reduced throughout most or all parts of the brain as a result of cardiac arrest [2–3], but in all instances, stroke ultimately involves dysfunction or loss of brain cells. However, there are significant differences in the mode of cell death between global and focal cerebral ischemia. It is suggested that brief periods of global cerebral ischemia in rodents and humans causes delayed neuronal death (DND) selectively in hippocampal CA1 pyramidal neurons [4–8]. Although DND exhibits distinct morphological features from apoptosis, the molecular machinery involved in the apoptotic process may contribute significantly to DND [9–10]. Whereas, in focal cerebral ischemia, most of the cells in the ischemic core

Venkata Prasuja Nakka and Anchal Gusain equally contributed to this work.

V. P. Nakka · A. Gusain · S. L. Mehta · R. Raghubir (✉)
Division of Pharmacology, Central Drug Research Institute, Chatter Manzil Palace, POB-173, Lucknow 226001, India
e-mail: raghubirr@yahoo.com

undergo necrosis, which is characterized by the sudden reduction of cellular energy and swelling and rupture of subcellular organelles. The rim of brain tissue that is hypoperfused surrounding the core is called ischemic penumbra, which has the capacity to recover if perfusion is improved [11–12]. Cell death in the penumbra is considered an active process largely dependent on the activation of cell death programs leading to apoptosis [13]. Available evidence suggests that activation of apoptotic pathways occur in the penumbra, both in the caspase-dependent and caspase-independent manner that may contribute to delayed ischemic cell death [14–18]. Yet, in the early stages of cerebral infarction, neurons of the so-called necrotic core display several characteristics of early apoptosis, which include cytoplasmic and nuclear condensations and activation-specific caspase cascades [19]. Although there is a clear indication of initiation of apoptotic pathway in the core, the complete morphological changes of apoptosis are not observed at the end stages of infarction. The abortion of apoptotic process in the core could be because of the severe impairment of energy levels that may cause a shift toward secondary necrosis from apoptosis [20]. In contrast, energy-dependent caspase activation cascades are observed in the penumbra, in which apoptosis can fully develop because of residual blood supply. On the other hand, activated caspases or calpains eventually cleave the ion pumps, such as plasma membrane calcium pump (PMCA) and Na^+/ Ca^{2+}exchanger (NCX), which results in the disruption of calcium (Ca^{2+}) homeostasis that can finally switch apoptotic signaling to necrosis [21–22]. Thus, it appears that necrosis is a more complex phenomenon, which can be linked to apoptosis. A quite simplistic view implies poor prospects regarding cell survival in the core of the cerebral infarction and therapeutic expectations to control cell death and cell survival in the penumbra. Many drugs aiming at excitotoxicity and oxidative stress have failed in clinical trials. Because, the pathology of ischemic stroke is complex and any approach targeting a single mechanism may not provide a general effective therapy for stroke patients. Hence, a detailed understanding of molecular mechanisms of apoptotic pathways may provide a more optimistic picture for future stroke treatments. Therefore, consistent with the significant role of apoptosis in the cerebral ischemia, this review focuses on the multiple mechanisms of cell death pathways involving key apoptotic regulatory molecules by emphasizing their role in the context of I/R injury.

Animal Models of Focal and Global Cerebral Ischemia

Focal and global cerebral ischemia represent diseases that are common in the human population. In this study, we briefly mention some of the well-established animal models of global or focal cerebral ischemia. For global ischemia, the modified four-vessel occlusion model (4-VO) developed by Pulsinelli and Buchan has been utilized by many investigators and has been well validated. The 4-VO model involves blocking of blood flow in both the carotid and vertebral arteries for a specific time. This technique is successful in approximately 50 to 75% animals, but the effects of ischemia vary between animal strains [23].The two-vessel occlusion (2-VO) model involves temporary occlusion of carotid arteries, which is also referred as "severe forebrain ischemia." In this model, bilateral common carotid artery occlusion is coupled with systemic hypotension to produce reversible forebrain ischemia. Global ischemia causes injury to selectively vulnerable brain areas such as the CA1 hippocampal region, the medium-sized neurons in the striatum, and the Purkinje cells in the cerebellum. The gerbil model of global ischemia has been widely used for investigations [24–25].

Middle cerebral artery occlusion (MCAO) is the most commonly used model to study focal cerebral ischemia, in which middle cerebral artery is occluded either transiently or permanently. MCAO can be induced by an intraluminal suture (nylon filament) or with a vascular clip. Focal ischemia causes injury predominantly to the cortex and the striatum. These MCAO models have been used extensively because of their relevance to human embolic stroke. Although focal cerebral ischemia has been studied in number of species including rat, dog, cat, rabbit, and baboon, among these, ischemic changes in rat and mice have been extensively investigated [2, 26–28].

Mechanisms of Cell Death After Cerebral Ischemia

It is pertinent to briefly outline the pathophysiology of stroke and possible mechanisms of cellular damage after ischemic insult, as this will help to streamline the involvement of various pathways and mode of cell death. Because we are focusing on apoptotic mechanisms at present, readers may consult our previous review for more details regarding excitotoxicity, oxidative stress, and inflammatory reactions after I/R [29].

Excitotoxicity and Acidosis

The I/R injury leads to depletion of energy stores leading to altered cell function by interrupting adenosine tri-phosphate (ATP)-dependent process, predominantly, the sodium/potassium ATPase (Na^+/K^+ATPase), leading to disruption of ionic gradients across the membranes. This causes an increase in extracellular K^+ and an influx of Na^+, chloride (Cl^-), and Ca^{2+} into the cells. The initial increase in extracellular K^+ concentration triggers depolarization and

reversal of the amino acid transporters. Under these conditions, both voltage-operated and receptor-operated Ca^{2+} channels are activated, leading to an elevation of free cytosolic Ca^{2+}. A massive release of excitatory amino acids, particularly glutamate, may derive both from reversal of glutamate transporters and from Ca^{2+}-dependent exocytosis [30]. Subsequently, binding of glutamate to ionotropic *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors promotes excessive Ca^{2+} influx, which triggers a range of downstream phospholipases and proteases that degrade membranes and proteins that are essential for cellular integrity. In addition, ionotropic glutamate receptors promote an excessive influx of Na^{+} with concomitant cell swelling and edema. Besides Ca^{2+}, imbalances in other ions are also important during ischemia. For instance, large amounts of zinc are stored in vesicles of excitatory neurons and released simultaneously upon depolarization. In addition, high intracellular Ca^{2+} levels cause mitochondrial Ca^{2+} overload, inhibition of ATP production, and huge breakdown of phospholipids, proteins, and nucleic acids by activation of Ca^{2+}-dependent phospholipases, proteases, and endonucleases. Moreover, the augmented intracellular Ca^{2+} further promotes increase in extracellular glutamate, thus, propagating the excitotoxicity [31]. The glutamate is released at high concentrations within the core and penumbral region of the ischemic zone. The activation of glutamate receptors in these areas leads to a massive influx of Ca^{2+}, which may activate a variety of catabolic processes and cause subsequent cell death [32]. Recently, it has been reported that glutamate-induced neuronal cell death is associated with apoptosis, as evidenced by characteristic fragmentation of DNA, morphological changes, activation of calpain, and caspase-3, as well as the upregulation and/or translocation of apoptosis-inducing factor (AIF) from mitochondria into cytosol and nuclei. Thus, it suggests that glutamate, at higher concentrations, may induce apoptosis, possibly by executing caspase-dependent and caspase-independent mechanisms [33]. On the other hand, Nicotera et al. demonstrated that activated caspase-3 cleave and inactivate the plasma membrane Ca^{2+} pump (PMCA) in neurons and nonneuronal cells undergoing apoptosis, which results in further intracellular Ca^{2+} overload. Expression of PMCA isoform that is not cleaved by caspases prevents the disturbance of Ca^{2+} handling and slows down the kinetics of apoptosis and markedly delays secondary necrosis, thus, shifting the balance of neuronal death from apoptosis to necrosis [21]. In addition, calpains cleave NCX, the major plasma membrane Ca^{2+}-extruding system during brain ischemia and in neurons undergoing excitotoxicity. Furthermore, overexpression of calpastatin (an endogenous calpain inhibitor) or the expression of NCX2 isoform, which is not cleaved by calpains, prevents Ca^{2+} overload and protects neurons from excitotoxic cell death. Conversely, suppression of NCX3 by RNA interference sensitizes neurons to Ca^{2+} overload and excitotoxicity [22]. Thus, proteolytic inactivation of NCX-driven neuronal Ca^{2+} extrusion might play an important role in delayed excitotoxic Ca^{2+} deregulation and neuronal death in cerebral ischemia.

Although it has been widely accepted that intracellular Ca^{2+} overload after ischemia is mainly mediated through glutamate-gated channels, the failure of glutamate antagonists in clinical trials has suggested that glutamate-independent mechanisms of Ca^{2+} entry during ischemia might also exist. Acidosis is a common feature of brain ischemia, where low pH is assumed to play an important role in the pathological process [34]. However, the mechanisms related to how acidosis leads to ischemic brain injury remains unclear. Xiong et al. have demonstrated that activation of Ca^{2+}-permeable acid-sensing ion channels (ASICs), in particular, the ASIC1a subunit, is mainly responsible for acidosis-mediated, glutamate receptor-independent, ischemic brain injury. Indeed, pharmacological inhibition of ASIC1a channels by amiloride and gene knockout of ASIC1a both protect the brain from ischemic injury even in the presence of NMDA blockade [35]. Furthermore, the specific coupling between NR2B subunit of NMDA receptor and ASIC1a through Ca^{+2}/calmodulin-dependent protein kinase II has suggested a new direction for therapeutic strategies against excitotoxic and acidosis-mediated ischemic damage [36]. Nevertheless, specific blockade of ASIC1a by the tarantula toxin psalmotoxin (PcTX) after MCAO has a therapeutic time window of up to 5 h, far beyond that of glutamate antagonists. Interestingly, PcTX, together with the NMDA blockade has prolonged the time window of effectiveness of NMDA blockade [37]. Thus, it indicates that combined inhibition of both glutamate-mediated exicitoxicity and ASIC1a-mediated acidosis may prove to be a novel neuroprotective strategy for stroke patients.

Oxidative Stress

Cerebral ischemia and reperfusion, in particular, are responsible for oxidative stress because of generation of free radicals [38], which culminate into deleterious effects during pathogenesis of ischemia [39–40]. Free radicals can cause membrane damage through peroxidation of unsaturated fatty acids in the phospholipids comprising the cell membrane [41]. Furthermore, they can also cause damage to fundamental cellular components (e.g., nucleic acid lesions, gene damage, gene repair activity), leading to subsequent cell death by necrotic or apoptotic mode [42].

The reactive oxygen species (ROS) that are particularly responsible in oxidative stress include nitric oxide ($NO^{\cdot}$)

and superoxide radical anion ($O2^{\cdot-}$). These two free radicals react with each other to form the powerful oxidant peroxynitrite ($ONOO^-$). Other important oxidant species are hydrogen peroxide (H_2O_2) and the hydroxyl free radical ($^{\cdot}OH$) [30]. Ischemia-induced NO overproduction is, in part, caused by increase in glutamate-mediated intracellular Ca^{2+} concentration, resulting in a calmodulin-dependent upregulation of nitric oxide synthase (NOS) [43]. The members of NOS family are large (~300 kDa) protein homodimers that catalyse the conversion of L-arginine first to *N*-hydroxyl-arginine, and then L-citrulline and $NO^{\cdot}$. NOS family comprises three types of nitric oxide synthases, i.e., NOS-1 to NOS-3. Cerebral ischemia causes a surge in NOS-1 (Ca^{2+}-dependent) activity in neurons and, possibly, glia also, and thought to be secondary to reversal of glutamate reuptake at synapses, activation of NMDA receptors, and resulting elevation of intracellular Ca^{2+}. Whereas increased NOS-3 activity in the vascular endothelium and an increase in NOS-2 activity in ischemic brain tissue probably derive from a range of infiltrating neutrophils and macrophages, activated microglia, and astrocytes. The activity of all the three forms of NOS has been reported to increase after I/R. The activity of NOS-1 and NOS-2 is largely deleterious after I/R, and their inhibition or inactivation, however, is neuroprotective [44]. Several of these ROS are produced at low levels during normal physiological conditions and are scavenged by endogenous antioxidant systems that includes superoxide dismutase (SOD), glutathione peroxidase, catalase, and anti-oxidant vitamins such as α-tocopherol and ascorbic acid. However, oxidative stress, particularly, becomes harmful in the brain, when the generation of ROS overrides the ability of the endogenous antioxidant system to eliminate excess ROS that subsequently leads to cellular damage (Fig. 1) [45]. After I/R, production of ROS overwhelms endogenous scavenging mechanisms and lead to many deleterious effects, including damage of membrane lipids, peroxidation of docosahexaenoic acid, a precursor of neuroprotective docosanoids proteins, cleavage of DNA during the hydroxylation of guanine, and methylation of cytosine. In addition to the harmful effects to cellular integrity, ROS can block mitochondrial respiration by inhibiting complex enzymes involved in the electron transport chain [46]. Moreover, oxidative stress facilitates mitochondrial transition pore formation, which causes increased release of inner and outer mitochondrial membrane space constituents including apoptosis-related proteins after I/R [47–48]. Furthermore, endoplasmic reticulum (ER) is also susceptible to oxidative stress, and oxidative injury to this organelle may be implicated in neuronal cell death after cerebral ischemia [49].

ROS activate various cell signaling pathways that are proposed to be involved in regulating cell survival/death, such as the p38, c-Jun N-terminal kinases (JNK), p53, extracellular signal-regulated kinases 1/2 (ERK1/2), and Akt pathways (see later discussion). In addition, ROS can also activate many transcription factors; particularly, the nuclear factor-kappa B (NF-κB) regulates cell death/survival known to be regulated by the redox state of the cell [50]. However, the activation of NF-κB block by antioxidant system via inhibiting phosphorylation of IκB in the serine residue. Moreover, oxidative stress induces the nuclear translocation of NF-κB without degradation of IκB [50, 51]. Nevertheless, oxidative damage does not occur in isolation but participates in the complex interplay between excitotoxicity, apoptosis, and inflammation in ischemia and reperfusion [52]. Because oxidative stress involves multiple post-ischemic cascades leading to cell death, therefore, effective strategies for the prevention/treatment of ischemic brain injury is likely to necessitate intervention at multiple mechanisms.

Inflammation

I/R injury-associated inflammatory reactions that occur at the blood–endothelium interface are extremely critical to the pathogenesis of tissue damage (Fig. 2).The major inflammatory mediators include cytokines, adhesion molecules, chemokines, and leukocytes. In cerebral ischemia, because of acute diminished CBF, the microvascular delivery of essential nutrients to the neurons is virtually prevented and, therefore, neuronal functioning is compromised. After the interruption of CBF, tissue injury begins with an inflammatory reaction that requires the infiltration of leukocytes, which are the cellular mediators of subsequent microvessel obstruction, edema formation, cellular necrosis, and tissue infarction [53]. Three classes of cell adhesion molecules including, i.e., selectins, integrins, and immunoglobulin super family, mediate leukocyte–endothelial cell interactions. Selectins are glycoproteins and comprised by P-, E-, and L-selectin. They mediate low-affinity leukocyte–endothelial cell interactions, thus, promoting the margination and rolling of leukocytes via binding carbohydrate residues [54–55]. It is suggested that selectins up-regulate in the endothelial cells and leukocytes after focal I/R [56–57]. This leads to neutrophil accumulation and subsequent injury to the brain. Moreover, the blockade of P- and E-selectin with antibodies [55, 57–59], use of an inhibitor of selectin (sCRsLex), and a synthetic oligopeptide [60] reduces polymorphonuclear (PMN) leukocyte infiltration and infarct volume, with improvement in neurologic function and CBF. Thus, leukocyte adhesion molecules may contribute to neutrophil accumulation and to ensuing reperfusion injury. However, a comprehensive understanding of the role of selectins in humans may help in preventing leukocyte rolling and adhesion, which is an early step toward brain infarction.

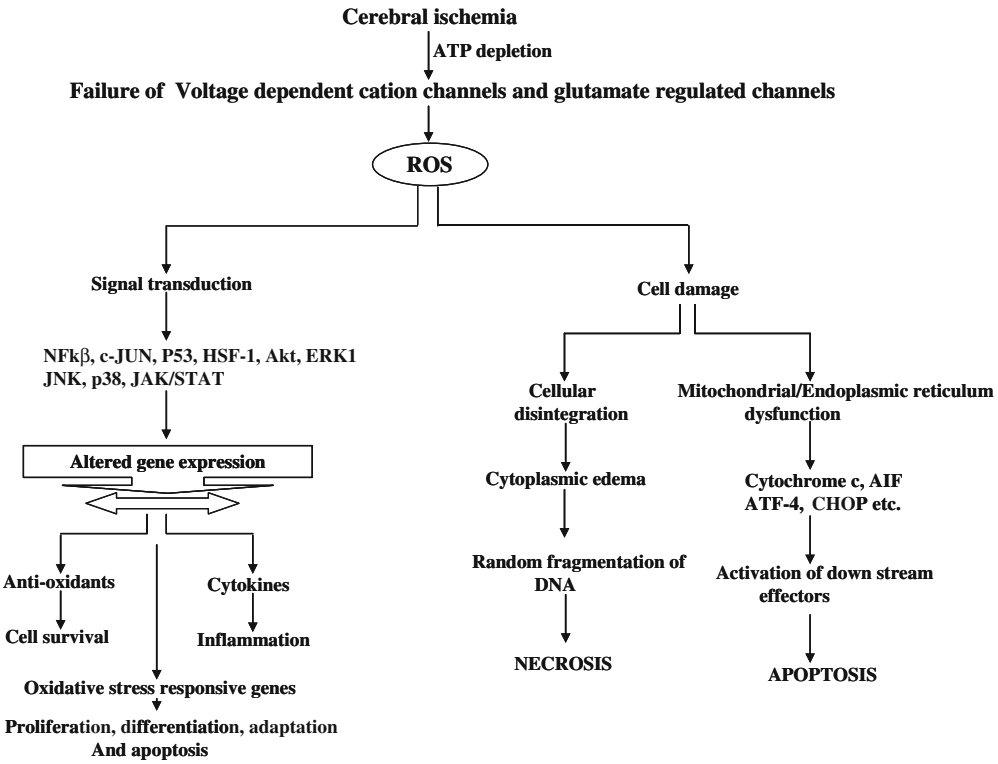

Fig. 1 Flow chart shows events that lead to inflammation and cerebral infraction upon ischemic insult (see text)

Moreover, migration of PMN leukocytes may also be mediated by interaction of adhesion molecules with leukocytes expressing β2-integrin CD11b/CD18 (Mac-1) and CD11a/CD18 (LFA-1) on their surface [61–63]. These cells get accumulated in the microvessels of the penumbral region and leads to additional disruption of microcirculation [64]. Interestingly, mice deficient in Mac-1 are less susceptible to I/R injury [65], and antibodies directed against CD18, CD11b, intracellular adhesion molecule-1 (ICAM-1), or Mac-1 reduce leukocytes accumulation and subsequent infarction after cerebral ischemia [66–69]. However, the animal data suggesting the crucial role of the β-2 integrin in cerebral ischemia does not show promise in human clinical trials using antibodies against Mac-1 [59].

The members of the immunoglobulin super gene family, comprising ICAM-1 and ICAM-2, platelet–endothelial cell adhesion molecule-1 (CD31), mucosal addressin cell adhesion molecule-1 (CD146), and vascular cell adhesion molecule-1 (CD106), are expressed on activated endothelial cells and play various roles in adhesion and serve as ligands for integrins. Among them all, ICAM-1 has raised significant interest as a mediator of leukocyte–endothelial cell adhesion and postischemic "no-reflow" phenomenon [64, 70]. As evident, the expression of ICAM-1 has been found to increase in both rodents [61] and human brains after I/R [71], and its increased expression may be linked to the accumulation of PMN leukocytes in microvessels of infarcted regions [72]. The functional importance of ICAM-1 is further demonstrated by the fact that ICAM-1-deficient mice have smaller infarction [73, 74]. Moreover, ICAM-1 protein knockdown and treatment with anti-ICAM-1 antibody reduces neutrophil accumulation, apoptosis, and ultimately, brain damage after cerebral ischemia [69, 75–76]. Interestingly, administration of anti-inflammatory agent ibuprofen showed a significant reduction in brain damage after MCAO [77].

However, the expression of cell adhesion molecules requires prerequisite intracellular mechanisms in which cytokines play a significant role. Cytokines, such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α) are produced by a variety of activated cell types, including endothelial cells, platelets, leukocytes, and fibroblasts, and serve as important mediators of inflammatory responses in cerebral ischemia [78, 79]. Existing evidence suggests that expression of IL-1 increases after transient or permanent cerebral ischemia in microglia, astrocytes, and neurons [80–82]. It has been demonstrated that IL-1 causes upregulation of E-selectin, ICAM-1, ICAM-2, and vascular cell adhesion molecule-1 on the surface of cerebral endothelial cells [83–87]. Similarly, the increased messenger RNA (mRNA) level of TNF-α has been detected in rats after focal cerebral ischemia and in the cerebrospinal fluid of ischemic stroke patients [88, 89]. Moreover, the blockade of IL-1 receptor decreased the number of necrotic neurons and brain damage after cerebral ischemia [86, 87]. Whereas, the blockade of TNF-α by TNF-binding proteins reduces the DNA fragmentation and brain damage after cerebral ischemia [90].

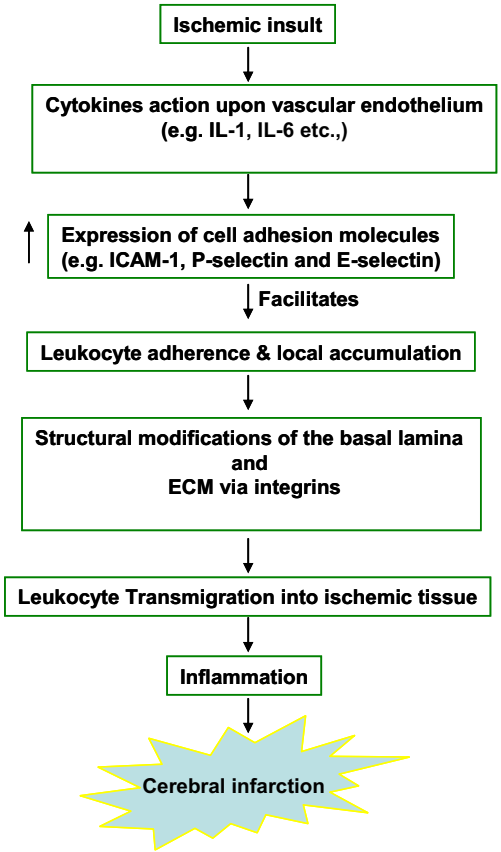

Fig. 2 Represents the pathways mediated by oxidative stress in cerebral ischemia. Imbalance between endogenous anti-oxidant system and ROS may be crucial for cell survival/death by causing dysfunction of subcellular organelles and subsequent activation of molecules that are involved in cell survival/death mechanisms that ultimately reflect the fate of the cell (see text for more details)

Matrix metalloproteinase (MMP) family is involved in the degradation, and remodeling of the extracellular matrix may also mediate breakdown of blood brain barrier (BBB) and is proinflammatory after transient focal cerebral ischemia. Increase in MMP-2 levels seems to cause an early opening of BBB, whereas increase in MMP-9 levels has been significantly associated with hemorrhagic transformation in nonhuman primates [91]. Similarly, increased expression of MMP-2 and MMP-9 are also observed in human stroke patients [92]. A recent study demonstrated that I/R lead to the release and activation of MMP-9 from neutrophils that are recruiting to the postischemic brain. This neutrophil-derived MMP-9 exhibits self-amplifying proinflammatory effects that trigger further neutrophil–endothelial adherence, neutrophil plugging of capillaries, and diapedesis into brain parenchyma. Therefore, specific inhibition of neutrophil degranulation or the activation of MMP-9 release by neutrophils may be a feasible therapeutic approach to limit secondary vascular and parenchymal injury after transient focal ischemia [93]. In addition, NF-κB, a dimeric transcription factor, is also involved in the regulation of inflammation. NF-κB heterodimer is composed of subunits Rel A (p65) and p50. NF-κB normally situate in the cytoplasm bound to its endogenous inhibitor protein, known as inhibitory kappa B (IκB). IκB kinase (IKK), an upstream, leads to phosphorylation of IκB at −32 and −36 serine residues. This liberates NF-κB and allows it to translocate to the nucleus and bind to κB sites, specific domains within the promoters of downstream genes, to activate their transcription. Many genes involved in inflammation contain functional κB sites, such as TNF-α, ICAM-1, cyclooxygenase-2 (COX-2), inducible NOS, and interleukin-6 (IL-6). It has been found that IKK and NF-κB activation correlate very well to the anti-inflammatory effect of mild hypothermia after experimental stroke [94]. However, the function of NF-κB in the stroke pathophysiology is yet to be clearly elucidated.

