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The kinder side of killer proteases: Caspase activation contributes to neuroprotection and CNS remodeling

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Abstract

Caspases are a family of cysteine proteases that are expressed as inactive zymogens and undergo proteolytic maturation in a sequential manner in which initiator caspases cleave and activate the effector caspases 3, 6 and 7. Effector caspases cleave structural proteins, signaling molecules, DNA repair enzymes and proteins which inhibit apoptosis. Activation of effector, or executioner, caspases has historically been viewed as a terminal event in the process of programmed cell death. Emerging evidence now suggests a broader role for activated caspases in cellular maturation, differentiation and other non-lethal events. The importance of activated caspases in normal cell development and signaling has recently been extended to the CNS where these proteases have been shown to contribute to axon guidance, synaptic plasticity and neuroprotection. This review will focus on the adaptive roles activated caspases in maintaining viability, the mechanisms by which caspases are held in check so as not produce apoptotic cell death and the ramifications of these observations in the treatment of neurological disorders.

Keywords

apoptosis; axon guidance; caspase; ischemic tolerance; neural plasticity; neuroprotection; preconditioning; proteasome; review

Introduction

Apoptosis or programmed cell death is defined by morphological changes including cell shrinkage, chromatin condensation, nuclear fragmentation, membrane blebbing, and apoptotic body formation. Integral to many forms of apoptosis are a family of cysteine proteases called caspases which are the mammalian homologues of the nematode cell death protein Ced-3. Caspases are synthesized as inactive precursors (or procaspases) which are constitutively expressed in virtually all mammalian cells.

Caspases are classified as either initiators or effectors depending on both structural aspects of the proteases as well as their role in programmed cell death. Initiator caspases have large N-terminal domains and are responsible for detecting and transducing apoptotic stimuli by cleaving and activating effector caspases. Effector caspases (caspases 3, 6 and 7) have shorter

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N terminal extensions and cleave downstream targets include structural proteins, inhibitors of apoptosis, DNA repair enzymes, cell cycle proteins and signal transduction molecules. Cleavage of these proteins results in the formation of membrane blebs, internucleosomal DNA degradation and other hallmark features of apoptosis.

The best understood series of events which result in effector caspase activation and apoptotic cell death are mediated by either a mitochondrial pathway or a death receptor pathway. The mitochondrial pathway can be activated by a variety of toxins, ionic dysfunction and subcellular relocalization of proapoptotic molecules. The key event in this pathway is the release of the electron carrier cytochrome *c* from the inner mitochondrial space into the cytosol.² Cytochrome *c* release is necessary but not sufficient for activation of downstream caspases;^{3–5} other mitochondrial proteins including Smac/Diablo AIF, HtrA2/Omi and EndoG may also be released to augment death.^{5–7} These proteins either cleave and inactivate inhibitor of apoptosis (IAP) proteins or cause nuclear degradation. These processes are required for cells to develop competence to die.^{6,7} Cytochrome *c* is one of several factors required for the formation of the apoptosome, a (d)ATP-dependent caspase activation and amplification complex⁸ which includes apoptosis protease activating factor (Apaf-1) and procaspase 9.⁹

Plasma membrane ‘death’ receptor pathways can be induced by a variety of extrinsic stimuli. These receptors belong to the tumor necrosis factor (TNF) super-family of cytokines involved in proliferation, differentiation and inflammation. TNF receptors are expressed on inflammatory, immune and microvasculature cells as well as on neurons and glia. TNF is produced mainly by activated macrophages and T cells in response to infection.¹⁰ Ligand binding to this receptor induces receptor trimerization and clustering at the plasma membrane. A death-inducing signaling complex is then formed through recruitment of cytosolic proteins in to close proximity of the cytoplasmic tail of TNF receptor, through the so-called death domains. Adaptor proteins bind with death receptors in order to create a death-inducing signaling complex which can include caspases (most notably caspases 8 and 10), kinases and structural proteins.¹¹ Receptor trimerization and DISC assembly results in initiator caspase cleavage, which directly activate caspase-3 and/or processes Bid into truncated Bid, which translocates to the mitochondria and elicits cytochrome *c* release.¹¹

Activation of caspase 3 by each of these pathways has historically been viewed as a terminal event in the cell death process. However, emerging evidence now suggests that not only can caspase 3 activation be held in check by a variety of cellular defense proteins, but also that activated caspases are essential for normal cell functioning including differentiation, process outgrowth and even neuroprotection.