Inflammatory Caspases: Switch to Apoptosis?

It is well known that caspase-1 plays a key role in inflammatory pathways, but the possible role of caspase-1 in neuronal cell death is not yet understood clearly. However, one study reported that upregulation of receptor-interacting protein-2 (Rip2/cardiac/Rick) mediates activation of caspase-1 in hypoxia and ischemia-induced neuronal cell death [95]. Furthermore, activated caspase-1 generates truncated Bid (tBid) that leads to the release of apoptogenic factors including cytochrome *c*, AIF, and Smac/Diablo from the mitochondria [95]. Thus, it suggests, cleavage of Bid as an important downstream effector of caspase-1 and provides evidence that caspase-1 may also be involved in mitochondrial-mediated apoptotic pathway [95]. On the other hand, Benchoua et al. [96] suggested that cathepsin B triggers the activation of proinflammatory caspases in focal cerebral ischemia. As activation of cathepsin B is observed concurrently to the activation of proinflammatory caspases, i.e., caspase-1 and caspase-11. Moreover, both of these caspase activation cascades colocalized with the cathepsin B in the cells of infarcted core. Furthermore, inhibition of cathepsin B protects cortical structures from ischemic damage [96]. Thus, it suggests that activated cathepsin B triggers the activation of proinflammatory caspases, and this pathway may probably be involved in promoting inflammatory responses and/or by amplifying the apoptotic process after cerebral ischemia.

Apoptosis

Programmed cell death (PCD) has a critical role during development and in normal cellular homeostasis. Kerr et al. [97] suggested that apoptosis is an energy-dependent, programmed form of cell death and described the morphological features as cell shrinkage, membrane blebbing,

chromatin condensation, and DNA fragmentation. However, dysregulation of this process is implicated in various diseases ranging from cancer and autoimmune disorders to neurodegenerative diseases and ischemia. Experimental animal models such as focal or global cerebral ischemia have suggested the involvement of cell survival/death signaling pathways in the pathogenesis of cerebral ischemia [98]. The morphological and biochemical evidences of apoptosis have been well documented in experimental animal models of ischemic brain injury. The most convincing morphological evidence of post-ischemic neuronal apoptosis was detected at early hours after the onset of an ischemic insult in the penumbra and during reperfusion [99–103].

Although PCD has often been equated with apoptosis, nonapoptotic forms of cell death also exist, which do not fulfill the criteria for apoptosis [104]. For instance, nonapoptotic forms of cell death, such as autophagy, necroptosis characterized by necrotic cell death morphology, and activation autophagy, have been described, which might contribute to delayed ischemic brain injury [105]. Nevertheless, PCD generally denotes any cell death that is mediated by the intracellular death program no matter what initiates it and whether or not it displays all of the characteristic features of apoptosis, but the term apoptosis retains its historical strength in describing a phenomenon with a set of characteristic features [106]. On the other hand, transient forebrain ischemia induces DND, selectively in the CA1 sector pyramidal cells of the hippocampus [9]. Although DND exhibits distinct morphological features, it shares some morphological aspects with apoptosis, i.e., formation of apoptotic bodies and chromatin condensation, but less as compared to typical apoptosis, etc. [107]. Moreover, the molecular machinery involved in apoptosis seems to play a significant role in DND. For example, activation of caspase-3 has been observed in mediating DND in the CA1 neurons of the hippocampus after ischemia, and inhibitors of caspase-3 have offered neuroprotection [9, 108]. However, detailed molecular mechanisms underlying DND in cerebral ischemia remains to be elucidated.

Caspase and Bcl-2 Family Members: The Crucial Regulators of Apoptotic Pathways

Caspases

The characteristic morphological changes observed by Kerr et al. are caused by a set of cysteine aspartic acid proteases (caspases) that are responsible for apoptosis in mammals [109]. So far, 14 mammalian caspases have been identified; at least eight of them play an important role during apoptosis [110]. There are two types of apoptotic caspases: initiators (e.g., caspase-8, caspase-9, and caspase-10) and executors (e.g., caspase-3 and caspase-7). The initiator caspases exist as monomers and interact with the other proteins by means of caspase activation and recruitment domain (CARD), or death effector domain (DED), which leads to their activation. Furthermore, activated initiator caspases cleave inactive forms of effector caspases, thereby, activating them. In turn, the activated effector caspases cleave other protein substrates in the cell, which contribute to the apoptotic process [111, 104].

The importance of caspases in developmental neuronal death is well established. Studies in caspase knockout mice have provided indisputable evidence for a crucial role of caspases in neuronal cell death. Caspase-3 and caspse-9 are the two major caspases involved in apoptosis that leads to neuronal cell death. Mice lacking both caspase-3 and caspase-9 show severe defects in neuronal cell death, such as lack of apoptosis in neuroepithelial progenitor cells during development [112, 113]. Similarly, apoptotic protease activating factor-1(Apaf-1)-null mice also exhibit reduced apoptosis in the brain during late embryonic development [114]. Thus, it suggests that mitochondrial-mediated apoptotic pathways may be involved in regulating neuronal cell death in the developing brain. In addition, a recent literature discusses a new role for caspases in ‘neuronal plasticity’ under physiological conditions, when activated at sublethal levels [115]. However, some caspases such as caspase-11 and caspase-12 get activated only under pathological conditions [116, 117]. As evident, cortical neurons deficient of caspase-12 are more resistant to apoptosis than the wild type, whereas caspase-12-deficient mice do not exhibit any obvious developmental or behavioral defects [117].

There is large body of evidence suggesting that cerebral ischemia can cause activation of caspases. For instance, upregulation and activation of caspases-3 have been found to precede neuronal cell death in animal models of focal and global cerebral ischemia [108, 118–120, 121–125]. Studies in humans have also provided the evidence of caspase activation after cerebral ischemia. For instance, increased level of procaspase-3 is observed within hours of strokes resulting from permanent atherothrombotic arterial occlusion, whereas both the activated caspase-3 and cleaved poly(ADP-ribose) polymerase (PARP) are detected only several days after cardiac arrest with reperfusion, suggesting that caspase-3 is likely to contribute for DND in clinical settings [126, 127].

Influence of Age on Caspase-Mediated Apoptosis

The ‘age aspect’ may play an important role in the regulation of apoptotic mechanisms after cerebral ischemia.

The key apoptotic regulatory molecules involved in caspase-mediated neuronal cell death such as caspase-3, Apaf-1, and Bax are found to be down-regulated during development and declines in parallel with the physiological PCD [128–131]. Accordingly, both the immature and mature brains subjected to hypoxia–ischemia has resulted into a dramatic increase in caspase-3 activity in immature brains as compared to slight increase in mature brain [128, 132]. Thus, it not only cast doubt on the role of caspase-mediated apoptosis in the adult brain but also indicates that additional or caspase-independent pathways (e.g., AIF) of neuronal cell death exist.

Bcl-2 Family

The Bcl-2 family of proteins plays a crucial role in the regulation of intracellular apoptotic signal transduction. This gene family includes both anti-apoptotic, e.g., Bcl-2, Bcl-xL, etc., and pro-apoptotic proteins, e.g., Bax, Bak, Bad, etc., that contain one or more Bcl-2 homology (BH) domains (Fig. 3). The major anti-apoptotic members Bcl-2 and Bcl-xL are localized on the mitochondrial outer membrane and to the ER and perinuclear membrane [133]. In the central nervous system, Bcl-2 is expressed at a high level during development and down-regulates after birth, whereas, in the peripheral nervous system, the expression of Bcl-2 is maintained throughout the life, and its deletion causes profound loss of peripheral motor, sensory, and sympathetic neurons after birth [134–136]. On the other hand, Bcl-xL is also expressed in the developing brain, and its expression continues to increase into adult life [137]. Mice lacking Bcl-xL exhibit massive apoptotic death of immature neurons throughout the developing nervous system [138]. Likewise, Bcl-w that is expressed at high levels in adult brain plays a significant role in maintaining sensory neuron survival at later stages as compared to Bcl-xL [139]. Thus, it appears that Bcl-2, Bcl-xL, and Bcl-w are crucial for the maintenance of neuronal survival. The Bcl-2 and Bcl-xL act by inhibiting pro-apoptotic Bcl-2 family members through heterodimerization, e.g., Bax [140, 141]. Bax is widely expressed in the nervous system [142], and Bax-deficient mice displayed increased number of neurons in superior cervical ganglia and facial nuclei. Furthermore, the Bax-deficient neonatal sympathetic and facial motor neurons are more resistant to cell death induced by nerve growth factor deprivation and axotomy, respectively. Thus, the activation of Bax appears to be crucial for neuronal cell death induced by trophic factor withdrawal and injury [143]. The interaction between Bax and Bcl-xL clearly regulates immature neuronal apoptosis during development, as the absence of Bax completely abrogates the increased neuronal apoptosis observed in the Bcl-xL-deficient nervous system [144]. Thus, Bax might be crucial for neuronal cell death during development.

In rodent models of stroke, post-ischemic alterations in the expression of Bcl-2 family members, including increased expression of Bax and reduced expression of Bcl-2 and Bcl-w are found both in the ischemic core and in the penumbra of the infarct [17]. Conversely, the increased expression of Bcl-2, Bcl-xL, or Bcl-w observed in ischemic areas may reflect an adaptive response of surviving neurons to ischemic insult [145–149]. An increased expression of Bim, BNip3, and Puma has been reported in ischemia, suggesting a possible role for BH3-only proteins in neuronal apoptosis [150–152]. In addition, the overexpression of BAG1 and Bcl-2, and deficiency in Bax and Bid have been shown to attenuate ischemia-induced damage [17, 153, 154]. These evidences clearly point that potentially effective treatment strategies that target the Bcl-2 family proteins may be beneficial.

Major Apoptotic Signaling Pathways Involving Key Regulatory Molecules

The major pathways involved in apoptotic signaling may be classified into extrinsic and intrinsic. A schematic view of apoptotic cascades employed by both extrinsic and intrinsic pathways is shown in Fig. 4.

Extrinsic Pathway

Death-Receptor-Mediated Apoptosis

Death receptors belong to tumor necrosis factor receptor (TNFR) super family and include Fas, also referred to as

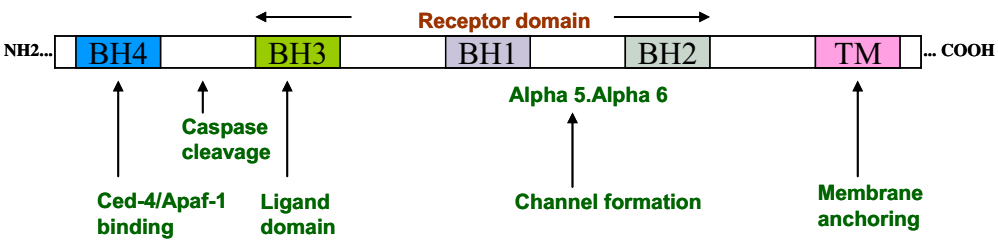

Fig. 3 Schematic illustration of functions of different domains of Bcl-2 family

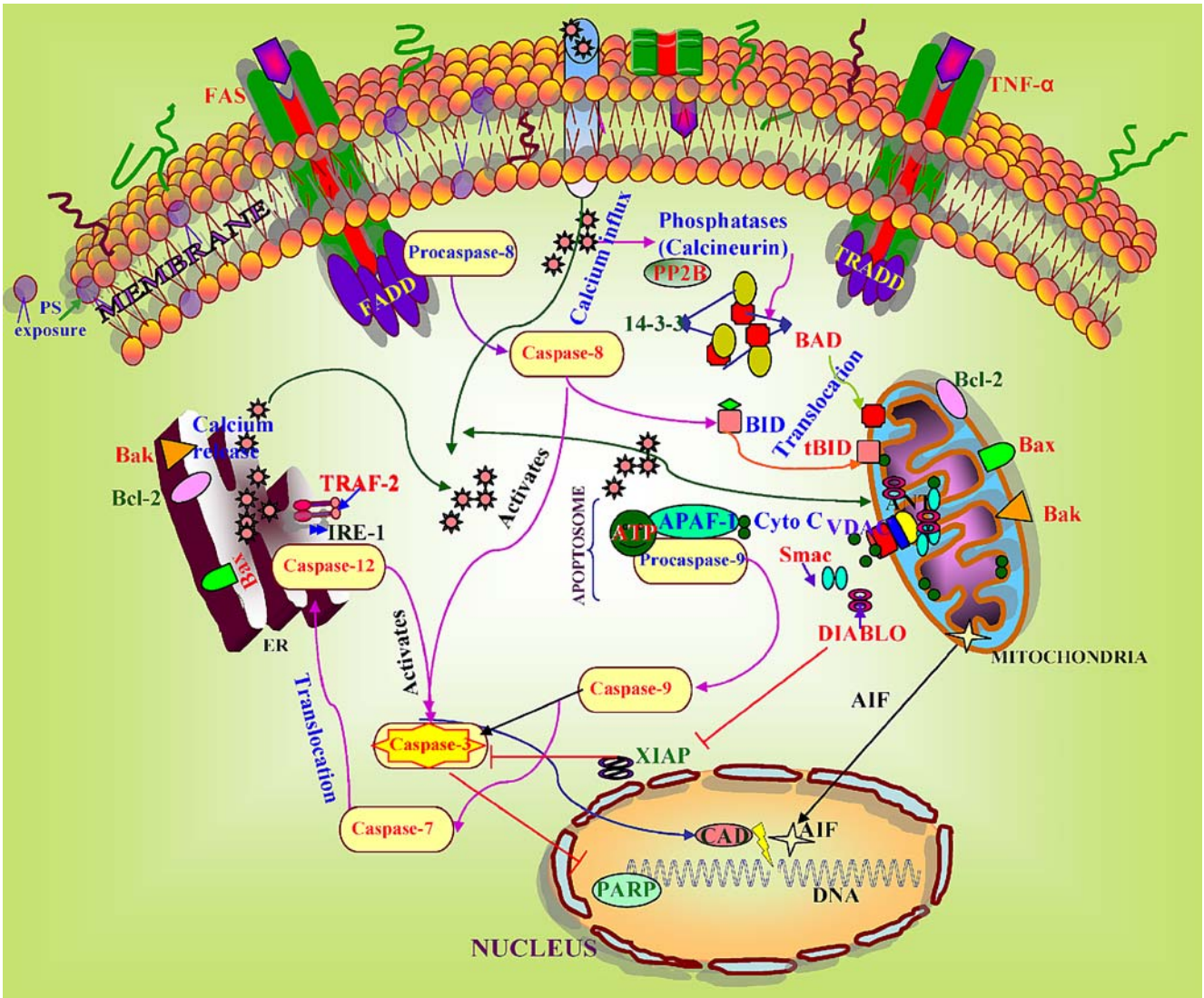

Fig. 4 Schematic overview showing the basic apoptotic signal transduction pathways (extrinsic and intrinsic). In the extrinsic pathway, upon stimulation, death receptor (e.g., Fas) ligation results in formation of multiprotein DISC that includes the receptor adaptor (e.g., FADD). DISC is the site of activation for procaspase-8, where it attains its active form by oligomerization. Caspase-8 can directly activate caspase-3, an effector caspase; on the other hand, it also cleaves proapoptotic Bid into tBid, which trnaslocates to mitochondria to execute apoptosis. In the intrinsic pathway, excessive influx of calcium into intracellular space causes disruption of normal homeostasis, which affects the function of subcellular organelles such as mitochondria and ER. Altered activity of protein phosphatase (e.g., calcineurin) causes translocation of BAD to promote mitochondrial apoptotic pathway. The release of cytochrome c from mitochondrial intermembrane space results in caspase-3 activation via apoptosome complex. The activity of caspases is negatively regulated by IAPs, XIAP, etc., whereas Smac/Diablo neutralizes their effect. The AIF releases from mitochondria and translocates to the nucleus. ER undergoes stress upon depletion of calcium from its lumen. ER stress induces activation of caspase12, which is specifically localized to the ER membrane and may be essential for ER stress-induced apoptosis with further activation of caspase-9 and caspase-3. Both the caspase-7 and tumor necrosis factor receptor-associated factor-2 (TRAF-2) activate caspase-12 in vitro. Moreover, efflux of calcium from ER might trigger secondary activation of mitochondria. Anti- and pro-apoptotic Bcl-2 family members are localized to both the mitochondria and the ER. Caspases cleave key structural components of the cytoskeleton and nucleus. The CAD is responsible for internucleosomal cleavage of DNA, which is a characteristic feature of apoptosis

CD95 or Apo1; and TNFR1, also referred to as p55 or CD120a, DR3, DR4, and DR5, all of which contain the death domain (DD) in their intracellular region to recruit downstream apoptotic proteins. The Fas has become paradigm for the study of death-receptor-mediated apoptosis [155]. Fas ligand (FasL) is a homotrimeric molecule, and each FasL trimer binds three Fas molecules. Upon binding to the trimeric FasL, Fas receptor forms micro-aggregates at the cell surface, allowing adaptor molecule Fas-associated death domain (FADD) to be recruited to its cytosolic tail by a multistep mechanism [156]. FADD contains a DED in its N-terminal region that interacts with

the DED in the prodomain of procaspase-8 and recruits caspase-8 to Fas. Fas, FADD, and procaspases-8 form death-inducing signaling complex (DISC), in which the initiator caspase-8 is activated. Procaspase-8 has weak proteolytic activity, which is enhanced by oligomerization within the DISC. Activated caspase-8 releases into the cytoplasm and initiates further downstream caspase cascade [157, 158].

The available evidence suggests that receptor-mediated activation of caspases may participate in ischemic brain damage. The activation of caspase-8 has been documented in some studies on experimental brain ischemia [159]. Release of TNF-α by neurons and glia [160–162] and upregulation of Fas mRNA and protein levels have been observed in the vulnerable areas after hypoxic ischemic injury. Moreover, a marked increase in the Fas-L expression is found in the penumbral region during early reperfusion after MCAO [17]. This strongly suggests that upregulation of death receptors may lead to assembly of DISC and the activation of procaspase-8 after focal cerebral ischemia [159, 163, 164]. Furthermore, BH3-only death-promoting factor, Bid, is involved in TNF/Fas family death-receptor-mediated extrinsic pathway. Activated caspase-8 leads to the cleavage of Bid to activated form, i.e., tBid. Then, the tBid targets to the mitochondrial membrane, and through conformational changes in Bax, it triggers cytochrome c release that may finally lead to activation of caspase-3 [165, 166].

Intrinsic Pathway

Mitochondria-Mediated Apoptosis

Mitochondria are involved in both the necrosis and apoptotic pathways, which depend on the severity of the insult or nature of the signaling pathways. Severe insults of cerebral ischemia render mitochondria dysfunctional for ATP production. Although caspase-dependent apoptosis requires ATP, a sudden decrease in ATP levels can alternatively induce necrotic cell death [167, 168]. In vitro studies demonstrate that various cellular or biochemical pathways are involved in mitochondrial-mediated apoptotic signaling. After death stimuli, mitochondria may become permeabilized, which causes the release of cytochrome c, procaspase-9, and Apaf-1 from its intermembrane space. In the presence of ATP/dATP, cytochrome c binds to the WD-40 domain of Apaf-1 and promotes Apaf-1 oligomerization, leading to the formation of Apaf-1 and cytochrome c multimeric complex. Procaspase-9 gets recruited to Apaf-1 and cytochrome c complex in 1:1 ratio through interaction between N-terminal CARD domain of Apaf-1 and that of caspase-9. Consequently, the procaspase-9 molecules approach each other and are activated by autocleavage. This holoenzyme is referred to as apoptosome, a very large complex of approximately 700 kDa [169–171] that initiates further downstream apoptotic cascade.

The involvement of mitochondria-mediated apoptotic pathway is supported by the observations of cytochrome c release from the mitochondria to the cytosol, both after transient and permanent cerebral ischemia [123, 172, 173–175]. Moreover, release of caspase-9 from the mitochondria [176], formation of caspase-9/Apaf-1 complex [177], and activation of capase-3 by caspase-9 [178] all strongly suggest the active role of mitochondria-mediated apoptotic pathway involving caspases after cerebral ischemia. Induction of internucleosomal DNA fragmentation is characteristic manifestation of apoptosis that occurs concurrently with neuronal cell death in vulnerable brain regions after ischemia [14, 179–181]. It has been suggested that caspase-3 may be the key executioner of nuclear degradation in ischemic neurons and contributes to DNA fragmentation, possibly by activation of caspase-activated deoxyribonuclease (CAD) [182–183]. The induction of deoxyribonuclease activity correlated with caspase-3 activation and caspase-3-mediated degradation of the inhibitor of caspase-activated deoxyribonuclease. Furthermore, inhibition of caspase-3-like activity diminishes CAD activation and prevents DNA fragmentation. Therefore, these results suggest that caspase-3-dependent CAD activity may play an important role in DNA fragmentation after I/R [184]. In addition, overexpression of superoxide dismutase 1 (SOD1) protects neurons against ischemic damage by attenuating mitochondrial release of cytochrome c and second Smac, which causes reduced caspase activation, hence, reduced apoptosis [185]. Thus, it appears that mitochondrial pathway of caspase activation may play an important role in neuronal cell death after ischemia. However, the activity of caspases is negatively regulated by IAPs, X-linked mammalian inhibitor of apoptosis protein (XIAP), etc., whereas Smac/Diablo neutralizes their effect.

On the other hand, the Bcl-2 family proteins play a crucial role in intracellular apoptotic signal transduction by regulating the mitochondrial membrane permeability. [133]. Increased levels of proapoptotic Bax and its translocation seems to be important for neuronal apoptosis [186–188]. A rapid translocation of cytosolic Bax to the mitochondria has been observed after cerebral ischemia, where it interacts with the mitochondrial adenine nucleotide translocator and the voltage-dependent anion channel. Moreover, the release of cytochrome c and caspase-9 from mitochondria coincides with the temporal and regional distribution of Bax [174]. Conversely, an extensive literature documents the capacity of anti-apoptotic Bcl-2 and Bcl-xL to block cell death after cerebral ischemia [189, 190]. Zhao et al. [191] demonstrated the overexpression of Bcl-2 to be protective against neuronal loss within the ischemic margin after experimental stroke, and this protection was accompanied

by reduced cytochrome c translocation to the cytosol and reduced activation of caspase-3. Similarly, overexpression of Bcl-xL inhibited mitochondrial hyperpolarization and cytochrome c release during neuronal apoptosis [192]. Thus, it strongly suggests the importance of Bcl-2 family proteins in the regulation of mitochondrial permeability after cerebral ischemia.

Bad also plays a critical role in the regulation of cell death/survival in cerebral ischemia. It resides as an inactive complex with the molecular chaperone 14–3–3 via the phosphorylation of four serine residues [193, 194]. During apoptotic stimuli, Bad gets dephosphorylated, dissociates from 14–3–3, and translocates to the outer mitochondrial membrane, where it subsequently dimerizes with Bcl-xL to promote mitochondrial cytochrome c release [193, 195, 196]. Such cascades of events are also observed in the early stages after MCAO [197]. However, some pathways inhibit the pro-apoptotic functions of Bad after cerebral ischemia. The transforming growth factor-β1 (TGF β1) suppresses Bad activity by phosphorylation of the Ser-112 residue of Bad and by the activation of ERK pathway [198]. In addition, Akt and PKA pathways are also involved in the phosphorylation of Bad and its binding to 14–3–3 in surviving neurons after I/R [199]. Interestingly, a recent report suggests that signal predominance varies from phosphatidylinositol-3-kinase (PI3-K)/Akt-mediated survival signaling to JNK-mediated death signaling (also involved in Bad activation) with the degree of neuronal damage after cerebral ischemia [200]. Thus, Bad seems to serve as a molecular switch between cell survival and cell death signaling pathways after cerebral ischemia.

ER-Mediated Apoptosis

ER is emerging as an important source of apoptotic signaling, as ER stress-induced apoptosis is associated with a range of diseases including neurodegenerative disorders, diabetes, cerebral ischemia, etc. [201]. However, the precise mechanism(s) of ER-mediated cell death signaling in cerebral ischemia is yet to be understood completely.

The ER is an intracellular organelle that plays an important role in the maintenance of intracellular Ca^{2+} homeostasis and proper folding of newly synthesized secretory and membranous proteins [202]. Cellular stress conditions, such as glucose deprivation, depletion of ER store Ca^{2+}, exposure to free radicals, and accumulation of unfolded/misfolded proteins, disrupt the proper function of ER and initiates unfolded protein response (UPR) to cope with this adverse situation [202–204]. UPR is characterized by the activation of three ER transmembrane effector proteins: PKR-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and the activating transcription factor-6 (ATF-6). In the physiological state, all three effectors bind to the ER chaperone glucose-regulated protein (GRP78) on their luminal domains; thus, GRP78 acts to suppress their activity [205, 206]. Under conditions of ER stress, when misfolded proteins accumulate in the ER lumen, GRP78 dissociates from the PERK, ATF-6, and IRE1, allowing their activation. The disassociated GRP78 binds to the unfolded proteins to assist in refolding. On the other hand, activated PERK phosphorylates eukaryotic initiation factor 2α (eIf2α) to avoid further accumulation of proteins by suppressing protein synthesis but also leads to the paradoxical increased translation of activating transcription factor-4 (ATF-4) and C/EBP homologous protein/growth arrest and DNA damage-inducible gene 153 (CHOP/GADD153) [207]. Expression of CHOP/GADD153 mRNA serves as a hallmark of ER stress, whereas both ATF-4 and CHOP/GADD153 are involved in apoptosis [207, 208–210]. Activated ATF6 (90 kDa) translocates to the golgi compartment, where it is split by proteases into the active form (50 kDa), and the activated ATF-6 translocates to the nucleus and binds the ER stress-response element (ERSE) promoter sequence and functions as transcription factor for ER-resident proteins such as GRP78, GRP94, etc. [211–213], whereas activated IRE1α (the ubiquitous isoform) cleaves a sequence of 26 bases from the coding region of x-box-binding protein-1 (XBP-1) mRNA and the transfer RNA ligase-gated transcript that leads to enhanced translation of XBP-1 protein, a transcription factor for ER-resident enzymes and chaperones. The XBP-1-binding protein is also an ERSE-binding transcription factor with overlapping binding specificity to ATF-6 [207]. In addition, active IRE1α has been shown to be involved in the activation of caspase-12 [214]. However, prolonged ER stress accompanied by failure of adaptive response may lead to apoptotic signaling.

An interesting hypothesis put forward that cerebral ischemia causes ER dysfunction, which may play a major role in the pathological process resulting in neuronal cell death [215]. Accumulation of unfolded proteins in the ER lumen [216], phosphorylation of eIf2α [217, 218], inhibition of protein synthesis [204, 219], depletion of Ca^{2+} from ER stores [202], activation of CHOP/GADD153 expression [220], and activation of caspase-12 [221, 222], all suggest the crucial role of ER in neuronal cell death signaling after cerebral ischemia. At present, exact signaling mechanisms underlying ER stress-induced apoptosis is poorly understood.

Although the activation of caspase-12 has been documented in rodent models of cerebral ischemia, the question of whether or not a human isoform of caspase-12 exists remains controversial [223]. However, despite the controversial role of caspase-12 in humans, a recent study demonstrated that ER stress and caspase-12 activation has been implicated in neurodegeneration in Creutzfeldt–Jakob-disease-affected individuals, and the activation of caspase-

12 correlates with the higher levels of ER molecular chaperones GRP58, GRP78, and GRP94 expression [224]. Moreover, human caspase-4, which plays a key role in ER stress-induced apoptosis, might functionally substitute for mouse caspase-12 in human system [225]. On the other hand, overexpression of CHOP/GADD153 induces apoptosis via inhibition of Bcl-2 [226, 227]. It is noteworthy that Bcl-2 family proteins modulate ER Ca^{2+} homeostasis and control cell death induced by ER stress agents [228]. Moreover, neurons lacking CHOP showed resistance to hypoxia–reoxygenation-induced apoptosis than the wild type [229]. Thus, it indicates that CHOP induction may be involved in ischemia-induced neuronal cell death pathway. Hence, there is an emerging need to study the basic molecular mechanisms underlying I/R-induced ER-mediated cell death/survival signaling pathways.

Although the apoptotic stimuli may undergo one of the above-mentioned pathways, these distinct pathways are cross-linked with each other. The death-receptor-mediated apoptotic pathway cross-link with mitochondrial-mediated apoptosis through cleavage of Bid by activating caspase-8. On the other hand, a close contact between ER and mitochondria appears to play an important role in the pathogenesis of neuronal cell death.

Cross-Talk Between ER and Mitochondria

The exposure of cells to apoptotic agents that disturb ER functions reveals a novel crosstalk between ER and mitochondria. Pretreatment with tunicamycin, a drug that blocks the ER-resident protein glycosylation, also affected cytochrome c release from the mitochondria, followed by caspase-3 activation and DNA fragmentation. Moreover, both cytochrome c released from the mitochondria and caspase-3 activation blockade occurs when cells are transfected with Bcl-2 specifically targeted to the ER compartment [230].

Recently, it has been demonstrated that a caspase cleavage product B-cell-associated protein 31 (BAP31), an integral membrane protein of ER, induces mitochondrial fission through ER Ca^{2+} signals, enhancing cytochrome c release to the cytosol [231]. BAP31 contains three predicted transmembrane domains, followed by a leucine zipper and a death effector domain-like (DED-L) region that associates with certain isoforms of procaspase-8 in the cytosol. Caspases cleave the cytosolic tail of BAP31 that exhibits apoptotic features, whereas overexpression of full-length BAP31 blocks the Fas-mediated apoptosis. In addition, BAP31 also binds to Bcl-2 and Bcl-xL [228]. Although the role of BAP31 in cerebral ischemia remains unexplored, these observations suggest that apoptotic cross-talk between the ER and the mitochondria might be associated with the pathological states of the brain.

The temporal profile of ER and mitochondrial dysfunction induced by transient cerebral ischemia suggests that ER dysfunction may be a process upstream of mitochondrial dysfunction. Phosphorylation of PERK and eIF2α during the early reperfusion after transient cerebral ischemia indicates ER dysfunction to be an early pathological process. Moreover, mitochondrial cytochrome c release has not seen before 2 h of reperfusion, implying that ER dysfunction does precede impairment of mitochondrial function [232]. However, a recent study on HeLa cell line has shown that, at early stages of apoptosis, cytochrome c translocates to the ER, where it selectively binds IP_3R, resulting in a sustained increase of cytosolic Ca^{2+} [233]. In agreement with the above observations, inositol 1,4,5-trisphospate receptor 1 (IP_3R1) and ryanodine receptors get activated after global cerebral ischemia by cytochrome c, resulting in Ca^{2+} efflux from ER [234]. Thus, it is possible that ER stress triggers a set of reactions leading to leakage of cytochrome c from the mitochondria that further stimulates ER receptors to release more Ca^{2+}, resulting in a positive feedback loop. Sanges and Marigo reported that ER stress inducers cause co-activation of AIF and caspase-12 and their subsequent re-distribution to the nucleus [235]. Furthermore, reduction in the AIF or caspase-12 expression by RNA interference revealed that AIF primarily controls apoptosis caused by changes in Ca^{2+} homeostasis but not necessary for protein misfolding apoptosis, whereas caspase-12 seems to regulate both AIF activation and PCD [235]. Thus, it suggests a novel apoptotic crosstalk between the ER and the mitochondria that might be linked to the pathogenesis of cerebral ischemia.

Other Intrinsic Apoptotic Regulators

Calpains

Calpains belong to cysteine proteases family, which may be involved in mediating both acute and chronic neuronal cell deaths along with caspases [236]. In the brain, the gating of post-synaptic glutamate receptors and other membrane channels trigger intracellular Ca^{2+} overload and calpain activation [237]. The mechanism of calpain-mediated signaling cascade induced by cerebral ischemia is still unclear. However, it has been demonstrated that calpains may be responsible for cleaving of procaspae-12 and loop of Bcl-xL, therefore, turning antiapoptotic Bcl-xL into proapoptotic. This suggests a novel apoptotic pathway involving Ca^{2+}-mediated calpain activation and crosstalk between calpain and caspase family [238]. In addition, calpains also process p53, amyloid-β peptide, and tau proteins (involved in Alzheimers), indicating their apoptotic regulatory role [239].

Moreover, several classes of synthetic calpain inhibitors have been used as potential therapeutic agents to prevent neurodegeneration in various models of central nervous system (CNS) injuries, including focal cerebral ischemia. Calpastatin is the only natural protein inhibitor of calpain, and transgenic mice overexpressing calpastatin have been useful for clarifying the role of the calpain system in brain injury and degeneration [240]. As limited knowledge is available on the role of calpain-mediated cell death, hence, extensive investigation using calpain inhibitors in vivo and proper understanding of calpains crosstalk with other apoptotic regulatory molecules will certainly help to unravel its key role after cerebral ischemia.

p53-Mediated Apoptosis

The p53 is a tumor suppressor gene involved in the regulation of apoptosis in several death paradigms. It plays an essential role in preventing the propagation of DNA-damaged cells and controlling aberrant cell cycle regulation during oncogenesis. If repair is not successful, p53 may initiate cell death by apoptosis in a dose-dependent manner, thus, preventing the propagation of genetic defects. Several reports strongly suggest that p53 has been implicated in the regulation of cell death after neuronal injury. For instance, mice lacking p53 exhibits significantly less ischemic damage than its wild type after MCAO [241]. In addition, enhanced expression of p53 has been observed in injured neurons before cell death induced by focal ischemia, excitotoxicity, and hypoxia, and also suggests its key role in ischemia-induced cell death [242].

Although p53 is one of the key modulator of cellular stress responses, the mechanisms by which p53 regulate apoptosis remains unclear. However, upregulation of Apaf-1 at both the transcription and translational levels in neurons undergoing p53-induced cell death suggests that Apaf-1 is a key transcriptional target for p53 after neuronal injury [243]. On the other hand, activated protein kinase C showed neuroprotection via blocking p53-mediated apoptosis in ischemic human brain endothelium. Moreover, it has normalized the Bax/Bcl-2 ratio and reduces the caspase-3 signaling [244]. In addition, Pifithriná, a specific synthetic inhibitor to p53, has shown to reduce the number of apoptotic cells in the ischemic brain by inhibiting the binding of p53 to its DNA sites because it reduced the expression of the p53-related gene p21WAF without changing the amount of p53 protein itself [245]. A recent study had demonstrated that p53 translocates to the mitochondria after transient global ischemia in rats, and this translocated p53 interacts with Bcl-xL and causes the release of cytochrome c. However, administration of Pifithriná reduces the release of cytochrome c from the mitochondria and subsequent apoptosis in CA1 neurons, suggesting that the mitochondrial p53 pathway is one of the novel mechanisms that may mediate neuronal cell death after ischemia [246].

Apoptosis-Inducing Factor

The AIF is a mitochondrial localized flavoprotein of ~67 kDa with NADH oxidase activity that is encoded by a nuclear gene. However, translocation of AIF from the mitochondria to the nucleus in which it induces large-scale (~50-kbp) DNA fragmentation and apoptosis independent of caspase activity [247, 248]. In the nervous system, AIF translocation occurs after a variety of toxic insults including NMDA-mediated excitotoxicity [249], global cerebral ischemia [250], focal cerebral ischemia [18, 251], and postnatal hypoxia–ischemia [252, 253].

Recent reports have provided evidence for a substantial role for AIF in ischemic brain damage. Plesnila et al. demonstrated that AIF released from mitochondria translocates to the nucleus at the early hours after focal cerebral ischemia and colocalized with DNA damage and apoptotic nuclear condensation in the ischemic penumbra. It is noteworthy that mitochondrial release of AIF that occurred several hours before cytochrom c release suggests that AIF mediates cell death independent of caspases [254]. Convincingly, intrathecal administration of Z-VAD.fmk, a pan caspase inhibitor, does prevent the caspase-mediated degradation of fodrin but has no significant effect on the mitochondrial release of AIF in neonatal brain damage after I/R [252]. Similarly, it has been found in several in vitro cell death models that neither pharmacological inhibition of caspases nor knockout of genes such as Apaf-1 or caspase-9 influenced the mitochondrio-nuclear translocation of AIF [255]. Furthermore, transfection of Bcl-2 via the herpes simplex virus in peri-infarct region of the brain not only blocks the translocation of AIF to the nucleus but also improves survival of cortical neurons after focal cerebral ischemia [256]. These data suggest that AIF releases from the mitochondria in a caspase-independent manner to relay and participate in cell death signaling after ischemia. Indeed, the downregulation of AIF protein levels in RNA interference-treated cultured neurons or in harlequin (Hq) mutant mice resulted in a significant reduction of neuronal cell death in experimental models of ischemia [257]. Therefore, caspase-independent cell death signaling may provide a promising novel target for therapeutic interventions in cerebral ischemia. However, the mechanism(s) of AIF-mediated cell death after cerebral ischemia remains ill-defined. Seong-woon Yu et al. [258] reported that activation of PARP-1 is the key step for AIF-mediated caspase-independent apoptosis, as pharmacological inhibition or genetic knockout of PARP-1 prevents the nuclear translocation of AIF. Consistent with the role of PARP-1-mediated cell death in I/R [259, 260], it appears that PARP-1/AIF pathway might play an important

role in mediating cell death after I/R. Further investigations required essentially to elucidate the precise mechanism(s) of AIF-mediated cell death in cerebral ischemia.

Mitogen-Activated Protein Kinases and Apoptosis

The mitogen-activated protein kinases (MAPKs) phosphorylate specific serine and threonine residues of target protein substrates and regulate a variety of cellular activities ranging from gene expression, mitosis, movement, metabolism, and programmed death [261]. Mammalian cells encode three major subfamilies of MAP kinases that include ERK, JNK or stress-activated protein kinases (SAPK), and p38 MAP kinases [174]. Upon stimulation, these three kinases relay signaling pathways that lead to various cellular responses (Fig. 5).

ERK-Mediated Apoptosis

The ERK regulate cellular responses to a variety of extracellular stimuli. The activation of ERK requires cell surface receptors such as tyrosine kinases (Trks) and G protein-coupled receptors that relay the activating signals to the Raf–MEK–ERK cascade through different small GTP-binding proteins such as Ras. Activated Raf (a MAPK kinase kinase) binds and phosphorylates MEK1/MEK2 (MAPK kinases), which, in turn, phosphorylates ERK1/2. Then ERK1/2 phosphorylates various substrates, including membrane proteins, nuclear proteins, transcription factors, and several MAPK-activated protein kinases. In the nervous system, ERK1/2 is critical for neuronal differentiation and plasticity, and modulates neuronal survival [262]. However, the recent evidence suggests that the activation of ERK1/2 also contributes to the cell death in animal models of ischemia-induced brain injury. For instance, increased levels of phosphorylated ERK are detected in the nuclei of cortical cells, and pretreatment with MEK1 inhibitor PD98059 blocks ERK phosphorylation and significantly reduces infarct volume after MCAO [263]. These results that are also confirmed by using other MEK1 inhibitors such as U0126 and SL327 suggest that ERK1/2 might associate with brain injury [264]. Furthermore, a recent

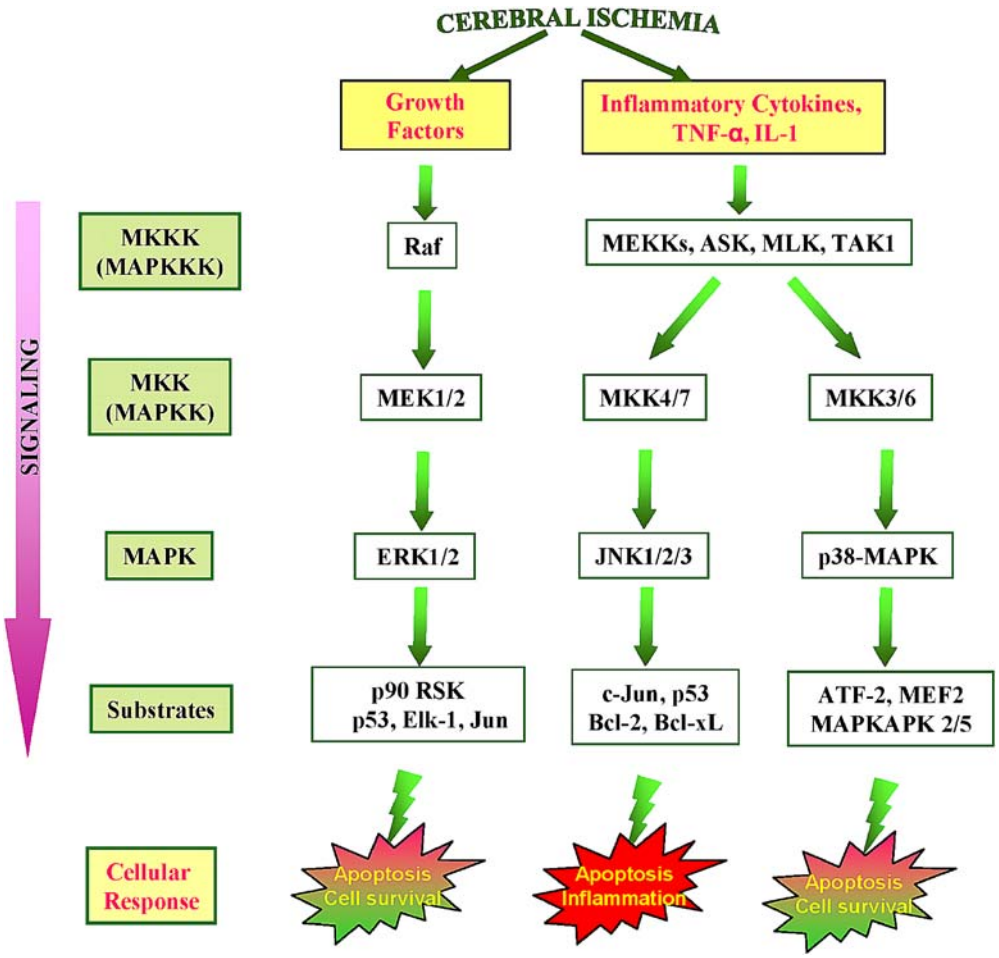

Fig. 5 The flow chart shows three conventional MAPKs (ERKs, JNKs, and p38) in relay of signaling pathways upon stimuli, the three tier regulatory cascade within each module (MAPKKK, MAPKK and MAPK levels) and the various cellular responses elicited by MAPK control (see text)

study suggests that ERK1/2 may play a key role in neuronal apoptosis induced by K^+ withdrawal [265]. In contrast, increased phospho-ERK1/2 staining was also detected in neurons that survived after ischemic brain injury, suggesting that ERK1/2 activation may also be involved in the cell survival [266, 267]. ERK1/2 appears to mediate antiapoptotic effects by direct action on its target kinase, p90Rsk2. The role of ERK1/2 in neuronal survival has been presented in an excellent review [268].

The ERK-induced apoptosis may be regulated via both extrinsic and intrinsic pathways. Recent studies showed that cerebral ischemia in mice induces ERK activation and IL-1 β expression, and inhibition of ERK activation by U0126 prevented increase in IL-1β mRNA [264]. These studies implicate ERK-mediated expression of death ligands and proinflammatory cytokines as an important mechanism in exacerbating of brain tissue injury. Consequently, ERK may act on mitochondria through Bax and/or p53. For instance, ERK may regulate apoptosis via direct phosphorylation of p53 at serine residue 15, and inactivation of ERK results in p53 dephosphorylation and inhibit apoptosis. Similarly, inhibition of ERK pathway causes decrease in Bax expression, suggesting that Bax and p53 may be important components in the ERK-mediated apoptotic signaling pathways [264]. Furthermore, overexpression of SOD1 resulted in the prominent reduction of phopho-ERK1/2 and apoptotic-related DNA fragmentation after transient MCAO [269]. Nevertheless, study of the mechanism of ERK-mediated apoptotic signaling and clarification of the precise understanding of ERK regulation in cerebral ischemia will help address the possibility of therapeutic strategies.

c-Jun N-terminal Kinase-Mediated Apoptosis

Emerging evidence suggests that activation of JNK, a member of the MAPK group signaling, may play an important role in ischemia-induced neuronal apoptosis [270]. After stress-induced activation, JNK allows phosphorylation of several transcription factors, particularly the c-Jun component of the transcription factor complex AP1, thus, triggering the expression of a number of apoptosis-regulatory proteins. In addition, JNK also directly enhances the proapoptotic activity of p53 but reverses the antiapoptotic function of Bcl-2 and Bcl-xL [271].The kinase family of JNK1 (SAPK-γ), JNK2 (SAPK-α), and JNK3 (SAPK-β) is encoded by three genes, and alternative splicing produces additional isoforms. JNK1 and JNK2 are expressed in all tissues and are required for normal brain development. Furthermore, JNK1 displays constitutive activity in the CNS and is required to maintain microtubule integrity in axons and dendrites in the adult animal [272]. In contrast, JNK3 is specifically implicated in promoting neuronal cell death, in that targeted deletion of JNK3 protects mice from brain injury after cerebral ischemia–hypoxia [273]. The downstream mechanism of JNK3-mediated apoptosis may include the induction of Bim and Fas and the mitochondrial release of cytochrome c [273]. Systemic administration of SP600125, a small molecule JNK-specific inhibitor, resluts in diminished JNK activity and reduced infarct volume after ischemia in a dose-dependent manner. Moreover, inhibition of JNK prevents mitochondrial translocation of Bax and Bim, release of cytochrome c and Smac, and activation of caspase-9 and caspase-3 [271], whereas activation of JNK causes serine phosphorylation of 14–3–3, thus, leading to disassociation of Bax from 14–3–3 and subsequent translocation to mitochondria [271].Thus, there is a growing evidence to substantiate the role of JNK as a critical cell death mediator in ischemic brain injury, possibly by executing one of the mechanisms that leads to cell death.

p38 Pathway

The p38 pathway is strongly activated by factors such as TNF-α and IL-1 β, which are known to be increased after stroke and have been shown to be involved in the mechanisms underlying ischemia-induced cell death. The p38 pathway plays an important role in transducing signals involved in cell survival, apoptosis, and inflammatory cytokine production. Sustained activation of p38 is shown to be associated with neuronal death/apoptosis [271]. However, selective p38 inhibitors promote survival. The p38 inhibition by SB203580 abolishes nitric oxide-induced cell death, cytochrome c release, and activation of caspase-3, indicating that p38 activation serves as an upstream mediator in the cell death process. Moreover, suppression of p38 activation by Bcl-2 suggests that death mediated by p38 might be involved in apoptosis [274]. It seems that the dynamic balance between growth factor-activated ERK and stress-activated JNK-p38 pathways may play an important role in determining the fate of a cell. As evident, withdrawal of nerve growth factor causes neuronal apoptosis that may be preceded by decreased ERK and increased p38/JNK activities [275].