The not-so-new news

Caspase activation is essential for differentiation, normal cell signaling and maturation

The apparently paradoxical role of the so-called killer proteases in mediating normal cell function has been described in a variety of non-neuronal systems. Caspase 3 activation is essential for terminal differentiation of lens cell precursors, erythrocytes, skeletal myoblasts, keratinocytes and monocyte-macrophage precursors^{12–15} as well as spermatid individualization and T cell activation.^{16,17} Moreover, non-lethal caspase activation in these processes can be elicited by either the mitochondrial or death receptor pathway.

The mitochondrial ‘death’ pathway is activated during the differentiation of monocytes into macrophages— a process in which cytochrome *c* is released and caspase 3 is activated. However, cellular substrates of caspase 3 such as poly(ADP-ribose)polymerase (PARP) remain intact during this process, suggesting that effector caspase activity is limited in scope and/or locally regulated.¹⁴ Interestingly, the individualization of *Drosophila* spermatids also requires

cytochrome *c* redistribution,¹⁶ although the gene encoding the apoptosis linked form of cytochrome *c* in this species does not appear to be required for cellular respiration.¹⁸

Recent work by Cauwels and colleagues has demonstrated that caspases can also be activated by death receptor pathways as an adaptive response to cell stress. Caspase inhibition sensitized mice to the lethal effect of recombinant TNF- α .¹⁹ The death-accelerating effect of caspase inhibition correlated with an increase in lipid peroxidation, and was significantly attenuated by antioxidants and inhibitors of phospholipase A₂. The authors speculated that caspases normally cleave and inactivate the reactive oxygen species (ROS) generating enzyme phospholipase A₂ and that by blocking caspase cleavage, they increase ROS production was increased, resulting in hastened necrotic cell death.

Caspase activation in neuronal tissue

Role in synaptic plasticity and growth cone guidance

Recent work has extended the role of activated caspases in adaptive cellular functions to CNS. Indeed, caspase 3, which is the most abundant cysteine protease in the brain, plays a critical role in axon guidance and synaptic plasticity.^{20–22} Axons navigate long distances to their targets using a succession of attractive and repulsive diffusible and substrate-bound molecular cues expressed throughout the brain.²³ Given the wide array of biochemical signals which are continually barraging expanding growth cones, it is perhaps not surprising that local signaling pathways within the growth cones provide rapid and critical regulation of neurite trajectory.

There is an extensive body of work concerning proteasome function and developmental processes such as cell fate and axon guidance. Intact proteasomal function is required for growth cone collapse and turning but not extension,²⁴ and the ubiquitin-proteasome system has shown to contribute to the rapid regulation of proteins critical for synaptic transmission, plasticity and morphological restructuring following BDNF application (Cline²⁵). In non-mammalian systems, the ubiquitin-proteasome system has been shown to be essential for axon pruning—a critical element for neural circuit refinement.²⁶

More recent work has revealed an association between proteasome function and caspase activation (see subsequent subsection: Proteasomal Regulation of Activated Caspases is Likely Critical to Limiting Protease Activity). Campbell and Holt have demonstrated that not only is local protein synthesis and proteasome mediated degradation within growth cones critical to neurite outgrowth, but that caspase 3 activation is essential for retinal growth cones to develop an appropriate chemotropic response.²⁰ The authors found significant amounts of activated caspase 3 is present in growth cones exposed to netrin-1 and LPA (which are responsible for turning and collapse respectively). Perhaps most surprisingly, this signaling pathway occurs very rapidly within the extending growth cone as increased caspase 3 cleavage was observed within 5 minutes of exposure to netrin-1 or LPA. Activated caspase 3 appears to be biologically active in this system as well as evidenced by increased cleavage of the protease substrate PARP.

Caspase-mediated protein degradation could serve as a critical signaling component to locally and rapidly allow cells (or parts of cells such as growth cones) to change responses to extrinsic signals. The finding that caspase 3 activation and proteasomal functioning is required for growth cone remodeling is intriguing not only from the standpoints of development and repair, but is particularly relevant to synaptic plasticity where BDNF has been shown to be critical to synaptic remodeling.^{27,28} It, however, remains to be determined if application of caspase inhibitors significantly alters BDNF mediated synaptic remodeling and circuit refinement.

Suggestive evidence exists that synaptic remodeling may also be mediated in part by non-lethal caspase activation. Preliminary work by Mattson and colleagues has shown that mice given

the broad spectrum caspase inhibitor zVAD one hour prior to testing demonstrated significantly impaired memory as assessed by Morris water maze spatial testing.²² Caspases substrates include a subset of AMPA-receptor subunits (GluR 1, 2, 3, 4), four different Cam kinases, over 9 PKC isoforms and PKC interacting proteins, MAP and tyrosine protein kinases all of which have been implicated in synaptic plasticity.²⁹ More detailed studies on this intriguing area are clearly needed to determine the extent, duration, localization of caspase activity in synaptic remodeling and learning paradigms.

Caspase activation in neuroprotection/ischemic tolerance

Ample evidence implicates caspase activation in the neurotoxicity associated with both acute and chronic neurological disorders. Cytochrome *c* has been shown to be released following cerebral ischemia *in vivo*,³⁰ and activation of the mitochondrial cell death pathway occurs following hypoxia *in vitro*.³¹ Moreover, caspase inhibitors are neuroprotective in *in vivo* and *in vitro* models of ischemia.^{32–36} We have, however, recently found that caspase activation also contributes to endogenous pathway which can protect against ischemic and excitotoxic injury.

The phenomenon of ischemic preconditioning is one in which exposure to a subtoxic insult renders tissue less vulnerable to subsequent severe insults (reviewed by Dirnagl *et al.*³⁷). Preconditioning is observed in brain, heart, liver, small intestine, skeletal muscle, kidney, and lung. Neuronal preconditioning can be evoked *in vivo* and *in vitro* by a variety of potentially noxious transient events such as cortical spreading depression, potassium depolarization, inhibition of oxidative phosphorylation, exposure to excitotoxins, beta amyloid, ceramide and cytokines, as well as brief episodes of hypoxia and ischemia (reviewed by Dirnagl *et al.*³⁷).

Emerging evidence suggests that the upregulation of pro-survival elements within preconditioned cells is dependent upon activation of signaling pathways associated with cell stress. For example, in cardiomyocytes and neurons, generation of ROS during preconditioning is critical for subsequent protection. Indeed, blockade of ROS production decreases the protection afforded by preconditioning.^{38–42} Metabolic dysfunction is also a likely contributor to preconditioning. Decline in the ATP/ADP ratio leads to opening of mitochondrial K_{ATP} channels,⁴³ and activation of these channels increases ROS generation.^{44,45} It has also been shown that neuroprotective doses of K_{ATP} openers may increase the release of cytochrome *c* from the mitochondria in neurons.⁴⁶

We recently reported that preconditioning by inhibition of oxidative phosphorylation *in vitro* or transient middle cerebral artery occlusion *in vivo* results in significant caspase 3 activation without inducing lethality. Moreover, caspase inhibition decreased preconditioning-induced neuroprotection. Our data support a mitochondrial caspase activation pathway as death receptor-linked caspase inhibitors do not block the expression of tolerance.⁴⁷ These observations do not, however, preclude the possibility that other preconditioning agents such as amyloid beta, ceramide or other stimuli may activate ‘death’ receptor pathways to evoke sub-toxic caspase 3 activation.