Inhibitor of Apoptosis Family of Proteins

The most well-recognized property of highly conserved IAP gene family is their ability to prevent apoptosis. It was first identified in insect cells infected by the baculovirus [276]. So far, in humans, the members of the IAP family include cIAP1, cIAP2, XIAP, NAIP (neuronal apoptosis inhibitory protein), survivin, and livin. Among them, XIAP appears to be the most potent direct caspase inhibitor both in vitro and in vivo [277]. All members of the family contain N-terminal baculovirus IAP repeat (BIR) domains,

and one conservative C-terminal RING domain (RING, really interesting novel gene). The BIR domains are zinc finger-like structures that can chelate zinc ions. These zinc fingers can bind to the surface of caspases so that the amino acid sequences or linkers between BIR domains can block the catalyzing grooves of caspases. As a result, IAP can protect a cell from apoptosis by inhibiting the activity of caspases. However, not all BIR-containing proteins are inhibitors of apoptosis. For instance, survivin, which contains only one BIR domain, may act as a regulator of mitosis rather than apoptosis. On the other hand, RING domain catalyzes the connection ubiquitin ligase with the RING domain or with other proteins. Thus, RING domain may possibly facilitate the degradation of caspases that bind to IAP [278, 279].

The expression and functions of IAPs is yet to be studied completely in the nervous system. However, existing data indicates that IAP family proteins can regulate cell demise in various neuropathological situations including I/R. For instance, adenovirus-mediated overexpression of XIAP inhibits cell death in the substantia nigra and cerebellar granules [280, 281]. The number of XIAP-positive cells significantly increased in the hippocampal CA1 region at 6, 12, and 24 h I/R, whereas slight increase in protein levels of XIAP observed at 12 and 24 h I/R [282].

However, the activity of IAP in mammals can be inhibited by Smac/Diablo, Omi/HtrA2, and GSPT1/eRF3. These proteins normally localized to the mitochondria and releases into the cytosol when apoptotic events ensue [277, 283]. Smac/Diablo inactivates XIAP via binding to its caspase-9 binding site [284]. Saito et al. [285] have reported that the XIAP pathway get activated upstream of the caspase cascade and the interaction of XIAP with Smac/Diablo and the caspases seems to be crucial in the regulation of neuronal cell death after cerebral ischemia. The XIAP level increased concurrently with the release of Smac/Diablo and activated caspase-9. Moreover, the binding of XIAP to Smac/Diablo and caspase-9 concurrent to the binding of Smac/Diablo to caspase-9. Furthermore, they have also reported that XIAP and Smac/Diablo signaling pathway is associated with oxidative stress [285].

A novel factor, X-linked mammalian inhibitor of apoptosis protein-associated factor 1 (XAF1), was found to antagonize endogenous XIAP by direct interaction. It expresses at low levels in neurons under normal conditions. Increased expression of XAF-1 triggers redistribution of XIAP from cytosol to the nucleus after focal cerebral ischemia [286]. It is suggested that the XIAP and XAF1 colocalizes to the nucleus in the penumbra at the early stage of ischemic cascade during neonatal hypoxia–ischemia [287]. The ratio between the endogenous levels of XIAP and XAF1 seems to be crucial in deciding the fate of the cell after ischemic insult [287].

Omi/HtrA2 is a recently described member of a novel family of serine proteases homologous to the *Escherichia coli* chaperone HtrA [288, 289]. In vitro studies demonstrated that Omi/HtrA2 cleaves various IAPs independent of caspases and the cleavage efficiency determined by its IAP-binding motif, AVPS. As a result IAPs lose their ubiquitin ligase activity on caspase substrates [290]. A recent study demonstrated that Omi/HtrA2 translocates from mitochondrial to the cytosolic compartment at early reperfusion after cerebral ischemia. Inhibition of caspases does not affect this translocation, whereas overexpression of SOD1 prevents it [291]. Moreover, treatment with ucf-101, a specific Omi/HtrA2 inhibitor, showed significant neuroprotection by reducing the number of TUNEL-positive cells and by attenuating the breakdown of XIAP [292].Thus, the IAP family proteins and their antagonists are promising potential targets to develop novel therapeutics for cerebral ischemia.

Heat Shock Proteins

Heat shock proteins (HSPs) including HSP70 are induced in the brain by a variety of pathological stimuli including ischemia [293–295]. Focal cerebral ischemia studies suggest that induction of HSP70 represents an endogenous protective mechanism that occurs in penumbral neurons but not in the core [296]. Selective overexpression of HSP70 protects the brain tissue against cerebral infarction in a mouse model of permanent focal ischemia [297]. Furthermore, magnetic resonance imaging (MRI) studies suggested that overexpression of HSP70 reduces overall lesion size and may limit the tissue damage within the lesion [298]. The protective mechanism of HSP70 has largely been believed to be related to its chaperone activity. However, recent studies have shown that its protective effect may also be because of its ability to inhibit apoptotic mechanisms. As evident, mice overexpressing HSP70 have fewer apoptotic cells and less DNA fragmentation after permanent focal cerebral ischemia. This was also associated with the reduction of early cytochrome c release from the mitochondria [299]. Conversely, mice lacking HSP70.1 gene also have increased infarction and apoptotic cell death after transient focal ischemia, which was also associated with the increased release of cytochrome c and subsequent activation of caspase-3 [300]. Furthermore, overexpression of HSP70 sequesters AIF and protects the neonatal brain subjected to hypoxia–ischemic brain injury [301]. Yet, other studies have also suggested that HSP70 modulates JNK-mediated apoptotic signaling via inhibiting JNK activity and dephosphorylation [302, 303]. Therefore, these studies strongly suggest the potential role of HSP70 in the regulation of apoptotic pathways in cerebral ischemia. However, understanding the detailed molecular mecha-

nisms underlying this protection might help to establish possible therapeutic strategies for stroke.

Autophagy

Autophagy is the major intracellular lysosome-mediated catabolic mechanism that is responsible for the bulk degradation and recycling of damaged or dysfunctional cytoplasmic components including subcellular organelles [304]. Autophagosomes are double-membraned cytoplasmic vesicles that are designed to engulf various cellular constituents, including cytoplasmic organelles. Autophagosomes fuse to lysosomes to become autolysosomes, where hydrolase enzymes digest sequestered cellular components. Autophagosomes and autolysosomes are formed during a process called macroautophagy (usually referred as 'autophagy'). It can be recognized at ultrastructural level by the appearance of intracellular double membrane vacuoles containing fragments of ER, mitochondria, and lysosomal hydrolases [305, 306].

Autophagy has been suggested both to protect cells from death by apoptosis and to act as a death-promoting mechanism (Fig. 6). Two major functions have been proposed for this process. First, autophagy is a short-term stress response under nutrient-starved conditions or amino-acid deficiency. By the lysosomal degradation of own cytoplasmic components, cells get substrates for both energy metabolism and vital protein synthesis; thus, it might be beneficial for cell survival. Next, it is suggested to play a role in type II or autophagic cell death. There are certain genes responsible for the formation of autophagosomes, e.g., Atg5 as covalent conjugate with Atg12 and beclin1. Surprisingly, beclin1 is deleted in many human tumors, and overexpression of this protein not only induces autophagy but also inhibits tumorigenecity. LC3, a mammalian homologue of ATg8, gets lipidated during autophagy, and this lipidated form serves as a good marker protein for autophagic vacuoles. The other autophagic proteins that include Atg7 and Atg3 assists for the LC3 lipidation and conjugation between Atg5 and Atg12 as well [307].

It has been demonstrated recently that treatment of SK-N-SH neuroblastoma cells with ER stressors markedly induced the formation of autophagosomes seen at the ultrastructural level. As an evident, LC3 labeled with green fluorescent protein was extensively induced in cells exposed to ER stress with conversion from LC3-I to LC3-II. Interestingly, the ER stress-induced autophagy is inhibited in the cells deficient of IRE1 or cells treated with JNK inhibitor, indicating that the IRE1–JNK pathway is required for autophagy activation after ER stress. In contrast, PERK-deficient and ATF6 knockdown cells exhibit autophagy after ER stress, similar to the wild-type cells. Disturbance of autophagy rendered cells vulnerable to ER stress, suggesting that autophagy plays important roles in cell survival after ER stress [308]. Although, the recent studies indicate the existence of autophagy in cerebral ischemia, the precise function of autophagy in this complex scenario, however, remains unclear. It has been recently proposed that oxidative and ER stresses in cerebral ischemia–hypoxia are potent stimuli of autophagy in neurons. They have also suggested that autophagosomes

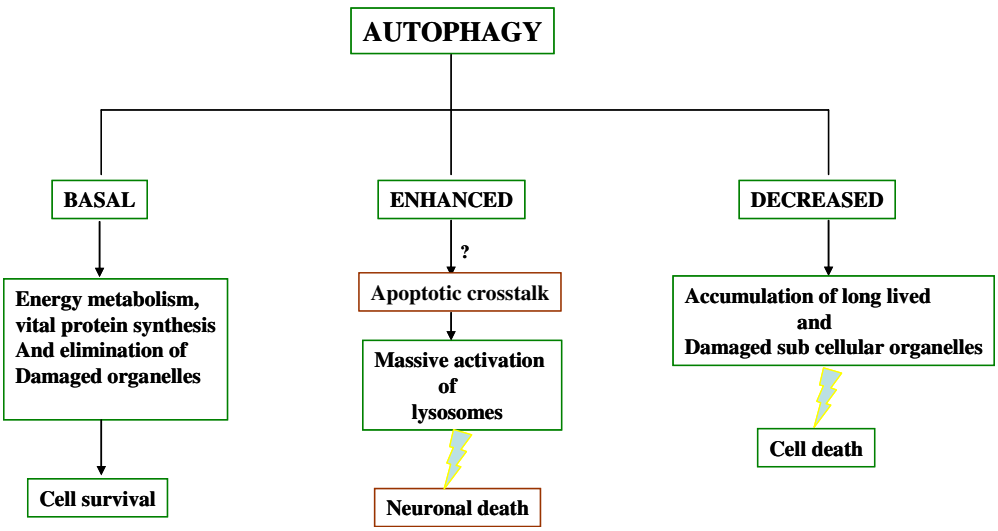

Fig. 6 A representation of how autophagy leads to cell survival/death of particular organism. Under starvation, basal autophagy serves as cell protective mechanism by degrading the cells' own cytosolic components as substrates for energy metabolism, etc. Decreased autophagy leads to cell loss by allowing damaged subcellular organelles and long lived proteins to accumulate and by failing to provide energy for cellular metabolism. Enhanced autophagy, however, leads to autophagic cell death, possibly, by crosstalk with cell-demising mechanisms such as apoptosis and necrosis

may have a shorter half-life in neurons and that a fraction of LC3 protein is degraded within autolysosomes, leading to a smaller detectable amount of LC3-II in the brain, while there are clear indications of on-going autophagy. Thus, they suggested finally that autophagy is an important modifier of cell death and survival, interacting with necrosis and apoptosis in determining the outcome and final morphology of deceased neurons. Furthermore, autophagy is evidenced by the increased levels of LC3-II in the adult brain after hypoxia–ischemia [128]. Thus, extensive investigations on the role of autophagy in regulating neuronal cell survival/death might help to develop new therapeutic strategies for cerebral ischemia [309].

Crosstalk of Autophagy and Apoptosis

The interplay between autophagy and apoptotic pathways is emerging as a crucial decision-making process in determining the initiation of programmed cell death. For instance, coupling of Atg5 to IFN-gamma leads to activation of autophagic death and activates extrinsic apoptotic pathways via the interaction with FADD and, possibly, caspase 8. Conversely, antiapoptotic signaling pathways suppress autophagy, e.g., the class I PI3K/Akt/TOR signaling pathway. Interestingly, Atg5 was first described as apoptosis-specific protein and found to be up-regulated in neurons during apoptosis activated by proteasomal inhibition. It seems that the Bcl-2-interacting domain of Beclin1 that serves as a point of autophagic and apoptotic pathways crosstalk during induction of autophagy. In addition, there are also few reports suggesting that pro-apoptotic members of Bcl-2 family members like Bax, Bid, and Bnip3 also constitute a point of crosstalk between the apoptotic and autophagic pathways [310]. Recent reports suggest that Bcl-2 inhibits autophagy through a direct interaction with the Beclin 1 autophagy protein and that the interaction between Bcl-2 and Beclin 1 may function as a rheostat that maintains autophagy at levels that are compatible with cell survival rather than cell death. In contrast, overexpression of Bcl-2 or Bcl-xL potentiates autophagy and autophagy gene-dependent death in MEFs with etoposide-induced apoptosis. However, the basis for the opposite effects of Bcl-2 family members on autophagy in different settings remains unclear. For instance, it is not clear whether Bcl-2 functions at the mitochondrial level to regulate autophagy, as autophagy inhibited by Bcl-2 is targeted to the ER but not to the mitochondria [311]. Although the mechanisms of autophagy and its crosstalk with apoptosis in cerebral ischemia awaits extensive investigation, consistent with the role of Bcl-2 family proteins in cerebral ischemia, perhaps, autophagy may play an important role in cell survival/death after ischemia.

Therapeutic Strategies

Thrombolytic Agents

Although tissue plasminogen activator (tPA) is the only approved therapy for acute stroke patients requiring thrombolysis, side effects such as hemorrhagic transformation, neurotoxic effects, and the narrow therapeutic time window counteract the positive effects of tPA in stroke patients [312]. The hemorrhagic transformation after tPA therapy in ischemic stroke may be mediated through the activation of MMP and disruption of the microvascular structure, as patients with elevated plasma MMP-9 levels are more likely to undergo hemorrhagic transformation after tPA [313]. Indeed, mice lacking tPA showed reduced MMP-9 levels in ischemic brain [314]. Interestingly, co-administration of tPA and MMP inhibitors reduces tPA-mediated mortality after focal cerebral ischemia in rats [315]. Thus, it appears that the combination therapy with tPA and MMP inhibitors may be a versatile therapeutic strategy, which may avoid tPA-mediated side effects. Furthermore, effective thrombolytic agent such as desmoteplase that lacks neurotoxic effects has become one of the most promising therapeutic approaches for stroke [316]. The desmoteplase in acute ischemic stroke study (DIAS) showed very encouraging results such as a low rate of symptomatic intracranial hemorrhage and post-ischemic outcome of stroke patients with a therapeutic time window of 3–9 h [317]. The beneficial effects of desmoteplase are further evaluated by the dose escalation of desmoteplase in acute stroke (DEDAS) study. DEDAS also confirmed that treatment with desmoteplase 3 to 9 h after ischemic stroke is safe and improves clinical outcome, especially in the patients fulfilling all MRI criteria [318]. However, most recently, Paion, in Aachen, Germany, announced the failure of phase III trials of desmoteplase, further setting back the ischemic stroke field. Microplasmin is another novel thrombolytic agent that may be useful in the treatment of ischemic stroke. Intravenous administration of microplasmin has shown to reduce ischemic brain damage and improvement in neurological function after thrombotic MCAO in rats [319]. Moreover, microplasmin has some advantages over other thrombolytic agents. As microplasmin attacks fibrin directly, whereas both tPA and desmoteplase act on fibrin through plasminogen. Furthermore, microplasmin showed significantly lower rate of hemorrhage as compared to tPA [320]. It is currently undergoing phase II trials.

Antioxidants

Edaravone (formerly known as MCI-186) has potent-free radical scavenging and antioxidant properties [321]. Re-

cently, a phase III, randomized, placebo-controlled, double-blind study at multicenters with edaravone demonstrated a significant improvement in neurological deficits in stroke patients, and edaravone has been used in patients with acute brain infarction since 2001 in Japan [322]. Edaravone has shown several protective effects against I/R-induced injury, including vascular endothelial cell injury, brain edema, DND, ER stress, infarct, and neurological deficits [323–328]. Moreover, a combination of edaravone with fibrinolytic agents and antithrombotics may offer future advantages by scavenging the free radicals associated with reperfusion injury. In addition, a free-radical-trapping agent NXY-059 (Cerovive) has shown to be neuroprotective in animal models of stroke. Administration of NXY-059 within 6 h after the onset of acute ischemic stroke significantly improved the primary outcome but did not significantly improve other outcome measures, including neurologic functioning as measured by the National Institutes of Health Stroke Scale (NIHSS) score [329]. However, NXY-059 disappointed, as it failed to replicate the positive results seen in the first phase III trials [330]. Hence, further research is required to assess the beneficial effects of NXY-059.

Anti-inflammatory Agents

Existing preclinical data suggest that inhibition of inflammatory mediators, iNOS and COX-2, is protective after cerebral ischemia. Pharmacological inhibition of iNOS reduces infarct volume by about 30% [331]. Whereas, mice lacking iNOS have smaller infarcts and better neurologic outcomes than the wild type [332]. Moreover, protection of post-ischemic brain by estrogen and progesterone appears through modulating iNOS expression [333, 334]. Recent reports suggest that prostaglandin E2EP1 receptors may be the downstream effectors responsible for neurotoxicity of COX-2 in ischemic stroke, and inhibitor of EP1 receptor has shown significant protection when administered 6 h after MCAO [335]. Fucoidin, an inhibitor of both P- and L-selectin, significantly reduced infarct size and improved neurological function in experimental stroke and reperfusion in rats [336]. Overexpression or treatment with IL-1 receptor antagonist (IL-1ra), an endogenous inhibitor of IL-1 reduces the infarct size [337, 338], whereas mice deficient of IL-1ra exhibited a significant increase in ischemic damage [339]. Inhibition of ICAM-1 expression by antisense oligonucleotides reduced infarct volume after MCAO in rats [340]. Similarly, inhibition of TNF-α expression by antisense oligo-deoxynucleotides is also neuroprotective after intracerebral hemorrhage [341]. Peroxisome proliferator-activated receptor (PPAR) belongs to the nuclear receptor superfamily and function as transcription factors in many important biological processes. Recently, PPAR gamma agonists have been shown to prevent inflammation and neuronal death after stroke and spinal cord injury [342–344]. In addition, agonists of PPAR-alpha and PPAR-delta have also been reported to be protective after cerebral ischemia [345–346]. These studies are important as PPAR-gamma agonist-induced neuroprotection may be mediated by the upregulation of glutamate transporters [347]. Interestingly, 3-aminobenzamide, a PARP inhibitor has shown to reduce the expression of MMP-9 and NF-κB, and neutrophil infiltration after cerebral ischemia in vivo, suggesting a novel role for PARP in inflammatory response [348].

Antiapoptotic Therapy

Inhibition of caspases is a noticeable target for the prevention of apoptosis in cerebral ischemia. Broad spectrum caspase inhibitors, like zVADfmk, BAF, or zDEVDfmk, have been shown to be neuroprotective in animal models of focal, global cerebral ischmia and neonatal hypoxic ischemic brain injury [349–352]. However, a later study suggests that these caspase inhibitors are more efficient after focal but not global cerebral ischemia [353]. A cocktail of caspase inhibitors YVADcmk, DEVDfmk, and IETD fmk has reduced the number of apoptotic cells after MCAO in rats [164]. Furthermore, a combination of isofurane and a specific caspase-8 inhibitor, z-IETD-fmk, causes sustained neuroprotection after focal cerebral ischemia in rats [354]. Thus, a combination therapy of caspase inhibitors along with other drugs that target different aspects of pathophysiology of cerebral ischemia would be a better approach than the administration of caspase inhibitors alone.

Fusion protein therapy, based on the use of small peptide sequences with neuroprotective properties fused with the protein transduction domain (PTD) from human immunodeficiency virus (HIV) transactivator of TAT [355–357], has also shown antiapoptotic potential. It is a non-invasive approach for in vivo studies and has several advantages that avoids the problems inherent in the use of viral vectors able to cross lipid membranes and can be administered systematically [190, 358, 359]. A number of fusion proteins with neuroprotective properties have been raised to date, including members of the Bcl-2 family and endogenous inhibitors of caspases. For instance, intraperitonial (i.p.) administration of a fusion protein containing the anti-apoptotic molecule Bcl-xL resulted in significant reduction of cerebral infarction after focal cerebral ischemia in mice [360]. Furthermore, topical application of fusion protein PTD–XIAP protected against cerebral ischemia by inhibiting apoptotic mechanisms and secondary regulation of transcription factors involved in neuronal survival [361]. Moreover, intraperitonial administration of PTD–BIR3–

RING fusion protein causes attenuation of cell death in vitro and decrease in apoptosis after cerebral ischemia in rat [362]. Thus, these findings encourage the development of a "fusion protein therapy" approach with anti-apoptotic molecules to reduce ischemic brain damage. Additionally, liposome-mediated site-specific delivery of a plasmid encoding human Bcl-2 protein have also shown significant reduction in the number of apoptotic cells in the infarct and penumbra area after MCAO in rats [363]. This represents a novel site-specific therapeutic approach for the treatment of stroke.

KR-31378, a novel benzopyran analog, and cilostazol, a type III phosphodiesterase inhibitor, have been shown to alter the expression levels of Bcl-2 and Bax after cerebral ischemia in rats [364–366]. Anilinoquinazoline (AQZ), a potent small molecule inhibitors of caspases-3, inhibits apoptosis induced by nerve growth factor withdrawal from differentiated PC12 cells [367]. Therefore, AQZs represent novel drug candidates to explore their antiapoptotic properties in animal models of cerebral ischemia. Minocycline, a tetracycline derivative with anti-inflammatory effect, has a property to inhibit caspase-1 and caspase-3, thus, preventing neuronal loss in vivo after global brain ischemia. In addition, minocycline offers neuroprotection by preventing p38 MAPK also [368]. PAN-811 is a small liphophilic compound (of 195 Da) belonging to the (*N*)-heterocyclic carboxaldehyde thiosemicarbazone (HCT) class of molecules. Intracerebral administration of PAN-811 post-ischemia significantly reduces infarct volume and inhibits necrotic and apoptotic cell death by reducing intracellular free Ca^{2+}, as well as scavenging free radicals. Besides, it also inhibits the downregulation of Bcl-2 and Bcl-xL but fail to suppress the Bax signal [369].