Cellular mechanisms to inactivate caspases

The observation that caspase 3 can be activated without causing large scale proteolysis of cellular substrates and apoptosis suggests that powerful intrinsic cell signaling mechanisms must be in place to limit the extent, location and/or duration of protease activation. Several signaling mechanism have been shown to block the activity of cleaved initiator caspases, notably the phosphorylation of cleaved caspase 9 by ERK2.⁴⁸ These pathways may provide important therapeutic targets for the treatment of cytotoxic injury. However, blockade of effector caspase activity and apoptosome assembly has only been observed with five groups

of proteins: calpains, calbindin, the Bcl-2 family, the inhibitors of apoptosis (IAPs) and members of the heat shock protein family (HSP)^{49,50} (see Figure 1).

Calpains

Calpains I and II are ubiquitously expressed calcium activated proteins which can both cleave and be cleaved by caspases.⁵¹ Calpains can both enhance and dampen caspase induced toxicity. N-terminal truncation of caspase-3 by calpain has been shown to inhibit caspase activation.^{52,53} Similarly, N-terminal truncation of caspase-9 by calpain I results in generation of a p35 peptide which is unable to activate caspase-3^{54,55} Calpains can also degrade several proapoptotic signaling proteins including Apaf 1⁵⁶ and Bax⁵⁷ suggesting that they are critical regulators of cell viability following caspases cleavage as and Bax.

Calbindin

The calcium binding protein, calbindin 28k, is critical for learning and memory and mice deficient in this protein have impaired spatial learning skills and fail to maintain long-term potentiation.^{58,59} Calbindin has also been shown to block apoptosis induced by a number of different stressors⁶⁰ and purified calbindin 28k can bind to and inhibit the action of activated caspase 3.^{49,61}

Bcl-2 family

The Bcl-2 family has been shown to play a complex and varied role in apoptotic cell death. Anti-apoptotic Bcl-2-family proteins suppress cytochrome *c* release from mitochondria, thereby protecting cells from apoptotic stresses (reviewed by Borner⁶²). Bcl_{XL} and Bcl-2 are thought to act, at least in part, by inhibiting pro-apoptotic members of the Bcl-2 family through heterodimerization. Several Bcl-2 family members are targets of caspase cleavage, a process which inactivates anti-apoptotic proteins or generates pro-apoptotic signaling proteins (see Table 1). Heterodimerization-independent activity of Bcl-2 has also been observed and may be mediated by inhibition of mitochondrial channels such as the VDAC.⁶³

There has been significant controversy over the mechanism of Bcl-2 family protection at the level of the apoptosome. Several investigators have reported that some of the antiapoptotic members of this family including Bcl_{XL} bind to Apaf-1 and decrease the proteolytic capability of the apoptosome.^{64,65} Others have not been able to recapitulate these observations although it does appear that Bcl-2 can interact with Apaf-1 if not directly, than through adaptor proteins such as K7 and Aven and likely other proteins which have yet to be identified.^{66,67}

The IAP family

The IAPs are a family of cytosolic proteins containing one to three characteristic baculoviral IAP repeat (BIR) domains. The BIR domains in XIAP, IAP1, and IAP2 bind activated caspases 3, 7 and 9 and functionally sequester them or target them to the proteasome for degradation via C' terminal RING finger domains which acts as an E3 ubiquitin ligase.^{68–71} c-IAP-1 and XIAP are also capable of undergoing negative self regulation via the E3-ubiquitin ligase activity associated with their C-terminal ring domains, which triggers autoubiquitination and targets these proteins to the proteasome.⁷⁰ Degradation of IAPs by Smac/Diablo and HtrA2/Omi does not induce cell death in and of itself, but rather enhances cellular vulnerability to apoptotic stimuli. Tight regulation of IAP expression is clearly required for cell viability as XIAP is also a caspase substrate under death-stimulatory conditions.⁷²

HSP family

The final group of proteins which can block activated caspase 3 signaling are the HSPs. These are highly conserved, abundantly expressed proteins with diverse functions including protein

complex assembly, trafficking, folding and targeting of damaged proteins to the proteasome.⁷³ HSPs are also capable of binding and sequestering activated caspases, APAF and AIF, making them particularly appealing targets for a role in limiting caspase activity.^{74–79} Small HSPs are induced by stress, are phosphorylation sensitive and are capable of oligomerization. HSP 27 has been shown to protect against apoptotic cell death downstream of cytochrome *c* release⁸⁰ and the small HSP alpha beta crystalline binds to the cleaved p24 fragment of caspase 3 before it undergoes maturation into a p20 subunit.⁸¹ HSC 70 is the most abundant HSP found in cells; it is expressed constitutively and is only mildly inducible⁸² whereas HSP 70 is the major inducible HSP found in all cells.⁸³ Not surprisingly, ischemic injury, ROS generation and injuries that induce protein denaturation increase HSP 70 protein expression (reviewed by Yenari *et al.*⁸³). Overexpression of HSP 70 protects the brain against glutamate toxicity, ischemia and oxidative injury.^{84–86} Upregulation of HSP 70 is critical to the neuroprotective pathway employed by preconditioned cells. That is, agents that block preconditioning (such as K_{ATP} channel blockers, caspase inhibitors and free radical spin traps) all block the induction of HSP 70.⁴⁷ The redistribution of chaperones to activated caspases and proteins that have been denatured by ROS is critical for the subsequent induction of HSP 70 and the expression of tolerance.⁴⁷ Taken together these data suggest that it is not so much the activation of caspases as it is the depletion of free pools of constitutively expressed HSPs by activated caspases that induces ischemic preconditioning.

Proteasomal regulation of activated caspases is likely critical to limiting protease activity

Proteins are targeted for degradation by the 26S proteasome via covalent attachment of multiple monomers of the 76 amino acid polypeptide ubiquitin. This process, called ubiquitination, takes place in a multistep reaction governed by four groups of enzymes; the ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), ubiquitin-protein ligases (E3)⁸⁷ and the ubiquitin chain assembly factors (E4).⁸⁸ The 26S proteasome is an ATP-dependent multisubunit complex found in both the cytoplasm and in the nucleus, and is the central proteolytic machinery of the ubiquitin/proteasome system. The 20S particle of the proteasome contains multiple subunits which possess chymotrypsin-like, trypsin-like and caspase-like cleavage sites.^{89,90}

Chronic proteasomal inhibition has been implicated in the pathogenesis of a number of neurological diseases.⁹¹ The role of the proteasome in maintaining cell viability is likely heavily dependent upon its ability to degrade proapoptotic as well as antiapoptotic proteins,^{92–94} a process which is dictated by currently ill-defined factors which dictate substrate specificity. In neuronal cells, proteasome inhibitors can, in some instances, protect against apoptosis by degrading caspases themselves,⁹⁵ by acting upstream of caspase activation,⁹⁶ by enhancing the degradation of such targets as BH-3 proapoptotic proteins⁹⁴ and cytotoxic kinases,⁹⁷ as well as by inducing the expression of molecular chaperones and enhancing MAPK phosphorylation.^{94,98,99} A fine balance must therefore exist to regulate cell fate decisions in response to alterations in proteasomal function and presentation of substrates.

The observation that proteasomal function must be maintained for synaptic strengthening and circuit refinement and that proteasome inhibitors block cleavage of caspase 3 suggests that anti-apoptotic proteins are likely targeted for degradation in a process which enhances non-lethal caspase activation.²⁰ However, it remains to be determined which proteins are degraded, and if and when, caspases are degraded as a part of a chemotropic response.

Limited cleavage of a subset of caspase substrates and spatial and temporal isolation of activated caspases may enhance cellular survival

Caspase activation is clearly an integral component of adaptive cellular responses; however, the question remains of why protease activation is necessary given the fine balance that must exist between maintaining normal cellular functioning and causing cell death. Two possibilities exist: first, limited caspase activation may enhance intrinsic cell survival pathways by inducing upregulation of cytoprotective proteins or second, caspases may selectively target proteins whose normal function could be deleterious to cell survival under stress. Indeed, there is support for both of these possibilities. We have found that limited caspase 3 activation by mild stressors is required for the upregulation of the cytoprotective protein HSP 70. Given that HSP 70 is capable of blocking both apoptotic and non-apoptotic cell death,⁸³ enhanced synthesis of this protein is clearly an adaptive response to limited caspase cleavage. It remains to be determined if similar adaptations are involved in processes such as neurite outgrowth and circuit refinement, although clearly some mechanism must be in place to limit the scope and duration of caspase activity.