Albumin

Albumin is another promising neuroprotective molecule under clinical investigation. It represents 55–62% of plasma proteins, and albumin solutions are currently used as plasma expander in the treatment of edema. Administration of albumin even 4 h after the onset of ischemia significantly improves neurological function and reduces brain edema and infarction [370]. Cerebroprotection by albumin is mediated by various mechanisms including enhanced microvascular perfusion and antioxidant properties. Moreover, albumin therapy reverses stagnation, thrombosis, and leukocyte adhesion within cortical venules in the reperfusion phase after focal ischemia further supports its utility in the treatment of acute ischemic stroke [371]. The albumin in ischemic stroke (ALIAS) dose escalation study showed that high-dose albumin up to 2.05 g/kg is well tolerated by patients with acute ischemic stroke without dose-limiting complications [372]. Furthermore, the neuroprotective effects of high-dose albumin in stroke patients is currently addressed in the multicenter ALIAS phase III study [373]. Disturbances of lung function are the major side effect of albumin treatment observed in clinical trials. However, use of combination therapy with low-dose albumin solutions and docosanoid derivates successfully reduces such side effects, and these protective effects are currently investigated in stroke patients [374]. Docosanoids are derivatives of the docosahexaenoic acid, which have been identified as major products of the oxidative membrane lipid degradation after cerebral ischemia [375]. The new docosahexaenoic acid derivate, 10,17S-docosatriene, inhibits infiltration of leukocytes followed by downregulation of NF-κB and COX-2 after cerebral ischemia [376].

Stem Cells and Neurogenesis

Stem cell implantation nowadays is one of the promising approaches for the treatment of ischemic stroke. Neuronal cells derived from a human teratocarcinoma cell line have promoted functional recovery when transplanted into the ischemic brain of laboratory animals. Furthermore, a small-scale open label clinical trial of neuroteratocarcinoma (NT_2N) transplantation in 12 patients with ischemic stroke showed that cell transplantation for stroke is generally safe and potentially feasible [377]. Because of the concerns that this line could evolve into tumors, therefore, focus has been made upon human neural stem cells. As a result, recently, some investigators generated clonal cell lines by genetic modification of somatic stem cells, and this cell line is promising to functional recovery from stroke [378]. On the other hand, focal cerebral ischemia in rats increases neurogenesis in the subventricular zone (SVZ), and newly generated neurons migrate toward ischemic boundary regions [379]. The concept of mobilization of endogenous stem cells has created a new hope for stroke patients. Recently, granulocyte colony-stimulating factor (G-CSF), a well-known stem cell mobilizing agent has gained much attention among stem cell researchers. Administration of G-CSF is neuroprotective, as it reduces infarct size after focal cerebral ischemia in rats [380]. The G-CSF has a dual activity beneficial for both counteracting the acute neuronal degeneration and to long-term plasticity after cerebral ischemia. It exhibits a strong apoptotic activity in mature neurons probably through its receptors, as the expression of these receptors are strongly up-regulated during cerebral ischemia. In addition, G-CSF stimulation of neural progenitor cells markedly improve functional recovery in models of cerebral ischemia and implicates neurogenesis as a mechanism underlying G-CSF's therapeutic benefits [381]. Likewise, treatment with erythropoietin (EPO) offers neuroprotection after cerebral ischemia, whose beneficial effects are largely attributed to decrease in neuronal apoptosis

[382]. Moreover, treatment of stroke with human recombinant EPO (rhEPO) increases synthesis of vascular endothelial growth factor and brain-derived neurotrophic factor that may be involved in angiogenesis and neurogenesis. Indeed, incubation of neurospheres derived from stroke SVZ with rhEPO showed enhanced neurogenesis [383]. Similar neuroprotective effects were also achieved with the new carbamylated derivative of EPO (CEPO) [383, 384]. Notably, in contrast to EPO, the CEPO do not affect hematopoiesis as EPO-induced hematopoiesis possibly enhances the risk of thrombosis [385]. Therefore, reduction of brain injury together with the enhancement of neurogenesis appears to be a better therapeutic strategy for cerebral ischemia. Furthermore, sildenafil, a phosphodiesterase type 5 (PDE5) inhibitor, augments neurogenesis and promotes functional recovery after MCAO in rats. Recently, it is reported that neurogenesis in the SVZ of aged rats decreases as compared with young rats and that delayed administration of sildenafil 7 days after MCAO enhances neurogenesis in ischemic brain and improve functional recovery in aged rats [386, 387].

Future Perspective

The recent investigations on the vital role of key apoptotic molecules in the modulation of apoptosis have inspired an increased interest among researchers. In recent years, a tremendous effort has been made to elucidate the role of these molecules in ischemia, paving ways for developing new therapeutic strategies. Many such agents targeting caspases and Bcl-2 family members have been evaluated for their neuroprotective efficacy in animal models of cerebral ischemia, but in spite their proven efficacy in animal models, several of these do not completely reiterate the efficacy and specificity in clinical settings. Mitochondria and ER regulate intrinsic apoptotic pathways by activating several pro/anti-apoptotic molecules. Furthermore, understanding the mechanisms of newly emerging pro/anti-apoptotic molecules such as Smac/Diablo, Omi/HtrA2, XAF1, AIF, etc. may be beneficial for therapeutic purposes. Moreover, apoptotic cross-talk between the ER and the mitochondria also suggests that therapeutic strategies should optimally be directed at multiple targets/mechanisms because inhibition of one such apoptotic pathway (either mitochondria- or ER-mediated) may augment an alternative one. Multiple cell death pathways may have common upstream initiators, whose identification might help in the development of new strategies for stroke therapy. For example, BAP31 mediates ER–mitochondria cross-talk via death-receptor-mediated pathway to induce apoptosis. Although, this aspect needs to be explored in cerebral ischemia, it might become a promising target. On the other hand, cytochrome c also has been implicated in ER–mitochondrial cross-talk. Furthermore, understanding the unresolved issues of alternate pathways like autophagy in cell death/survival regulation may suggest some new direction. Therefore, it is indispensable that optimal neuroprotective approaches should include either combination treatment strategies directed toward multiple cell death pathways or the use of a single compound that may affect more than one cell death mechanism. Thus, comprehensive knowledge regarding multiple cell death mechanisms involving key apoptotic regulatory molecules may provide insight for the development of better therapeutic strategies aiming at cerebral ischemia.

Acknowledgment Mr. Venkata Prasuja Nakka, Ms. Anchal Gusain, and Mr. Suresh L Mehta received Senior Research Fellowship from the Council of Scientific and Industrial Research, New Delhi, India.

References

1. Eng HL, Dalkara T, Moskowitz MA (2003) Mechanisms, challenges and opportunities in stroke. Nat Neurosci 4:399–415
2. Smith WS (2004) Pathophysiology of focal cerebral ischemia: a therapeutic perspective. J Vasc Interv Radiol 15:S3–S12
3. Traystman RJ (2003) Animal models of focal and global cerebral ischemia. ILAR 44:85–95
4. Kirino T (1982) Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res 230:57–69
5. Pulsinelli WA, Brierley JB, Plum F (1982) Temporal profile of neuronal damage in a model of transient forebrain ischemia. Ann Neurol 11:491–498
6. Bonnekoh P, Barbier A, Oschlies U, Hossmann KA (1990) Selective vulnerability in the gerbil hippocampus: morphological changes after 5-min ischemia and long survival times. Acta Neuropathol (Berl) 80:18–25
7. Petito CK, Feldmann E, Pulsinelli WA, Plum F (1987) Delayed hippocampal damage in humans following cardiorespiratory arrest. Neurology 37:1281–1286
8. Horn M, Schlote W (1992) Delayed neuronal death and delayed neuronal recovery in the human brain following global ischemia. Acta Neuropathol (Berl) 85:79–87
9. Kirino T (2000) Delayed neuronal death. Neuropathology 20: S95–S97
10. Lipton P (1999) Ischemic cell death in brain neurons. Physiol Rev 79:1431–1568
11. Astrup J, Siesjö BK, Symon L (1981) Thresholds in cerebral ischemia—the ischemic penumbra. Stroke 12:723–725
12. Ginsberg MD (2003) Adventures in the pathophysiology of brain ischemia: penumbra, gene expression, neuroprotection: the 2002 Thomas Willis lecture. Stroke 34:214–223
13. Ferrer I (2006) Apoptosis: future targets for neuroprotective strategies. Cerebrovasc Dis 2:9–20
14. Linnik MD, Zobrist RH, ad Hatfield MD (1993) Evidence supporting a role for programmed cell death in focal cerebral ischemia in rats. Stroke 24:2002–2009
15. Charriaut-Marlangue C, Margaill I, Represa A, Popovici T, Plotkine M, Ben Ari Y (1996) Apoptosis and necrosis after reversible focal ischemia: an in situ DNA fragmentation analysis. J Cereb Blood Flow Metab 16:186–194

16. Li Y, Chopp M, Jiang N, Zaloga C (1995) In situ detection of DNA fragmentation after focal cerebral ischemia in mice. Brain Res Mol Brain Res 28:164–168
17. Ferrer I, Planas AM (2003) Signaling of cell death and cell survival following focal cerebral ischemia: life and death struggle in the penumbra. J Neuropathol Exp Neurol 62:329–339
18. Ferrer I, Friguls B, Dalfó E, Justicia C, Planas AM (2003) Caspase-dependent and caspase-independent signalling of apoptosis in the penumbra following middle cerebral artery occlusion in the adult rat. Neuropathol Appl Neurobiol 29:472–481
19. Benchoua A, Guegan C, Couriaud C, Hosseini H, Sampaio N, Morin D, Onteniente B (2001) Specific caspase pathways are activated in the two stages of cerebral infarction. J Neurosci 21:7127–7134
20. Nicotera P, Leist M, Ferrando-May E (1998) Intracellular ATP, a switch in the decision between apoptosis and necrosis. Toxicol Lett 102–103:139–142
21. Schwab BL, Guerini D, Didszun C, Bano D, Ferrando-May E, Fava E, Tam J, Xu D, Xanthoudakis S, Nicholson DW, Carafoli E, Nicotera P (2002) Cleavage of plasma membrane calcium pumps by caspases: a link between apoptosis and necrosis. Cell Death Differ 9:818–831
22. Bano D, Young KW, Guerin CJ, Lefeuvre R, Rothwell NJ, Naldini L, Rizzuto R, Carafoli E, Nicotera P (2005) Cleavage of the plasma membrane Na^{+2}/Ca^{2+} exchanger in excitotoxicity. Cell 120:275–285
23. Pulsinelli WA, Buchan AM (1988) The four-vessel occlusion rat model: method for complete occlusion of vertebral arteries and control of collateral circulation. Stroke 19:913–914
24. Eklof B, Siesjo BK (1972a) The effect of bilateral carotid artery ligation upon acid-base parameters and substrate levels in the rat brain. Acta Physiol Scand 86:528–538
25. Eklof B, Siesjo BK (1972b) The effect of bilateral carotid artery ligation upon the blood flow and the energy state of the rat brain. Acta Physiol Scand 86:155–165
26. Tamura A, Graham DI, McCulloch J, Teasdale GM (1981) Focal cerebral ischaemia in the rat: 1. Description of technique and early neuropathological consequences following middle cerebral artery occlusion. J Cereb Blood Flow Metab 1:53–60
27. Bederson JB, Pitts LH, Tsuji M, Nishimura MC, Davis RL, Bartkowski H (1986) Rat middle cerebral artery occlusion: evaluation of the model and development of a neurologic examination. Stroke 17:472–476
28. Longa EZ, Weinstein PR, Carlson S, Cummins R (1989) Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 20:84–91
29. Mehta SL, Manhas N, Raghubir R (2007) Molecular targets in cerebral ischemia for developing novel therapeutics. Brains Res Rev 54:34–66
30. Moroa MA, Almeida A, Bolanos JP, Lizasoain I (2005) Mitochondrial respiratory chain and free radical generation in stroke. Free Radic Biol Med 39:1291–1304
31. Eng HL, Dalkara T, Moskowitz MA (2003) Mechanisms, challenges and opportunities in stroke. Nat Neurosci 4:399–415
32. Beal MF (1992) Mechanisms of excitotoxicity in neurologic diseases. FASEB J 6:3338–3344
33. Zhang YM, Bhavnani BR (2006) Glutamate-induced apoptosis in neuronal cells is mediated via caspase-dependent and independent mechanisms involving calpain and caspase-3 proteases as well as apoptosis inducing factor (AIF) and this process is inhibited by equine estrogens. BMC Neurosci 7:49
34. Xiong ZG, Chu XP, Simon RP (2006) Ca^{2+}-permeable acid-sensing ion channels and ischemic brain injury. J Membr Biol 209:59–68
35. Xiong ZG, Zhu XM, Chu XP, Minami M, Hey J, Wei WL, MacDonald JF, Wemmie JA, Price MP, Welsh MJ, Simon RP (2004) Neuroprotection in ischemia: blocking calcium permeable acid-sensing ion channels. Cell 118:687–698
36. Gao J, Duan B, Wang DG, Deng XH, Zhang GY, Xu L, Xu TL (2005) Coupling between NMDA receptor and acid-sensing ion channel contributes to ischemic neuronal death. Neuron 48:635–646
37. Pignataro G, Simon RP, Xiong ZG (2007) Prolonged activation of ASIC1a and the time window for neuroprotection in cerebral ischaemia. Brain 130:151–158
38. Nita DA, Nita V, Spulber S, Moldovan M, Popa DP, Zagrean AM, Zagrean L (2001) Oxidative damage following cerebral ischemia depends on reperfusion- a biochemical study in rat. J Cell Mol Med 5:163–170
39. Lewen A, Matz P, Chan PH (2000) Free radical pathways in CNS injury. J Neurotrauma 17:871–890
40. Chan PH (2001) Reactive oxygen radicals in signaling and damage in the ischemic brain. J Cereb Blood Flow Metab 21:2–14
41. Halliwell B, Gulteridge JMC (eds) (1989) Free radicals in biology and medicine, 2nd edn. Oxford, Clarendon
42. Liu PK (2003) Ischemia–reperfusion-related repair deficit after oxidative stress: implications of faulty transcripts in neuronal sensitivity after brain injury. J Biomed Sci 10:4–13
43. Warner DS, Sheng H, Batinic-Haberle I (2004) Oxidants, antioxidants and the ischemic brain. J Exp Biol 207:3221–3231
44. Love S (1999) Oxidative stress in brain ischemia. Brain Pathol 9:119–131
45. Chong ZZ, Li F, Maiese K (2005) Oxidative stress in the brain: novel cellular targets that govern survival during neurodegenerative disease. Prog Neurobiol 75:207–246
46. Yamamoto T, Maruyama W, Kato Y, Yi H, Shamoto-Nagai M, Tanaka M, Sato Y, Naoi M (2002) Selective nitration of mitochondrial complex I by peroxynitrite: involvement in mitochondria dysfunction and cell death of dopaminergic SH-SY5Y cells. J Neural Transm 109:1–13
47. Kim GW, Kondo T, Noshita N, Chan PH (2002) Manganese superoxide dismutase deficiency exacerbates cerebral infarction after focal cerebral ischemia/reperfusion in mice: implications for the production and role of superoxide radicals. Stroke 33:809–815
48. Murakami K, Kondo T, Kawase M, Li Y, Sato S, Chen SF, Chan PH (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
49. Hayashi T, Saito A, Okuno S, Ferrand-Drake M, Doddand RL, Chan PK (2005) Damage to the endoplasmic reticulum and activation of apoptotic machinery by oxidative stress in ischemic neurons. J Cereb Blood Flow Metab 25:41–53
50. Chan PH (2001) Reactive oxygen radicals in signaling and damage in the ischemic brain. J Cereb Blood Flow Metab 21:2–14
51. Crack PJ, Taylor JM (2005) Reactive oxygen species and the modulation of stroke. Free Radic Biol Med 38:1433–1444
52. McCulloch J, Dewar D (2001) A radical approach to stroke therapy. Proc Natl Acad Sci USA 98:10989–10999
53. Huang J, Upadhyay UM, Tamargo RJ (2006) Inflammation in stroke and focal cerebral ischemia. Surgical Neurol 66:232–245
54. Fassbender K, Mossner R, Motsch L, Kischka U, Grau A, Hennerici M (1995) Circulating selectin- and immunoglobulin-type adhesion molecules in acute ischemic stroke. Stroke 26:1361–1364
55. Huang J, Kim LJ, Mealey R, Marsh HC Jr, Zhang Y, Tenner AJ, Connolly ES Jr, Pinsky DJ (1999) Neuronal protection in stroke by an sLex-glycosylated complement inhibitory protein. Science 285:595–599
56. Okada Y, Copeland BR, Mori E, Tung MM, Thomas WS, del Zoppo GJ (1994) P-selectin and intercellular adhesion molecule-1 expression after focal brain ischemia and reperfusion. Stroke 25:202–211

57. Huang J, Choudhri TF, Winfree CJ, McTaggart RA, Kiss S, Mocco J, Kim LJ, Protopsaltis TS, Zhang Y, Pinsky DJ, Connolly ES Jr (2000) Postischemic cerebrovascular E-selectin expression mediates tissue injury in murine stroke. Stroke 31:3047–3053
58. Mayadas TN, Johnson RC, Rayburn H, Hynes RO, Wagner DD (1993) Leukocyte rolling and extravasation are severely compromised in P selectin-deficient mice. Cell 74:541–554
59. Mocco J, Choudri T, Huang J, Harfeldt E, Efros L, Klingbeil C, Vexler W, Hall W, Zhang Y, Mack W, Popilskis S, Pinsky DJ, Connolly ES (2002) HuEP5C7 as a humanized monoclonal anti-E/P Selectin neurovascular protective strategy in a blinded placebo-controlled trial of nonhuman primate stroke. Circ Res 91:907–914
60. Morikawa E, Zhang SM, Seko Y, Toyoda T, Kirino T (1996) Treatment of focal cerebral ischemia with synthetic oligopeptide corresponding to lectin domain of selectin. Stroke 27:951–955
61. Zhang RL, Chopp M, Zaloga C, Zhang ZG, Jiang N, Gautam SC, Tang WX, Tsang W, Anderson DC, Manning AM (1995a) The temporal profiles of ICAM-1 protein and mRNA expression after transient MCA occlusion in the rat. Brain Res 682:182–188
62. Lindsberg PJ, Carpen O, Paetau A, Karjalainen-Lindsberg ML, Kaste M (1996) Endothelial ICAM-1 expression associated with inflammatory cell response in human ischemic stroke. Circulation 94:939–945
63. Fiszer U, Korczak-Kowalska G, Palasik W, Korlak J, Gorski A, Czlonkowska A (1998) Increased expression of adhesion molecule CD18 (LFA-1beta) on the leukocytes of peripheral blood in patients with acute ischemic stroke. Acta Neurol Scand 97:221–224
64. del Zoppo GJ, Schmid-Schonbein GW, Mori E, Copeland BR, Chang CM (1991) Polymorphonuclear leukocytes occlude capillaries following middle cerebral artery occlusion and reperfusion in baboons. Stroke 22:1276–1283
65. Soriano SG, Coxon A, Wang YF, Frosch MP, Lipton SA, Hickey PR, Mayadas TN (1999) Mice deficient in Mac-1 (CD11b/CD18) are less susceptible to cerebral ischemia/ reperfusion injury. Stroke 30:134–139
66. Mori E, Del Zoppo GJ, Chambers JD, Copeland BR, Arfors KE (1992) Inhibition of polymorphonuclear leukocyte adherence suppresses no-reflow after focal cerebral ischemia in baboons. Stroke 23:712–718
67. Chen H, Chopp M, Zhang RL, Bodzin G, Chen Q, Rusche JR, Todd RF (1994) Anti-CD11b monoclonal antibody reduces ischemic cell damage after transient focal cerebral ischemia in rat. Ann Neurol 35:458–463
68. Chopp M, Zhang RL, Chen H, Li Y, Jiang N, Rusche JR (1994) Postischemic administration of an anti-Mac-1 antibody reduces ischemic cell damage after transient middle cerebral artery occlusion in rats. Stroke 25:869–875
69. Zhang RL, Chopp M, Jiang N, Tang WX, Prostak J, Manning AM, Anderson DC (1995b) Anti-intercellular adhesion molecule-1 antibody reduces ischemic cell damage after transient but not permanent middle cerebral artery occlusion in the Wistar rat. Stroke 26:1438–1442
70. Hallenbeck JM, Dutka AJ (1990) Background review and current concepts of reperfusion injury. Arch Neurol 47:1245–1254
71. Shyu KG, Chang H, Lin CC (1997) Serum levels of intercellular adhesion molecule-1 and E-selectin in patients with acute ischaemic stroke. J Neurol 244:90–93
72. Stenberg PE, Shuman MA, Levine SP, Bainton DF (1984) Redistribution of alpha-granules and their contents in thrombin-stimulated platelets. J Cell Biol 98:748–760
73. Soriano SG, Lipton SA, Wang YF, Xiao M, Springer TA, Gutierrez-Ramos JC, Hickey PR (1996) Intercellular adhesion molecule-1-deficient mice are less susceptible to cerebral ischemia–reperfusion injury. Ann Neurol 39:618–624
74. Connolly ES Jr, Winfree CJ, Springer TA, Naka Y, Liao H, Yan SD, Stern DM, Solomon RA, Gutierrez-Ramos JC, Pinsky DJ (1996) Cerebral protection in homozygous null ICAM-1 mice after middle cerebral artery occlusion. Role of neutrophil adhesion in the pathogenesis of stroke. J Clin Invest 97:209–216
75. Chopp M, Li Y, Jiang N, Zhang RL, Prostak J (1996) Antibodies against adhesion molecules reduce apoptosis after transient middle cerebral artery occlusion in rat brain. J Cereb Blood Flow Metab 16:578–584
76. Vemuganti R, Dempsey RJ, Bowen KK (2004) Inhibition of intercellular adhesion molecule-1 protein expression by antisense oligonucleotides is neuroprotective after transient middle cerebral artery occlusion in rat. Stroke 35:179–184
77. Antezana DF, Clatterbuck RE, Alkayed NJ, Murphy SJ, Anderson LG, Frazier J, Hurn PD, Traystman RJ, Tamargo RJ (2003) High-dose ibuprofen for reduction of striatal infarcts during middle cerebral artery occlusion in rats. J Neurosurg 98:860–866
78. Becker KJ (2001) Targeting the central nervous system inflammatory response in ischemic stroke. Curr Opin Neurol 14:349–353
79. Del Zoppo G, Ginis I, Hallenbeck JM, Iadecola C, Wang X, Feuerstein GZ (2000) Inflammation and stroke: putative role for cytokines, adhesion molecules and iNOS in brain response to ischemia. Brain Pathol 10:95–112
80. Liu T, McDonnell PC, Young PR, White RF, Siren AL, Hallenbeck JM, Barone FC, Feurestein GZ (1993) Interleukin-1 beta mRNA expression in ischemic rat cortex. Stroke 24:1746–1750
81. Buttini M, Sauter A, Boddeke HW (1994) Induction of interleukin-1 beta mRNA after focal cerebral ischaemia in the rat. Brain Res Mol Brain Res 23:126–134
82. Zhang Z, Chopp M, Goussev A, Powers C (1998) Cerebral vessels express interleukin 1beta after focal cerebral ischemia. Brain Res 784:210–217
83. Weller A, Isenmann S, Vestweber D (1992) Cloning of the mouse endothelial selectins. Expression of both E- and P-selectin is inducible by tumor necrosis factor alpha. J Biol Chem 267:15176–15183
84. Yoshimoto T, Houkin K, Tada M, Abe H (1997) Induction of cytokines, chemokines and adhesion molecule mRNA in a rat forebrain reperfusion model. Acta Neuropathol (Berl) 93:154–158
85. Zhang RL, Chopp M, Jiang N, Tang WX, Prostak J, Manning AM, Anderson DC (1995) Anti-intercellular adhesion molecule-1 antibody reduces ischemic cell damage after transient but not permanent middle cerebral artery occlusion in the Wistar rat. Stroke. 26:1438–1442
86. Garcia JH, Liu KF, Relton JK (1995b) Interleukin-1 receptor antagonist decreases the number of necrotic neurons in rats with middle cerebral artery occlusion. Am J Pathol 147:1477–1486
87. Relton JK, Martin D, Thompson RC, Russell DA (1996) Peripheral administration of interleukin-1 receptor antagonist inhibits brain damage after focal cerebral ischemia in the rat. Exp Neurol 138:206–213
88. Liu T, Clark RK, McDonnell PC, Young PR, White RF, Barone FC, Feuerstein GZ (1994) Tumor necrosis factor-alpha expression in ischemic neurons. Stroke 25:1481–1488
89. Zaremba J, Skrobanski P, Losy J (2001) Tumour necrosis factor-alpha is increased in the cerebrospinal fluid and serum of ischaemic stroke patients and correlates with the volume of evolving brain infarct. Biomed Pharmacother 55:258–263
90. Nawashiro H, Martin D, Hallenbeck JM (1997) Neuroprotective effects of TNF binding protein in focal cerebral ischemia. Brain Res 778:265–271