As to the hypothesis that caspases cleave potentially deleterious signaling proteins, there is significant support for this as well. Substrates such as phospholipase A2, which generates ROS, and the DNA repair enzyme PARP, which depletes cellular ATP levels, are cleaved in some systems as part of the non-apoptotic functions of caspases. Indeed, caspase mediated PARP cleavage appears to be important for ischemic preconditioning¹⁰⁰ as well as other forms of neuroprotection⁸² and the chemotropic response.²⁰ Table 1 summarizes substrates whose cleavage by caspases results in potentially adaptive cellular responses. Equally, if not more, important to the substrates which are cleaved by caspases in these adaptive processes are the substrates which are spared. Cleavage of substrates such as DNA fragmentation factor, c140 replication factor and other proteins integral to maintaining cell viability are routinely spared in neuronal and non-neuronal cells in which caspase activation is required for normal cell functioning.¹⁰¹ Table 2 is a summary of proteins which appear to be critical for maintaining viability and therefore must remain intact or cells will die.

There appears to be great specificity in the location, duration and interaction of caspases with proteolytic targets which suggests that caspase activity is limited in scope and/or locally regulated. This specificity can be obtained at a number of levels including the requirement for post translational modification of some caspase substrates for efficient cell death execution. For instance, the lamin intermediate filament proteins which underlie the nuclear membrane and mediate membrane interactions with chromatin must be first phosphorylated by protein kinase C to be efficiently degraded by caspases.¹⁰² The complex relationships between kinases, phosphatases and other post translational modification enzymes and caspases are only beginning to be understood. A further example of this complexity is the recent observation that caspases cleave protein phosphatase 2A¹⁰³ which increases this enzyme's ability to dephosphorylate targets such as MAPK;^{104,105} however the MAP kinase kinase (MEKK1) becomes constitutively active when cleaved by caspases.¹⁰⁶

When caspase activation is non-lethal and adaptive, the activation may be limited to discrete subcellular compartments. For example, the process of spermatid individualization requires activation of the drosophila caspase homologue drICE, but protease cleavage was only observed in a cytoplasmic compartment that is eliminated during this maturational process.¹⁶ Similarly, activated caspase-3 is essential for the maturation of megakaryocytes and immunohistochemical support exists for protease activation in puncta within and around mitochondria. In contrast, caspase mediated apoptosis in these cells results in diffuse caspase activation throughout the cell.¹⁰⁷ In addition to the spatial isolation of activated caspases, most studies suggest that temporal restriction of caspase activity is essential for maintaining cell

viability. Indeed, caspases are only transiently activated during erythroid differentiation process, and caspase 8 mRNA expression is appreciably downregulated in monocytes undergoing differentiation.¹⁰⁸ We have observed caspase 3 cleavage within 6 hours of preconditioning stress *in vitro* and *in vivo*, but that this activity is substantially less than that evoked by the apoptogen staurosporin (McLaughlin and Aizenman, unpublished observations), and that caspase activity is rapidly dampened following preconditioning.⁴⁷

Ramification of use of caspase inhibitors in neurological disorders

Caspases cleave a number of proteins associated with chronic neurodegenerative diseases including amyloid precursor protein, presenillins, *parkin*, *huntingtin*, dentatorubral pallidoluysian atrophy protein, androgen receptor and *atrophin-1* (reviewed by Fischer *et al.* 29). With the notable exception of the presenillins and *parkin*,¹⁰⁹ cleavage of these substrates results in generation of a toxic fragment and may contribute to the formation of protein aggregates and proteasomal dysfunction.