91. Latour LL, Kang DW, Ezzeddine MA, Chalela JA, Warach S (2004) Early blood brain barrier disruption in human focal brain ischemia. Ann Neurol 56:468–477
92. Horstmann S, Kalb P, Koziol J, Gardner H, Wagner S (2003) Profiles of matrix metalloproteinases, their inhibitors, and laminin in stroke patients: influence of different therapies. Stroke 34:2165–2170
93. Gidday JM, Gasche YG, Copin JC, Shah AR, Perez RS, Shapiro SD, Chan PH, Park TS (2005) Leukocyte-derived matrix metalloproteinase-9 mediates blood-brain barrier breakdown and is proinflammatory after transient focal cerebral ischemia. Am J Physiol Heart Circ Physiol 289:H558–H568
94. Wang Q, Tang XN, Yenari MA (2007) The inflammatory response in stroke. J Neuroimmunol 184:53–68
95. Zhang W, Wang X, Narayanan M, Zhang Y, Huo C, Reed JC, Friedlander RM (2003) Fundamental role of the Rip2/caspase-1 pathway in hypoxia and ischemia-induced neuronal cell death. Proc Natl Acad Sci U S A 100:16012–16017
96. Benchoua A, Braudeau J, Reis A, Couriaud C, Onteniente B (2004) Activation of proinflammatory caspases by cathepsin B in focal cerebral ischemia. J Cereb Blood Flow Metab 24:1272–1279
97. Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239–257
98. Sugawara T, Fujimura M, Noshita N, Kim GW, Saito A, Hayashi T, Narasimhan P, Maier CM, Chan PH (2004) Neuronal death/survival signaling pathways in cerebral ischemia. NeuroRx 1: 17–25
99. Li Y, Chopp M, Jiang N, Zhang ZG, Zaloga C (1995) Induction of DNA fragmentation after 10 to 120 minutes of focal cerebral ischemia in rats. Stroke 26:1252–1257
100. Charriaut-Marlangue C, Margaill I, Represa A, Popovici T, Plotkine M, Ben-Ari Y (1996) Apoptosis and necrosis after reversible focal ischemia: an in situ DNA fragmentation analysis. J Cereb Blood Flow Metab 16:186–194
101. Fujimura M, Morita-Fujimura Y, Murakami K, Kawase M, Chan PH (1998) Cytosolic redistribution of cytochrome c after transient focal cerebral ischemia in rats. J Cereb Blood Flow Metab 18:1239–1247
102. Li Y, Chopp M, Powers C, Jiang N (1997) Apoptosis and protein expression after focal cerebral ischemia in rat. Brain Res 765: 301–312
103. Li Y, Powers C, Jiang N, Chopp M (1998) Intact, injured, necrotic and apoptotic cells after focal cerebral ischemia in the rat. J Neurol Sci 156:119–132
104. Bredesen DE, Rao RV, Mehlen P (2006) Cell death in the nervous system. Nature 443:796–802
105. Degterev A, Huang Z, Boyce M, Jagtap P, Mizushima N, Cuny GD, Mitchinson TJ, Moskowitz MA, Yuan J (2005) Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. Nat Chem Biol 1:112–119
106. Sastry PS, Rao KS (2000) Apoptosis and the nervous system. J. Neurochem 74:1–20
107. Nitatori T, Sato N, Waguri S, Karasawa Y, Araki H, Shibanai K, Kominami E, Uchiyama Y (1995) Delayed neuronal death in the CA1 pyramidal cell layer of the gerbil hippocampus following transient ischemia is apoptosis. J Neurosci 15:1001–1011
108. Chen J, Nagayama T, Jin K, Stetler RA, Zhu RL, Graham SH, Simon RP (1998) Induction of caspase-3-like protease may mediate delayed neuronal death in the hippocampus after transient cerebral ischemia. J Neurosci 18:4914–4928
109. Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, Yuan J (1996) Human ICE/CED-3 protease nomenclature. Cell 87:171
110. Shi Y (2002) Mechanisms of caspase activation and inhibition during apoptosis. Mol Cell 9:459–470
111. Fischer U, Janicke RU, Schulze-Osthoff K (2003) Many cuts to ruin: a comprehensive update of caspase substrates. Cell Death Differ 10:76–100
112. Kuida K, Zheng TS, Na S, Kuan CY, Yang D, Karasuyama H, Rakic P, Flavell RA (1996) Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. Nature 384:368–372
113. Kuida K, Haydar TF, Kuan CY, Gu Y, Taya C, Karasuyama H, Su MSS, Rakic P, Flavell RA (1998) Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. Cell 94:325–337
114. Cecconi F, Alvarez-Bolado G, Meyer BI, Roth KA, Gruss P (1998) Apaf1 (CED-4 homolog) regulates programmed cell death in mammalian development. Cell 94:727–737
115. Mattson MP (2007) Mitochondrial regulation of neuronal plasticity. Neurochem Res 32:707–715
116. Kang SJ, Wang S, Hara H, Peterson EP, Namura S, Amin-Hanjani S, Huang Z, Srinivasan A, Tomaselli KJ, Thornberry NA, Moskowitz MA, 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):51
117. Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, Yuan J (2000) Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. Nature 403: 98–103
118. Namura S, Zhu J, Fink K, Endres M, Srinivasan A, Tomaselli KJ, Yuan J, Moskowitz MA (1998) Activation and cleavage of caspase-3 in apoptosis induced by experimental cerebral ischemia. J Neurosci 18:3659–3668
119. Hermann DM, Kilic E, Hata R, Hossmann KA, Mies G (2001) Relationship between metabolic dysfunctions, gene responses and delayed cell death after mild focal cerebral ischemia in mice. Neuroscience 104:947–955
120. Niwa M, Hara, A, Iwai T, Wang S, Hotta K, Mori H, Uematsu T (2001) Caspase activation as an apoptotic evidence in the gerbil hippocampal CA1 pyramidal cells following transient forebrain ischemia. Neurosci Lett 300:103–106
121. Ni B, Wu X, Su Y, Stephenson D, Smalstig EB, Clemens J, Paul SM (1998) Transient global forebrain ischemia induces a prolonged expression of the caspase-3 mRNA in rat hippocampal CA1 pyramidal neurons. J Cereb Blood Flow Metab 18:248–256
122. Davoli MA, Fourtounis J, Tam J, Xanthoudakis S, Nicholson D, Robertson GS, Ng GY, Xu D (2002) Immunohistochemical and biochemical assessment of caspase-3 activation and DNA fragmentation following transient focal ischemia in the rat. Neuroscience 115:125–136
123. Ouyang YB, Tan Y, Comb M, Liu CL, Martone ME, Siesjo BK, Hu BR (1999) Survival and death-promoting events after transient cerebral ischemia: phosphorylation of Akt, release of cytochrome C, and activation of caspase-like proteases. J Cereb Blood Flow Metab 19:1126–1135
124. Gillardon F, Kiprianova I, Sandkuhler J, Hossmann KA, Spranger M (1999a) Inhibition of caspases prevents cell death of hippocampal CA1 neurons, but not impairment of hippocampal long-term potentiation following global ischemia. Neuroscience 93:1219–1222
125. Gillardon F, Bottiger B, Schmitz B, Zimmermann M, Hossmann KA (1997) Activation of CPP-32 protease in hippocampal neurons following ischemia and epilepsy. Brain Res Mol Brain Res 50:16–22
126. Love S, Barber R, Srinivasan A, Wilcock GK (2000a) Activation of caspase-3 in permanent and transient brain ischaemia in man. Neuro Report 11:2495–2499
127. Love S, Barber R, Wilcock GK (2000b) Neuronal death in brain infarcts in man. Neuropathol Appl Neurobiol 26:2055–2066
128. Zhu C, Wang X, Xu F, Bahr BA, Shibata M, Uchiyama Y, Hagberg H, Blomgren K (2005) The influence of age on

apoptotic and other mechanisms of cell death after cerebral hypoxia–ischemia. Cell Death Diff 12:162–176
129. Ota K, Yakovlev AG, Itaya A, Kameoka M, Tanaka Y, Yoshihara K (2002) Alteration of apoptotic protease-activating factor-1 (APAF-1)-dependent apoptotic pathway during development of rat brain and liver. J Biochem (Tokyo) 131:131–135
130. Yakovlev AG, Ota K, Wang G, Movsesyan V, Bao WL, Yoshihara K, Faden AI (2001) Differential expression of apoptotic protease-activating factor-1 and caspase-3 genes and susceptibility to apoptosis during brain development and after traumatic brain injury. J Neurosci 21:7439–7446
131. Vekrellis K, McCarthy MJ, Watson A, Whitfield J, Rubin LL, Ham J (1997) Bax promotes neuronal cell death and is down-regulated during the development of the nervous system. Development 124:1239–1249
132. Gill R, Soriano M, Blomgren K, Hagberg H, Wybrecht R, Miss MT, Hoefer S, Adam G, Niederhauser O, Kemp JA, Loetscher H (2002) Role of caspase-3 activation in cerebral ischemia-induced neurodegeneration in adult and neonatal brain. J Cereb Blood Flow Metab 22:420–430
133. Yuan J, Yankner BA (2000) Apoptosis in the nervous system. Nature 407:802–809
134. Merry DE, Korsmeyer SJ (1997) Bcl-2 gene family in the nervous system. Annu Rev Neurosci 20:245–267
135. Michaelidis TM, Sendtner M, Cooper JD, Airaksinen MS, Holtmann B, Meyer M, Thoenen H (1996) Inactivation of bcl-2 results in progressive degeneration of motoneurons, sympathetic and sensory neurons during early postnatal development. Neuron 17:75–89
136. Merry DE, Veis DJ, Hickey WF, Korsmeyer SJ (1994) bcl-2 protein expression is wide spread in the developing nervous system and retained in the adult PNS. Development 120:301–311
137. González-García M, García I, Ding L, O'Shea S, Boise LH, Thompson CB, Núñez G (1995) Bcl-x is expressed in embryonic and postnatal neural tissues and functions to prevent neuronal cell death. Proc Natl Acad Sci U S A 92:4304–4308
138. Motoyama N, Wang F, Roth KA, Sawa H, Nakayama K, Nakayama K, Negishi I, Senju S, Zhang Q, Fujii S et al (1995) Massive cell death of immature hematopoietic cells and neurons in Bcl-x-deficient mice. Science 267:1506–1510
139. Middleton G, Wyatt S, Ninkina N, Davies AM (2001) Reciprocal developmental changes in the roles of Bcl-w and Bcl-x(L) in regulating sensory neuron survival. Development 128:447–457
140. Oltvai ZN, Milliman CL, Korsmeyer SJ (1993) Bcl-2 heterodimerizes in vivowith a conserved homolog, Bax, that accelerates programmed cell death. Cell 74:609–619
141. Sedlak TW, Oltvai ZN, Yang E, Wang K, Boise LH, Thompson CB, Korsmeyer SJ (1995) Multiple Bcl-2 family members demonstrate selective dimerizations with Bax. Proc Natl Acad Sci U S A 92:7834–7838
142. Deckwerth TL, Elliott JL, Knudson CM, Johnson EM Jr, Snider WD, Korsmeyer SJ (1996) BAX is required for neuronal death after trophic factor deprivation and during development. Neuron 17:401–411
143. Yuan J, Yankner BA (2000) Apoptosis in the nervous system. Nature 407:802–809
144. Shindler KS, Latham CB, Roth KA (1997) Bax deficiency prevents the increased cell death of immature neurons in bcl-x-deficient mice. J Neurosci 17:3112–3119
145. O'Reilly LA, Print C, Hausmann G, Moriishi K, Cory S, Huang DC, Strasser A (2001) Tissue expression and subcellular localization of the pro-survival molecule Bcl-w. Cell Death Differ 8:486–494
146. Guegan C, Ceballos-Picot I, Nicole A, Kato H, Onteniente SB (1998) Recruitment of several neuroprotective pathways after permanent focal ischemia in mice. Exp Neurol 154:371–380
147. Minami M, Jin KL, Li W, Nagayama T, Henshall DC, Simon RP (2000) Bcl-w expression is increased in brain regions affected by focal cerebral ischemia in the rat. Neurosci Lett 279:193–195
148. Yan C, Chen J, Chen D, Minami M, Pei W, Yin XM, Simon RP (2000) Overexpression of the cell death suppressor Bcl-w in ischemic brain: implications for a neuroprotective role via the mitochondrial pathway. J Cereb Blood Flow Metab 20:620–630
149. Ferrer I, Lopez E, Blanco R, Rivera R, Ballabriga J, Pozas E, Marti E (1998) Bcl-2, Bax, and Bcl-x expression in the CA1 area of the hippocampus following transient forebrain ischemia in the adult gerbil. Exp Brain Res 121:167–173
150. Okuno S, Saito A, Hayashi T, Chan PH (2004) The c-Jun N-terminal protein kinase signaling pathway mediates Bax activation and subsequent neuronal apoptosis through interaction with Bim after transient focal cerebral ischemia. J Neurosci 24:7879–7887
151. Schmidt-Kastner R, Aguirre-Chen C, Kietzmann T, Saul I, Busto R, Ginsberg MD (2004) Nuclear localization of the hypoxia-regulated pro-apoptotic protein BNIP3 after global brain ischemia in the rat hippocampus. Brain Res 1001:133–142
152. Reimertz C, Kögel D, Rami A, Chittenden T, Prehn JH (2003) Gene expression during ER stress-induced apoptosis in neurons: induction of the BH3-only protein Bbc3/PUMA and activation of the mitochondrial apoptosis pathway. J Cell Biol 162:587–597
153. Gibson ME, Han BH, Choi J, Knudson CM, Korsmeyer SJ, Parsadanian M, Holtzman DM (2001) BAX contributes to apoptotic-like death following neonatal hypoxia–ischemia: evidence for distinct apoptosis pathways. Mol Med 7:644–655
154. Kermer P, Digicaylioglu MH, Kaul M, Zapata JM, Krajewska M, Stenner-Liewen F, Takayama S, Krajewski S, Lipton SA, Reed JC (2003) BAG1 over-expression in brain protects against stroke. Brain Pathol 13:495–506
155. Conradt B, Horvitz HR (1998) The C. elegans protein EGL-1 is required for programmed cell death and interacts with the Bcl-2-like protein CED-9. Cell 93:519–529
156. Algeciras-Schimnich A, Shen L, Barnhart BC, Murmann AE, Burkhardt JK, Peter ME (2002) Molecular ordering of the initial signaling events of CD95. Mol Cell Biol 22:207–220
157. Ashkenazi A, Dixit VM (1998) Death receptors: signaling and modulation. Science 281:1305–1308
158. Sasaki C, Kitagawa H, Zhang WR, Warita H, Sakai K, Abe K (2000) Temporal profile of cytochrome c and caspase-3 immunoreactivities and TUNEL staining after permanent middle cerebral artery occlusion in rats. Neurol Res 22:223–228
159. Velier JJ, Ellison JA, Kikly KK, Spera PA, Barone FC, Feuerstein GZ (1999) Caspase-8 and caspase-3 are expressed by different populations of cortical neurons undergoing delayed cell death after focal stroke in the rat. J Neurosci 19:5932–5941
160. Rupalla K, Allegrini PR, Sauer D, Wiessner C (1998) Time course of microglia activation and apoptosis in various brain regions after permanent focal cerebral ischemia in mice. Acta Neuropathol (Berl) 96:172–178
161. Botchkina GI, Geimonen E, Bilof ML, Villarreal O, Tracey KJ (1999) Loss of NF-kappaB activity during cerebral ischemia and TNF cytotoxicity. Mol Med 5:372–381
162. Sairanen T, Carpen O, Karjalainen-Lindsberg ML, Paetau A, Turpeinen U, Kaste M, Lindsberg PJ (2001) Evolution of cerebral tumor necrosis factor alpha production during human ischemic stroke. Stroke 32:1750–1758
163. Gross A, Yin XM, Wang K, Wei MC, Jockel J, Milliman C, Erdjument-Bromage H, Tempst P, Korsmeyer SJ (1999) Caspase cleaved BID targets mitochondria and is required for cytochrome c release, while BCL-XL prevents this release but not tumor necrosis factor-R1/Fas death. J Biol Chem 274:1156–1163
164. Krupinski J, Lopez E, Marti E, Ferrer I (2000) Expression of caspases and their substrates in the rat model of focal cerebral ischemia. Neurobiol Dis 7:332–342

165. Li H, Zhu H, Xu CJ, Yuan J (1998) Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell 94:491–501
166. Luo X, Budihardjo I, Zou H, Slaughter C, Wang X (1998) Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell 94:481–490
167. Leist M, Single B, Castoldi AF, Kuhnle S, Nicotera P (1997) Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. J Exp Med 185:1481–1486
168. Schinzel AC, Takeuchi O, Huang Z, Fisher JK, Zhou Z, Rubens J, Hetz C, Danial NN, Moskowitz MA, Korsmeyer SJ (2005) Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia. Proc Natl Acad Sci U S A 102:12005–12010
169. Cain K, Brown DG, Langlais C, Cohen GM (1999) Caspase activation involves the formation of the aposome, a large (approximately 700 kDa) caspase activating complex. J Biol Chem 274:22686–22692
170. Kim HE, Du F, Fang M, Wang X (2005) Formation of apoptosome is initiated by cytochrome c-induced dATP hydrolysis and subsequent nucleotide exchange on Apaf-1. Proc Natl Acad Sci U S A 102:17545–17550
171. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X (1997) Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91:479–489
172. Fujimura M, Morita-Fujimura Y, Murakami K, Kawase M, Chan PH (1998) Cytosolic redistribution of cytochrome c after transient focal cerebral ischemia in rats. J Cereb Blood Flow Metab 18:1239–1247
173. Perez-Pinzon MA, Xu GP, Born J, Lorenzo J, Busto R, Rosenthal M, Sick TJ (1999) Cytochrome C is released from mitochondria into the cytosol after cerebral anoxia or ischemia. J Cereb Blood Flow Metab 19:39–43
174. Cao G, Minami M, Pei W, Yan C, Chen D, O'Horo C, Graham SH, Chen J (2001) Intracellular Bax translocation after transient cerebral ischemia: implications for a role of the mitochondrial apoptotic signaling pathway in ischemic neuronal death. J Cereb Blood Flow Metab 21:321–333
175. Sugawara T, Fujimura M, Morita-Fujimura Y, Kawase M, Chan PH (1999) Mitochondrial release of cytochrome c corresponds to the selective vulnerability of hippocampal CA1 neurons in rats after transient forebrain ischemia. J Neurosci 19:RC39
176. Krajewski S, Krajewska M, Ellerby LM, Welsh K, Xie Z, Deveraux QL, Salvesen GS, Bredesen DE, Rosenthal RE, Fiskum G, Reed JC (1999) Release of caspase-9 from mitochondria during neuronal apoptosis and cerebral ischemia. Proc Natl Acad Sci U S A 96:5752–575
177. Cao G, Luo Y, Nagayama T, Pei W, Stetler RA, Graham SH, Chen J (2002) Cloning and characterization of rat caspase-9: implications for a role in mediating caspase-3 activation and hippocampal cell death after transient cerebral ischemia. J Cereb Blood Flow Metab 22:534–546
178. Mouw G, Zechel JL, Zhou Y, Lust WD, Selman WR, Ratcheson RA (2002) Caspase-9 inhibition after focal cerebral ischemia improves outcome following reversible focal ischemia. Metab Brain Dis 17:143–151
179. MacManus JP, Hill IE, Preston E, Rasquinha I, Walker T, Buchan AM (1995) Differences in DNA fragmentation following transient cerebral or decapitation ischemia in rats. J Cereb Blood Flow Metab 15:728–737
180. Chen J, Jin K, Chen M, Pei W, Kawaguchi K, Greenberg DA, Simon RP (1997) Early detection of DNA strand breaks in the brain after transient focal ischemia: implications for the role of DNA damage in apoptosis and neuronal cell death. J Neurochem 69:232–245
181. Kihara S, Shiraishi T, Nakagawa S, Toda K, Tabuchi K (1994) Visualization of DNA double strand breaks in the gerbil hippocampal CA1 following transient ischemia. Neurosci Lett 175:133–136
182. Graham SH, Chen J (2001) Programmed cell death in cerebral ischemia. J Cereb Blood Flow Metab 21:99–109
183. Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S (1998) A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. Nature 391:43–50
184. Luo Y, Cao G, Pei W, O, Horo C, Graham SH, Chen J (2002) Induction of caspase-activated deoxyribonuclease activity after focal cerebral ischemia and reperfusion. J Cereb Blood Flow Metab 22:15–20
185. Sugawara T, Noshita N, Lewe'n A, Gasche Y, Ferrand-Drake M, Fujimura M, Morita-Fujimura Y, Chan PH (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
186. Krajewski S, Mai JK, Krajewska M, Sikorska M, Mossakowski MJ, Reed JC (1995) Upregulation of bax protein levels in neurons following cerebral ischemia. J Neurosci 15:6364–6376
187. Putcha GV, Deshmukh M, Johnson EM Jr (1999) BAX translocation is a critical event in neuronal apoptosis: regulation by neuroprotectants, BCL-2, and caspases. J Neurosci 19: 7476–7485
188. Sulejczak D, Czarkowska-Bauch J, Macias M, Skup M (2004) Bcl-2 and Bax proteins are increased in neocortical but not in thalamic apoptosis following devascularizing lesion of the cerebral cortex in the rat: an immunohistochemical study. Brain Res 1006:133–149
189. Linnik MD, Zahos P, Geschwind MD, Federoff HJ (1995) Expression of bcl-2 from a defective herpes simplex virus-1 vector limits neuronal death in focal cerebral ischemia. Stroke 26:1670–1674
190. Asoh S, Ohsawa I, Mori T, Katsura K, Hiraide T, Katayama Y, Kimura M, Ozaki D, Yamagata K, Ohta S (2002) Protection against ischemic brain injury by protein therapeutics. Proc Natl Acad Sci U S A 99:17107–17112
191. Zhao H, Yenari MA, Cheng D, Sapolsky RM, Steinberg GK (2003) Bcl-2 overexpression protects against neuron loss within the ischemic margin following experimental stroke and inhibits cytochrome c translocation and caspase-3 activity. J Neurochem 85:1026–1036
192. Poppe M, Reimertz C, Düssmann H, Krohn AJ, Luetjens CM, Böckelmann D, Nieminen AL, Kögel D, Prehn JH (2001) Dissipation of potassium and proton gradients inhibits mitochondrial hyperpolarization and cytochrome c release during neural apoptosis. J Neurosci 21:4551–4563
193. Zha J, Harada H, Yang E, Jockel J, Korsmeyer SJ (1996) Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14–3–3, not Bcl-xL. Cell 87:619–628
194. Datta SR, Katsov A, Hu L, Petros A, Fesik SW, Yaffe MB, Greenberg ME (2000) 14–3–3 proteins and survival kinases cooperate to inactivate BAD by BH3 domain phosphorylation. Mol Cell 6:41–51
195. Zhang L, Chen J, Fu H (1999) Suppression of apoptosis signal-regulating kinase 1-induced cell death by 14–3–3 proteins. Proc Natl Acad Sci U S A 96:8511–8515
196. Hsu YT, Youle RJ (1997) Nonionic detergents induce dimerization among members of the Bcl-2 family. J Biol Chem 272:13829–13834
197. Saito A, Hayashi T, Okuno S, Ferrand-Drake M, Chan PH (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
198. Zhu Y, Yang GY, Ahlemeyer B, Pang L, Che XM, Culmsee C, Klumpp S, Krieglstein J (2002) Transforming growth factor-beta 1 increases bad phosphorylation and protects neurons against damage. J Neurosci 22:3898–3909
199. Chan PH (2005) Mitochondrial dysfunction and oxidative stress as determinants of cell death/survival in stroke. Ann Acad Sci 1042:203–209
200. Kamada H, Nito C, Endo H, Chan PH (2007) Bad as a converging signaling molecule between survival PI3-K/Akt and death JNK in neurons after transient focal cerebral ischemia in rats. J Cereb Blood Flow Metab 27:521–533
201. Shiraishi H, Okamoto H, Yoshimura A, Yoshida H (2006) ER stress-induced apoptosis and caspase-12activation occurs downstream of mitochondrial apoptosis involving Apaf-1. J Cell Sci 119:3958–3966
202. Paschen W, Doutheil J (1999) Disturbances of the functioning of endoplasmic reticulum: a key mechanism underlying neuronal cell injury? J Cereb Blood Flow Metab 19:1–18
203. Ma Y, Hendershot LM (2001) The unfolding tale of the unfolded protein response. Cell 107:827–830
204. DeGracia DJ, Kumar R, Owen CR, Krause GS, White BC (2002) Molecular pathways of protein synthesis inhibition during brain reperfusion: implications for neuronal survival or death. J Cereb Blood Flow Metab 22:127–141
205. Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D (2000) Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat Cell Biol 2:326–332
206. Shen J, Chen X, Hendershot L, Prywes R (2002) ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals. Dev Cell 3:99–111
207. Degracia DJ, Montie HL (2004) Cerebral ischemia and the unfolded protein response. J Neurochem 91:1–8
208. Zinszner H, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H, Stevens JL, Ron D (1998) CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev 12:982–995
209. Oyadomari S, Mori M (2004) Roles of CHOP/GADD153 in endoplasmic reticulum stress. Cell Death Differ 11:381–389
210. Hayashi T, Saito A, Okuno S, Ferrand-Drake M, Doddand RL, Chan PK (2005) Damage to the endoplasmic reticulum and activation of apoptotic machinery by oxidative stress in ischemic neurons. J Cereb Blood Flow Metab 25:41–53
211. Haze K, Yoshida H, Yanagi H, Yura T, Mori K (1999) Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. Mol Biol Cell 10:3787–3799
212. Roy B, Lee AS (1999) The mammalian endoplasmic reticulum stress response element consists of an evolutionarily conserved tripartite structure and interacts with a novel stress-inducible complex. Nucleic Acids Res 27:1437–1443
213. Wang S, Longo FM, Chen J, Butman M, Graham SH, Haglid KG, Sharp FR (1993) Induction of glucose regulated protein (grp78) and inducible heat shock protein (hsp70) mRNAs in rat brain after kainic acid seizures and focal ischemia. Neurochem Int 23:575–582
214. Yoneda T, Imaizumi K, Oono K, Yui D, Gomi F, Katayama T, Tohyama M (2001) Activation of caspase-12, an endoplastic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. J Biol Chem 276:13935–13940
215. Paschen W (1996) Disturbances of calcium homeostasis within the endoplasmic reticulum may contribute to the development of ischemic-cell damage. Med Hypotheses 47:283–288
216. Hu BR, Martone ME, Jones YZ, Liu CL (2000) Protein aggregation after transient cerebral ischemia. J Neurosci 20: 3191–3199
217. Althausen S, Mengesdorf T, Mies G, Olah L, Nairn AC, Proud CG, Paschen W (2001) Changes in the phosphorylation of initiation factor eIF- 2alpha, elongation factor eEF-2 and p70 S6 kinase after transient focal cerebral ischaemia in mice. J Neurochem 78:779–787
218. Kumar R, Azam S, Sullivan JM, Owen C, Cavener DR, Zhang P, Ron D, Harding HP, Chen JJ, Han A, White BC, Krause GS, DeGracia DJ (2001) Brain ischemia and reperfusion activates the eukaryotic initiation factor 2alpha kinase, PERK. J Neurochem 77:1418–1421
219. Nowak TS Jr, Fried RL, Lust WD, Passonneau JV (1985) Changes in brain energy metabolism and protein synthesis following transient bilateral ischemia in the gerbil. J Neurochem 44:487–494
220. Paschen W, Gissel C, Linden T, Althausen S, Doutheil J (1998) Activation of gadd153 expression through transient cerebral ischemia: evidence that ischemia causes endoplasmic reticulum dysfunction. Brain Res Mol Brain Res 60:115–122
221. Mouw G, Zechel JL, Gamboa J, Lust WD, Selman WR, Ratcheson RA (2003) Activation of caspase-12, an endoplasmic reticulum resident caspase, after permanent focal ischemia in rat. Neuroreport 14:183–186
222. Shibata M, Hattori H, Sasaki T, Gotoh J, Hamada J, Fukuuchi Y (2003) Activation of caspase-12 by endoplasmic reticulum stress induced by transient middle cerebral artery occlusion in mice. Neuroscience 118:491–499
223. Szegezdi E, Fitzgerald U, Samali A (2003) Caspase-12 and ER-stress-mediated apoptosis: the story so far. Ann N Y Acad Sci 1010:186–194
224. Hetz C, Russelakis-Carneiro M, Maundrell K, Castilla J, Soto C (2003) Caspase-12 and endoplasmic reticulum stress mediate neurotoxicity of pathological prion protein. EMBO J 22:5435–5445
225. Hitomi J, Katayama T, Eguchi Y, Kudo T, Taniguchi M, Koyama Y, Manabe T, Yamagishi S, Bando Y, Imaizumi K, Tsujimoto Y, Tohyama M (2004) Involvement of caspase-4 in endoplasmic reticulum stress-induced apoptosis and Aβ-induced cell death. J Cell Biol 165:347–356
226. McCullough KD, Martindale JL, Klotz LO, Aw T, Holbrook NJ (2001) Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state. Mol Cell Biol 21:1249–1259
227. Zinszner H, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H, Stevens JL, Ron D (1998) CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev 12:982–995
228. Xu C, Bailly-Maitre B, Reed JC (2005) Endoplasmic reticulum stress: cell life and death decisions. J Clin Invest 115:2656–2664
229. Tajiri S, Oyadomari S, Yano S, Morioka M, Gotoh T, Hamada J-I, Ushio Y, Mori M (2004) Ischemia-induced neuronal cell death is mediated by the endoplasmic reticulum stress pathway involving CHOP. Cell Death Differ 11:403–415
230. Putney JW Jr, Ribeiro CM (2000) Signaling pathways between the plasma membrane and endoplasmic reticulum calcium stores. Cell Mol Life Sci 57:1272–1286
231. Rutter GA, Rizzuto R (2000) Regulation of mitochondrial metabolism by ER Ca^{2+} release: an intimate connection. Trends Biochem Sci 25:215–221
232. Hacki J, Egger L, Monney L, Conus S, Rosse T, Fellay I, Borner C (2000) Apoptotic crosstalk between the endoplasmic reticulum and mitochondria controlled by Bcl-2. Oncogene 19:2286–2295
233. Boehning D, Patterson RL, Sedaghat L, Glebova NO, Kurosaki T, Snyder SH (2003) Cytochrome c binds to inositol (1,4,5) trisphosphate receptors, amplifying calcium-dependent apoptosis. Nat Cell Biol 5:1051–1061

234. Beresewicz M, Kowalczyk JE, Zablocka B (2006) Cytochrome c binds to inositol (1,4,5) trisphosphate and ryanodine receptors in vivo after transient brain ischemia in gerbils. Neurochem Int 48:568–571
235. Sanges D, Marigo V (2006) Cross-talk between two apoptotic pathways activated by endoplasmic reticulum stress: differential contribution of caspase-12 and AIF. Apoptosis 11:1629–1641
236. Chan SL, Mattson MP (1999) Caspase and calpain substrates: roles in synaptic plasticity and cell death. J Neurosci Res 58:167–190
237. Orrenius S, Zhivotovsky B, Nicotera P (2003) Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol 4: 552–565
238. Nakagawa T, Yuan J (2000) Cross-talk between two cysteine protease families: activation of caspase-12 by calpain in apoptosis. J Cell Biol 50:887–894
239. Raynaud F, Marcilhac A (2006) Implication of calpain in neuronal apoptosis. FEBS J 273:3437–3443
240. Garcıa-Bonilla L, Burda J, Pineiro D, Ayuso I, Gomez-Calcerrada M, Salinas M (2006) Calpain-induced proteolysis after transient global cerebral ischemia and ischemic tolerance in a rat model. Neurochem Res 31:1433–1441
241. Renolleau S, Benjelloun N, Ben-Ari Y, Charriaut-Marlangue C (1997) Regulation of apoptosis-associated proteins in cell death following transient focal ischemia in rat pups. Apoptosis 2: 368–376
242. McGahan L, Hakim AM, Robertson GS (1998) Hippocampal Myc and p53 expression following transient global ischemia. Brain Res Mol Brain Res 56:133–145
243. Fortin A, Sean P, Jason C, MacLaurin G, Kushwaha N, Hickman ES, Thompson CS, Hakim A, Albert PR, Cecconi F, Helin K, Park DS, Slack RS (2001) APAF1 is a key transcriptional target for p53 in the regulation of neuronal cell death. J Cell Biol 155: 207–216
244. Cheng T, Liu D, Griffin JH, Fernández JA, Castellino F, Rosen ED, Fukudome K, Zlokovic BV (2003) Activated protein C blocks p53-mediated apoptosis in ischemic human brain endothelium and is neuroprotective. Nat Med 9:338–342
245. Leker RR, Aharonowiz M, Greig NH, Ovadia H (2004) The role of p53-induced apoptosis in cerebral ischemia: effects of the p53 inhibitor pifithrinά. Exp Neurol 187:478–486
246. Endo H, Kamada H, Nito C, Nishi T, Chan PH (2006) Mitochondrial translocation of p53 mediates release of cytochrome c and hippocampal CA1 neuronal death after transient global cerebral ischemia in rats. J Neurosci 26:7974–7983
247. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Costantini P, Loeffler M, Larochette N, Goodlett DR, Aebersold R, Siderovski DP, Penninger JM, Kroemer G (1999) Molecular characterization of mitochondrial apoptosis-inducing factor. Nature 397:441–446
248. Daugas E, Susin SA, Zamzami N, Ferri KF, Irinopoulou T, Larochette N, Prevost MC, Leber B, Andrews D, Penninger J, Kroemer G (2000) Mitochondrio-nuclear translocation of AIF in apoptosis and necrosis. FASEB J 14:729–739
249. Wang H, Yu SW, Koh DW, Lew J, Coombs C, Bowers W, Federoff HJ, Poirier GG, Dawson TM, Dawson VL (2004) Apoptosis-inducing factor substitutes for caspase executioners in NMDA-triggered excitotoxic neuronal death. J Neurosci 24:10963–10973
250. Cao G, Clark RS, Pei W, Yin W, Zhang F, Sun FY, Graham SH, Chen J (2003) Translocation of apoptosis-inducing factor in vulnerable neurons after transient cerebral ischemia and in neuronal cultures after oxygen-glucose deprivation. J Cereb Blood Flow Metab 23:1137–1150
251. Komjati K, Mabley JG, Virag L, Southan GJ, Salzman AL, Szabo C (2004) Poly (ADP-ribose) polymerase inhibition protect neurons and the white matter and regulates the translocation of apoptosisinducing factor in stroke. Int J Mol Med 13:373–382
252. Zhu C, Qiu L, Wang X, Hallin U, Cande C, Kroemer G, Hagberg H, Blomgren K (2003) Involvement of apoptosis-inducing factor in neuronal death after hypoxia–ischemia in the neonatal rat brain. J Neurochem 86:306–317
253. Matsumori Y, Hong SM, Aoyama K, Fan Y, Kayama T, Sheldon RA, Vexler ZS, Ferriero DM, Weinstein PR, Liu J (2005) Hsp70 overexpression sequesters AIF and reduces neonatal hypoxic/ischemic brain injury. J Cereb Blood Flow Metab 25:899–910
254. Plesnila N, Zhu C, Culmsee C, Groger M, Moskowitz MA, Blomgren K (2004) Nuclear translocation of apoptosis-inducing factor after focal cerebral ischemia. J Cereb Blood Flow Metab 24:458–466
255. Cande C, Vahsen N, Garrido C, Kroemer G (2004) Apoptosis-inducing factor (AIF): caspase-independent after all. Cell Death Differ 11:591–595
256. Zhao H, Yenari MA, Cheng D, Barreto-Chang OL, Sapolsky RM, Steinberg GK (2004) Bcl-2 transfection via herpes simplex virus blocks apoptosis-inducing factor translocation after focal ischemia in the rat. J Cereb Blood Flow Metab 24:681–692
257. Culmsee C, Zhu C, Landshamer S, Becattini B, Wagner E, Pellechia M, Blomgren K, Plesnila N (2005) Apoptosis-inducing factor triggered by poly (ADP-ribose) polymerase and Bid mediates neuronal cell death after oxygen-glucose deprivation and focal cerebral ischemia. J Neurosci 25:10262–10272
258. Yu SW, Wang H, Poitras MF, Coombs C, Bowers WJ, Federoff HJ, Poirier GG, Dawson TM, Dawson VL (2002) Mediation of poly (adp-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science 297:259–263
259. Endres M, Wang ZQ, Namura S, Waeber C, Moskowitz MA (1997) Ischemic brain injury is mediated by the activation of poly(ADP-ribose)polymerase. J Cereb Blood Flow Metab 17:1143–1151
260. Eliasson MJ, Sampei K, Mandir AS, Hurn PD, Traystman RJ, Bao J, Pieper A, Wang ZQ, Dawson TM, Snyder SH (1997) Poly (ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. Nat Med 3:1089–1095
261. Johnson GL, Lapadat R (2002) Mitogen-activated protein kinase pathways mediated by erk, jnk, and p38 protein kinases. Science 298:1911–1912
262. Cheung ECC, Slack RS (2004) Emerging role for erk as a key regulator of neuronal apoptosis. Science`s stke. 251:PE45
263. Alessandrini A, Namura S, Moskowitz MA, 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
264. Zhuang S, Schnellmann RG (2006) A death-promoting role for extracellular signal-regulated kinase. J Pharmacol Exp Ther 319:991–997
265. Subramaniam S, Zirrgiebel U, Von Bohlen Und Halbach O, Strelau J, Laliberte C, Kaplan DR, Unsicker K (2004) ERK activation promotes neuronal degeneration predominantly through plasma membrane damage and independently of caspase-3. J Cell Biol 165:357–369
266. Irving EA, Barone FC, Reith AD, Hadingham SJ, 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
267. Namura S, Iihara K, Takami S, Nagata I, Kikuchi H, Matsushita K, Moskowitz MA, Bonventre JV, 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
268. Hetman M, Gozaz A (2004) Role of extracellular signal regulated kinases 1 and 2 in neuronal survival. Eur J Biochem 271:050–2055
269. Noshita N, Sugawara T, Hayashi T, Lewén A, Omar G, Chan PH (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 22:923–7930
270. Irving EA, Bamford M (2002) Role of mitogen- and stress-activated kinases in ischemic injury. J Cereb Blood Flow Metab 22:631–647
271. Gao Y, Signore AP, Yin W, Cao G, Yin X, Sun F, Luo Y, Graham SH, Chen J (2005) Neuroprotection against focal ischemic brain injury by inhibition of c-Jun N-terminal kinase and attenuation of the mitochondrial apoptosis-signaling pathway. J Cereb Blood Flow Metab 25:694–712
272. Shackelford DA, Yeh RY (2006) Modulation of ERK and JNK activity by transient forebrain ischemia in rats. J Neurosci Res 83:476–488
273. Kuan C-Y, Whitnarsh A, Yang DD, Lioa G, Schloemer A, Dong C, Bao J, Banasiak K, Haddad GG, Flavell RA, Davis R, Rakic P (2003) A critical role of neural-specific JNK3 for ischemic apoptosis. Proc Natl Acad Sci U S A 100:15184–15189
274. Cheng A, Chan SL, Milhavet O, Wang S, Mattson MP (2001) p38 map kinase mediates nitric oxide-induced apoptosis of neural progenitor cells. J Biol Chem 276:43320–43327
275. Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME (1995) Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 270:1326–1331
276. Clem RJ, Fechheimer M, Miller LK (1991) Prevention of apoptosis by a baculovirus gene during infection of insect cells. Science 254:1388–1390
277. Wilkinson JC, Wilkinson AS, Scott FL, Csomos RA, Salvesen GS, Duckett CS (2004) Neutralization of Smac/Diablo by inhibitors of apoptosis (IAPs). J Biol Chem 279:51082–51090
278. Fan T, Han L, Cong R, Liang J (2005) Caspase family proteases and apoptosis. Acta Biochimica et Biophysica Sinica 37:719–727
279. Yang Y, Fang S, Jensen JP, Weissman AM, Ashwell JD (2000) Ubiquitin protein ligase activity of IAPs and their degradation in proteasomes in response to apoptotic stimuli. Science 288:874–877
280. Eberhardt O, Coelln RV, Kugler S, Lindenau J, Rathke-Hartlieb S, Gerhardt E, Haid S, Isenmann S, Gravel C, Srinivasan A, Bahr M, Weller M, Dichgans J, Schulz JB (2000) Protection by synergistic effects of adenovirus-mediated x-chromosome-linked inhibitor of apoptosis and glial cell line-derived neurotrophic factor gene transfer in the 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine model of Parkinson's disease. J Neurosci 15:9126–9134
281. Simons M, Beinroth S, Gleichmann M, Liston P, Korneluk RG, MacKenzie AE, Bahr M, Klockgether T, Robertson GS, Weller M, Schulz JB (1999) Adenovirus-mediated gene transfer of IAPs delays apoptosis of cerebellar granule neurons. J Neurochem 72:292–301
282. Siegelin MD, Kossatz LS, Winckler J, Rami A (2005) Regulation of XIAP and Smac/DIABLO in the rat hippocampus following transient forebrain ischemia. Neurochem Int 46:41–51
283. Vaux DL, Silke J (2003) Mammalian mitochondrial IAP binding proteins. Biochem Biophys Res Commun 304:499–504
284. Costantini P, Bruey JM, Castedo M, Métivier D, Loeffler M, Susin SA, Ravagnan L, Zamzami N, Garrido C, Kroemer G (2002) Pre-processed caspase-9 contained in mitochondria participates in apoptosis. Cell Death Differ 9:82–88
285. Saito A, Hayashi T, Okuno S, Nishi T, Chan PH (2004) Oxidative stress is associated with xiap and Smac/Diablo signaling pathways in mouse brains after transient focal cerebral ischemia. Stroke 35:1443–1448
286. Siegelin M, Touzani O, Toutain J, Liston P, Ramia A (2005) Induction and redistribution of XAF1, a new antagonist of XIAP in the rat brain after transient focal ischemia. Neurobiol Dis 20:509–518
287. Wang X, Zhu C, Wang X, Hagberg H, Korhonen L, Sandberg M, Lindholm D, Blomgren K (2004) X-linked inhibitor of apoptosis (XIAP) protein protects against caspase activation and tissue loss after neonatal hypoxia–ischemia. Neurobiol Dis 16:179–189
288. Faccio L, Fusco C, Chen A, Martinotti S, Bonventre JV, Zervos AS (2000) Characterization of a novel human serine protease that has extensive homology to bacterial heat shock endoprotease htra and is regulated by kidney ischemia. J Biol Chem 275: 2581–2588
289. Savopoulos JW, Carter PS, Turconi S, Pettman GR, Karran EH, Gray CW, Ward RV, Jenkins O, Creasy CL (2000) Expression, purification, and functional analysis of the human serine protease HtrA2. Protein Expr Purif 19:227–234
290. Yang Q, Church-Hajduk R, Ren J, Newton ML, Du C (2003) Omi/HtrA2 catalytic cleavage of inhibitor of apoptosis (IAP) irreversibly inactivates IAPs and facilitates caspase activity in apoptosis. Genes Dev 17:1487–1496
291. Saito A, Hayashi T, Okuno S, Nishi T, Chan PH (2004) Modulation of the Omi/HtrA2 signaling pathway after transient focal cerebral ischemia in mouse brains that over express SOD1. Mol Brain Res 127:89–95
292. Althaus J, Siegelin MD, Dehghani F, Cilenti L, Zervos AS, Rami A (2007) The serine protease Omi/HtrA2 is involved in XIAP cleavage and in neuronal cell death following focal cerebral ischemia/reperfusion. Neurochem Intl 50:172–180
293. Nowak TS Jr (1993) Synthesis of heat shock/stress proteins during cellular injury. Ann NY Acad Sci 679:142–156
294. Sharp FR, Kinouchi H, Koistinaho J, Chan PH, Sagar SM (1993) HSP70 heat shock gene regulation during ischemia. Stroke 24: I72–175
295. Yenari MA, Giffard RG, Sapolsky RM, Steinberg GK (1999) The neuroprotective potential of heat shock protein 70 (HSP70). Mol Med Today 5:525–531
296. Weinstein PR, Hong S, Sharp FR (2004) Molecular identification of the ischemic penumbra. Stroke 35:2666–2670
297. Rajdev S, Hara K, Kokubo Y, Mestril R, Dillmann W, Weinstein PR, Sharp FR (2000) Mice overexpressing rat heat shock protein 70 are protected against cerebral infarction. Ann Neurol 47: 782–791
298. Van der Weerd L, Lythgoe MF, Badin RA, Valentim LM, Akbar MT, de Belleroche JS, Latchman DS, Gadian DG (2005) Neuroprotective effects of HSP70 overexpression after cerebral ischaemia-An MRI study. Exp Neurol 195:257–266
299. Tsuchiya D, Hong S, Matsumori Y, Shiina H, Kayama T, Swanson RA, Dillman WH, Liu J, Panter SS, Weinstein PR (2003) Overexpression of rat heat shock protein 70 is associated with reduction of early mitochondrial cytochrome c release and subsequent DNA fragmentation after permanent focal ischemia. J Cereb Blood Flow Metab 23:718–727
300. Lee SH, Kwon HM, Kim YJ, Lee KM, Kim M, Yoon BW (2004) Effects of Hsp70.1 gene knockout on the mitochondrial apoptotic pathway after focal cerebral ischemia. Stroke 35: 2195–2199
301. Matsumori Y, Hong SM, Aoyama K, Fan Y, Kayama T, Sheldon RA, Vexler ZS, Ferriero DM, Weinstein PR, Liu J (2005) Hsp70 overexpression sequesters AIF and reduces neonatal hypoxic/ischemic brain injury. J Cereb Blood Flow Metab 25: 899–910
302. Park HS, Lee JS, Huh SH, Seo JS, Choi EJ (2001) Hsp72 functions as a natural inhibitory protein of c-Jun N-terminal kinase. EMBO J 20:446–456
303. Meriin AB, Yaglom JA, Gabai VL, Zon L, Ganiatsas S, Mosser DD, Zon L, Sherman MY (1999) Protein-damaging stresses activate c-Jun N-terminal kinase via inhibition of its dephosphorylation: a novel pathway controlled by HSP72. Mol Cell Biol 19:2547–2555

304. Klionsky DJ, Emr SD (2000) Autophagy as a regulated pathway of cellular degradation. Science 290:1717–1721
305. Tsujimoto Y, Shimizu S (2005) Another way to die: autophagic programmed cell death. Cell Death Differ 12:528–1534
306. Yuan J, Lipinski M, Degterev A (2003) Diversity in the mechanisms of neuronal cell death. Neuron 40:401–413
307. Eskelinen EL, Jekyll D, Hyde M (2005) Autophagy can promote both cell survival and cell death. Cell Death Differ 12:1468–1472
308. Ogata M, Hino S, Saito A, Morikawa K, Kondo S, Kanemoto S, Murakami T, Taniguchi M, Tanii I, Yoshinaga K, Shiosaka S, Hammarback JA, Urano F, Imaizumi K (2006) Autophagy is activated for cell survival after endoplasmic reticulum stress. Mol Cell Biol 26:9220–9231
309. Adhami F, Schloemer A, Kuan C (2007) The roles of Autophagy in cerebral ischemia. Autophagy 3:42–44
310. Hamacher-Brady A, Brady NR, Gottlieb RA (2006) The interplay between pro-death and pro-survival signaling pathways in myocardial ischemia/reperfusion injury: apoptosis meets autophagy. Cardiovasc Drugs Ther 20:445–462
311. Levine B, Yuan J (2005) Autophagy in cell death: an innocent convict? J Clin Invest 115:2679–2688
312. Lebeurrier N, Vivien D, Ali C (2004) The complexity of tissue-type plasminogen activator: can serine protease inhibitors help in stroke management? Expert Opin Ther Targets 8:309–320
313. Montaner J, Molina CA, Monasterio J, Abilleira S, Arenillas JF, Ribo M, Quintana M, Alvarez-Sabin J (2003) Matrix metalloproteinase-9 pretreatment level predicts intracranial hemorrhagic complications after thrombolysis in human stroke. Circulation 107:598–603
314. Wang X, Lee SR, Arai K, Lee SR, Tsuji K, Rebeck GW, Lo EH (2003) Lipoprotein receptor-mediated induction of matrix metalloproteinase by tissue plasminogen activator. Nat Med 9:1313–1317
315. Pfefferkon T, Rosenberg GA (2003) Closure of the blood–brain barrier by matrix metalloproteinase inhibition reduces rtPA-mediated mortality in cerebral ischemia with delayed reperfusion. Stroke 34:2025–2030
316. Reddrop C, Moldrich RX, Beart PM, Farso M, Liberatore GT, Howells DW, Petersen KU, Schleuning WD, Medcalf RL (2005) Vampire bat salivary plasminogen activator (desmoteplase) inhibits tissue-type plasminogen activator-induced potentiation of excitotoxic injury. Stroke 36:1241–1246
317. Hacke W, Albers G, Al-Rawi Y, Bogousslavsky J, Davalos A, Eliasziw M, Fischer M, Furlan A, Kaste M, Lees KR, Soehngen M, Warach S (2005) The desmoteplase in Acute Ischemic Stroke Trial (DIAS): a phase II MRI-based 9-hour window acute stroke thrombolysis trial with intravenous desmoteplase. DIAS Study Group Stroke 36:66–73
318. Furlan AJ, Eyding D, Albers GW, Al-Rawi Y, Lees KR, Rowley HA, Sachara C, Soehngen M, Warach S, Hacke W (2006) Dose escalation of desmoteplase for acute ischemic stroke (DEDAS) evidence of safety and efficacy 3 to 9 hours after stroke onset. Stroke 37:1227–1231
319. Suzuki Y, Chen F, Ni Y, Marchal G, Collen D, Nagai N (2004) Microplasmin reduces ischemic brain damage and improves neurological function in a rat stroke model monitored with MRI. Stroke 35:2402–2406
320. Chen F, Suzuki Y, Nagai N, Sun X, Wang H, Yu J, Marchal G, Ni Y (2007) Microplasmin and tissue plasminogen activator: comparison of therapeutic effects in rat stroke model at multiparametric MR imaging. Radiology 244:429–438
321. Watanabe T, Yuki S, Egawa M, Nishi H (1994) Protective effects of MCI-186 on cerebral ischemia: possible involvement of free radical scavenging and antioxidant actions. J Pharmacol Exp Ther 268:1597–1604
322. The Edaravone Acute Brain Infarction Study Group (2003) Effect of a novel free radical scavenger, edaravone (MCI-186), on acute brain infarction: randomized, placebo-controlled, double-blind study at multicenters. Cerebrovasc Dis 15:222–229
323. Watanabe T, Morita I, Nishi H, Murota S (1988) Preventive effect of MCI-186 on 15-HPETE induced vascular endothelial cell injury in vitro. Prostaglandins Leukot Essent Fat Acids 33:81–87
324. Abe K, Yuki S, Kogure K (1988) Strong attenuation of ischemic and postischemic brain edema in rats by a novel free radical scavenger. Stroke 19:480–485
325. Yamamoto T, Yuki S, Watanabe T, Mitsuka M, Saito K, Kogure K (1997) Delayed neuronal death prevented by inhibition of increased hydroxyl radical formation in a transient cerebral ischemia. Brain Res 762:240–242
326. Toyoda K, Fujii K, Kamouchia M, Nakane H, Arihiro S, Okada Y, Ibayashi S, Iida M (2004) Free radical scavenger, edaravone, in stroke with internal carotid artery occlusion. J Neurol Sci 221: 11–17
327. Qi X, Okuma Y, Hosoi T, Nomura Y (2004) Edaravone protects against hypoxia/ischemia-induced endoplasmic reticulum dysfunction. J Pharmacol Exp Ther 311:388–393
328. Mizuno A, Umemura K, Nakashima M (1998) Inhibitory effect of MCI-186, a free radical scavenger, on cerebral ischemia following rat middle cerebral artery occlusion. Gen Pharmacol 30:575–578
329. Lees KR, Zivin JA, Ashwood T, Davalos A, Davis SM, Diener H, Grotta J, Lyden P, Shuaib A, Hardemark H, Wasiewski WW (2006) NXY-059 for acute ischemic stroke. N Engl J Med 354:588–600
330. Fisher M (2007) Advances in stroke 2006. Stroke 38:214–215
331. Iadecola C, Zhang F, Xu X (1995) Inhibition of inducible nitric oxide synthase ameliorates cerebral ischemic damage. Am J Physiol 268:R286–R292
332. Zhao X, Haensel C, Araki E, Ross ME, Iadecola C (2000) Gene-dosing effect and persistence of reduction in ischemic brain injury in mice lacking inducible nitric oxide synthase. Brain Res 872:215–218
333. Coughlan T, Gibson C, Murphy S (2005) Modulatory effects of progesterone on inducible nitric oxide synthase expression in vivo and in vitro. J Neurochem 93:932–942
334. Park EM, Cho S, Frys KA, Glickstein SB, Zhou P, Anrather J, Ross ME, Iadecola C (2006) Inducible nitric oxide synthase contributes to gender differences in ischemic brain injury. J Cereb Blood Flow Metab 26:392–401
335. Kawano T, Anrather J, Zhou P, Park L, Wang G, Frys KA, Kunz A, Cho S, Orio M, Iadecola C (2006) Prostaglandin E2 EP1 receptors: downstream effectors of COX-2 neurotoxicity. Nat Med 12:225–229
336. Ruehl ML, Orozco JA, Stoker MB, McDonagh PF, Coull BM, Ritter LS (2002) Protective effects of inhibiting both blood and vascular selectins after stroke and reperfusion. Neurol Res 24:226–232
337. Mulcahy NJ, Ross J, Rothwell NJ, Loddick SA (2003) Delayed administration of interleukin-1 receptor antagonist protects against transient cerebral ischaemia in the rat. Br J Pharmacol 140:471–476
338. Yang GY, Zhao YJ, Davidson BL, Betz AL (1997) Over-expression of interleukin-1 receptor antagonist in the mouse brain reduces ischemic brain injury. Brain Res 751:181–188
339. Pinteaux E, Rothwell NJ, Boutin H (2006) Neuroprotective actions of endogenous interleukin-1 receptor antagonist (IL-1ra) are mediated by glia. Glia 53:551–556
340. Vemuganti R, Dempsey RJ, Bowen KK (2004) Inhibition of intercellular adhesion molecule-1 protein expression by antisense oligonucleotides is neuroprotective after transient middle cerebral artery occlusion in rat. Stroke 35:179–184
341. Mayne M, Ni W, Yan HJ, Xue M, Johnston JB, Del Bigio MR, Peeling J, Power C (2001) Antisense oligodeoxynucleotide

inhibition of tumor necrosis factor-alpha expression is neuroprotective after intracerebral hemorrhage. Stroke 32:240–248
342. Luo Y, Yin W, Signore AP, Zhang F, Hong Z, Wang S, Graham SH, Chen J (2006) Neuroprotection against focal ischemic brain injury by the peroxisome proliferator-activated receptor-g agonist rosiglitazone. J Neurochem 97:435–448
343. Tureyen K, Kapadia R, Bowen KK, Satriotomo I, Liang J, Feinstein DL, Vemuganti R (2007) Peroxisome proliferator-activated receptor-gamma agonists induce neuroprotection following transient focal ischemia in normotensive, normoglycemic as well as hypertensive and type-2 diabetic rodents. J Neurochem 101:41–45
344. Park SW, Yi JH, Miranpuri G, Satriotoma I, Bowen K, Resnick DK, Vemuganti R (2007) Thiazolidinedione class of peroxisome proliferator-activated receptor gamma agonists prevents neuronal damage, motor dysfunction, myelin loss, neuropathic pain, and inflammation after spinal cord injury in adult rats. J Pharmacol Exp Ther 320:1002–1012
345. Deplanque D, Gelé P, Pétrault O, Six I, Furman C, Bouly M, Nion S, Dupuis B, Leys D, Fruchart JC, Cecchelli R, Staels B, Duriez P, Bordet R (2003) Peroxisome proliferator-activated receptor-alpha activation as a mechanism of preventive neuroprotection induced by chronic fenofibrate treatment. J Neurosci 23:6264–6271
346. Iwashita A, Muramatsu Y, Yamazaki T, Muramoto M, Kita Y, Yamazaki S, Mihara K, Moriguchi A, Matsuoka N (2007) Neuroprotective efficacy of the peroxisome proliferator-activated receptor delta-selective agonists in vitro and in vivo. J Pharmacol Exp Ther 320:1087–1096
347. Romera C, Hurtado O, Mallolas J, Pereira MP, Morales JR, Romera A, Serena J, Vivancos J, Nombela F, Lorenzo P, Lizasoain I, Moro MA (2007) Ischemic preconditioning reveals that GLT1/EAAT2 glutamate transporter is a novel PPAR gamma target gene involved in neuroprotection. J Cereb Blood Flow Metab 27:1327–1338
348. Koh SH, Chang D-I, Kim H-T, Kim J, Kim M-H, Kim KS, Bae I, Kim H, Kim DW, Kim SH (2005) Effect of 3-aminobenzamide, PARP inhibitor, on matrix metalloproteinase-9 level in plasma and brain of ischemic stroke model. Toxicol 214:131–139
349. Loddick SA, MacKenzie A, Rothwell NJ (1996) An ICE inhibitor, z-VAD-DCB attenuates ischaemic brain damage in the rat. NeuroReport 7:1465–1468
350. Hara H, Friedlander RM, Gagliardini V, Ayata C, Fink K, Huang Z, Shimizu-Sasamata M, Yuan J, Moskowitz MA (1997) Inhibition of interleukin 1 β-converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. Proc Natl Acad Sci U S A 94:2007–2012
351. Endres M, Namura S, Shimizu-Sasamata M, Waeber C, Zhang L, Gómez-Isla T, Hyman BT, Moskowitz MA (1998) Attenuation of delayed neuronal death after mild focal ischemia in mice by inhibition of the caspase family. J Cereb Blood Flow Metab 18:238–247
352. Cheng Y, Deshmukh M, D, Costa A, Demaro JA, Gidday JM, Shah A, Sun Y, Jacquin MF, Johnson EM, Holtzman DM (1998) Caspase inhibitor affords neuroprotection with delayed administration in a rat model of neonatal hypoxic-ischemic brain injury. J Clin Investig 101:1992–1999
353. Li H, Colbourne F, Sun P, Zhao Z, Buchan AM (2000) Caspase inhibitors reduce neuronal injury after focal but not global cerebral ischemia in rats. Stroke 31:176–182
354. Inoue S, Davis DP, Drummond JC, Cole DJ, Patel PM (2006) The combination of isoflurane and caspase 8 inhibition results in sustained neuroprotection in rats subject to focal cerebral ischemia. Anesth Analg 102:1548–1555
355. Frankel AD, Pabo CO (1988) Cellular uptake of the tat protein from human immunodeficiency virus. Cell 55:1189–1193
356. Green M, Loewenstein PM (1988) Autonomous functional domains of chemically synthesized human immunodeficiency virus tat trans-activator protein. Cell 55:1179–1188
357. Fawell S, Seery J, Daikh Y, Moore C, Chen LL, Pepinsky B, Barsoum J (1994) Tat-mediated delivery of heterologous proteins into cells. Proc Natl Acad Sci U S A 91:664–668
358. Schwarze SR, Ho A, Vocero-Akbani A, Dowdy SF (1999) In vivo protein transduction: delivery of a biologically active protein into the mouse. Science 285:1569–1572
359. Kilic U, Kilic E, Dietz GP, Bahr M (2003) Intravenous TAT-GDNF is protective after focal cerebral ischemia in mice. Stroke 34:1304–1310
360. Kilic E, Dietz GP, Hermann DM, Bahr M (2002) Intravenous TAT-Bcl-xL is protective after middle cerebral artery occlusion in mice. Ann Neurol 52:617–622
361. Guegan C, Braudeau J, Couriaud C, Dietz GP, Lacombe P, Bahr M, Nosten-Bertrand M, Onteniente B (2006) PTD-XIAP protects against cerebral ischemia by anti-apoptotic and transcriptional regulatory mechanisms. Neurobiol Dis 22:177–186
362. Fan YF, Lu CZ, Xie J, Zhao YX, Yang GY (2006) Apoptosis inhibition in ischemic brain by intraperitoneal PTD-BIR3-RING (XIAP). Neurochem Int 48:50–59
363. Cao YJ, Shibata T, Rainov NG (2002) Liposome-mediated transfer of the bcl-2 gene results in neuroprotection after in vivo transient focal cerebral ischemia in an animal model. Gene Ther 9:415–419
364. Hong KW, Kim KY, Lee JH, Shin HK, Kwak YG, Kim SO, Lim H, Yoo SE (2002) Neuroprotective effect of (2S,3S,4R)-*N*″-cyano-*N*-(6-amino-3, 4-dihydro-3-hydroxy-2-methyl-2-dimethoxymethyl-2H-benzopyran-4-yl)-*N*′-benzylguanidine(KR-31378), a benzopyran analog, against focal ischemic brain damage in rats. J Pharmacol Exp Ther 301:210–216
365. Kim KY, Lee JH, Park JH, Yoo MA, Kwak YG, Kim SO, Yoo SE, Hong KW (2004) Anti-apoptotic action of (2S,3S,4R)-*N*″-cyano-*N*-(6-amino-3,4-dihydro-3-hydroxy-2-methyl-2-dimethoxymethyl-2H-benzopyran-4-yl)-*N*′-benzylguanidine (KR-31378) by suppression of the phosphatase and tensin homolog deleted from chromosome 10 phosphorylation and increased phosphorylation of casein kinase2/Akt/ cyclic AMP response element binding protein via maxi-K channel opening in neuronal cells. Eur J Pharmacol 497:267–277
366. Choi JM, Shin HK, Kim KY, Lee JH, Hong KW (2002) Neuroprotective effect of cilostazol against focal cerebral ischemia via antiapoptotic action in rats. J Pharmacol Exp Ther 300:787–793
367. Scott CW, Sobotka-Briner C, Wilkins DE, Jacobs RT, Folmer JJ, Frazee WJ, Bhat RV, Ghanekar SV, Aharony D (2003) Novel small molecule inhibitors of caspase-3 block cellular and biochemical features of apoptosis. J Pharmacol ExpTher 304: 433–440
368. D'Mello SR, Chin PC (2005) Treating neurodegenerative conditions through the understanding of neuronal apoptosis. Curr Drug Targets CNS Neurol Disord 4:3–23
369. Jiang ZG, Lu XC, Nelson V, Yang X, Pan W, Chen RW, Lebowitz MS, Almassian B, Tortella FC, Brady RO, Ghanbari HA (2006) A multifunctional cytoprotective agent that reduces neurodegeneration after ischemia. Proc Natl Acad Sci U S A 103:1581–1586
370. Belayev L, Liu Y, Zhao W, Busto R, Ginsberg MD (2001) Human albumin therapy of acute ischemic stroke: marked neuroprotective efficacy at moderate doses and with a broad therapeutic window. Stroke 32:553–560
371. Belayev L, Pinard E, Nallet H, Seylaz J, Liu Y, Riyamongkol P, Zhao W, Busto R, Ginsberg MD (2002) Albumin therapy of transient focal cerebral ischemia: in vivo analysis of dynamic microvascular responses. Stroke 33:1077–1084

372. Ginsberg MD, Hill MD, Palesch YY, Ryckborst KJ, Tamariz D (2006) The ALIAS pilot trial. A ose-escalation and safety study of albumin therapy for acute ischemic stroke-I: physiological responses and safety results. Stroke 37:2100–2106
373. Palesch YY, Hill MD, Ryckborst KJ, Tamariz D, Ginsberg MD (2006) The ALIAS pilot trial. A dose-escalation and safety study of albumin therapy for acute ischemic stroke-II: neurological outcome and efficiency analysis. Stroke 37:2107–2114
374. Belayev L, Marcheselli VL, Khoutorova L, Rodriguez de Turco EB, Busto R, Ginsberg MD, Bazan NG (2005) Docosahexaenoic acid complexed to albumin elicits high-grade ischemic neuroprotection. Stroke 36:118–123
375. Bazan NG (2005) Synaptic signaling by lipids in the life and death of neurons. Mol Neurobiol 31:219–230
376. Marcheselli VL, Hong S, Lukiw WJ, Tian XH, Gronert K, Musto A, Hardy M, Gimenez JM, Chiang N, Serhan CN, Bazan NG (2003) Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression. J Biol Chem 278:43807–43817
377. Nelson PT, Kondziolka D, Wechsler L, Goldstein S, Gebel J, DeCesare S, Elder EM, Zhang PJ, Jacobs A, McGrogan M, Lee VM, Trojanowski JQ (2002) Clonal human (hNT) neuron grafts for stroke therapy: neuropathology in a patient 27 months after implantation. Am J Pathol 160:1201–1206
378. Gilman S (2006) Pharmacologic management of ischemic stroke: relevance to stem cell therapy. Exp Neurol 199:28–33
379. Zhang R, Zhang Z, Wang L, Wang Y, Gousev A, Zhang L, Ho KL, Morshead C, Chopp M (2004) Activated neural stem cells contribute to stroke-induced neurogenesis and neuroblast migration toward the infarct boundary in adult rats. J Cereb Blood Flow Metab 24:441–448
380. Schäbitz WR, Kollmar R, Schwaninger M, Juettler E, Bardutzky J, Schölzke MN, Sommer C, Schwab S (2003) Neuroprotective effect of granulocyte colony-stimulating factor after focal cerebral ischemia. Stroke 34:745–751
381. Schneider A, Krüger C, Steigleder T, Weber D, Pitzer C, Laage R, Aronowski J, Maurer MH, Gassler N, Mier W, Hasselblatt M, Kollmar R, Schwab S, Sommer C, Bach A, Kuhn HG, Schäbitz WR (2005) The hematopoietic factor G-CSF is a neuronal ligand that counteracts programmed cell death and drives neurogenesis. J Clin Invest 115:2083–2098
382. Siren AL, Fratelli M, Brines M, Goemans C, Casagrande S, Lewczuk P, Keenan S, Gleiter C, Pasquali C, Capobianco A (2001) Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress. Proc Natl Acad Sci U S A 98: 4044–4049
383. Wang L, Zhang Z, Wang Y, Zhang R, Chopp M (2004) Treatment of stroke with erythropoietin enhances neurogenesis and angiogenesis and improves neurological function in Rats. Stroke 35:1732–1737
384. Villa P, Van Beek J, Larsen AK, Gerwien J, Christensen S, Cerami A, Brines M, Leist M, Ghezzi P, Torup L (2007) Reduced functional deficits, neuroinflammation, and secondary tissue damage after treatment of stroke by nonerythropoietic erythropoietin derivatives. J Cereb Blood Flow Metab 27: 552–563
385. Leist M, Ghezzi P, Grasso G, Bianchi R, Villa P, Fratelli M, Savino C, Bianchi M, Nielsen J, Gerwien J, Kallunki P, Larsen AK, Helboe L, Christensen S, Pedersen LO, Nielsen M, Torup L, Sager T, Sfacteria A, Erbayraktar S, Erbayraktar Z, Gokmen N, Yilmaz O, Cerami-Hand C, Xie QW, Coleman T, Cerami A, Brines M (2004) Derivatives of erythropoietin that are tissue protective but not erythropoietic. Science 305:239–242
386. Zhang R, Wang Y, Zhang L, Zhang Z, Tsang W, Lu M, Chopp M (2002) Sildenafil (Viagra) induces neurogenesis and promotes functional recovery after stroke in rats. Stroke 33:2675–2680
387. Zhang RL, Zhang Z, Zhang L, Wang Y, Zhang C, Chopp M (2006) Delayed treatment with sildenafil enhances neurogenesis and improves functional recovery in aged rats after focal cerebral ischemia. J Neurosci Res 83:1213–1219