Similar pathology may contribute to an inherited form of Parkinson's disease caused by a mutation in the *parkin* gene. *Parkin* is an E3 ubiquitin ligase and caspases substrate which when cleaved (or mutated in a familial form of PD) results in loss of protein function and inefficient cellular ubiquitin conjugating activity.^{110,111} Loss of *parkin*-mediated ubiquitination likely contributes to the formation of protein aggregates which are important to the neuropathological changes associated with the disease. Caspase cleavage of *parkin* may also contribute to the pathology of PD in sporadic forms of the disease.^{110,111}

In spite of the strong evidence suggesting that caspase activity is an integral component of the neurotoxicity observed in a number of chronic neurodegenerative diseases, there is at least some suggestive evidence that the sustained cell stress in chronic degenerative diseases may lead to enhanced survival to acute stressors, an adaptation that may be akin to preconditioning cells. Indeed, mice expressing exon 1 of the human Huntington gene are resistant to intrastriatal injections of quinolinic acid, dopamine or 6-hydroxydopamine.¹¹² Given the increasingly complex role of caspase activation in normal cell functioning and adaptation to stress, coupled with the very real risks of autoimmune disease and formation of cancerous tumors, the possibility of long term systemic delivery of caspase inhibitors for these types of chronic diseases is untenable. However, tissue-specific delivery of selective inhibitors for treatment of acute degenerative events such as seizures, head trauma, hypoxia or stroke is currently considered to be more realistic and has shown impressive preliminary proof of principle observations.^{114–116} In events involving large scale neurological compromise, loss of the 'kinder' effects of killer proteases (such as mediating reinnervation and upregulating prosurvival factors) is much less of a consideration than the immediate need to block massive neuronal apoptosis.

The clinically gray area of these recently reported adaptive consequences of caspase activation falls in the potentially over-aggressive treatment of events such as transient ischemic attacks or mild neurological surgeries with antioxidants and protease inhibitors which may, in fact, dampen the initiation of appropriate and adaptive cellular defenses. Indeed, clinical reports suggest that transient ischemic attacks confer a more favorable prognosis on patient who suffer subsequent strokes^{117,118} although this observation is not without controversy.¹¹⁹ Ischemic preconditioning is already used in conjunction with procedures such as angioplasty and coronary bypass, and patients with a prior history of angina sustain smaller infarcts and increased survival following myocardial infarction (reviewed by Carroll and Yellon¹²⁰). These findings call into question the traditional models of cell stress management and offer the potential of identifying an entirely new class of therapeutic targets.

Conclusions

The neuroadaptive changes which neurons undergo during circuit formation and refinement, and when mounting cell stress responses make them especially vulnerable to death. Inappropriate regulation of caspase activation during these events could lead to apoptosis. As the signaling pathways responsible for limiting caspase activation and maintaining adaptive proteasomal functioning become more fully understood, novel targets for protection from focal stroke and brain injury will likely be identified. The development of pharmacological preconditioning agents could provide more efficacious and safe methods to protect the brain in individuals at high-risk of stroke as supplements given prior to invasive cerebral surgery or following transient ischemic attacks. Moreover, some of the interventional strategies being developed to treat patients with chronic neurodegenerative diseases or patients subject to invasive neurological procedures should be critically evaluated given that blockade of cell death pathways may actually prove deleterious to regeneration and viability in some circumstances.

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Figure 1.

Mechanisms of attenuating caspase activity. (A) Caspases are normally proteolytically processed into p10 and p20 subunits following removal of an amino terminal cap. Calpain proteolysis of caspases results in formation of an aberrant, functionally inactive p35 subunit. (B) Activation of the mitochondrial cell death pathway causes release of cytochrome *c* and other sequestered proteins into the cytosol and amplification of caspase cleavage via apoptosome assembly. Caspase activity can be limited by molecules (such as Bcl-2 as depicted in this diagram) competing with procaspases and cytochrome *c* for binding directly or indirectly via adaptor proteins to Apaf. (C) Activated caspases are bound by intracellular defense proteins such as calbindin as well as members of the IAP and HSP family which limits their interaction

with substrates. Caspases are likely degraded follow protein binding via ubiquination and proteasomal cleavage, a process which has been well characterized with activated caspase interaction with the IAP family.

Table 1

Caspase substrates that can be cleaved with non-lethal or adaptive consequences

	Name/function	Effect of cleavage	Reference
Acinus/gelsolin	Cytoskeletal/structural proteins	maybe important for maturational processes involving enucleation	14
Apaf-1	Apoptosis protease activating factor	↓ Apoptosome mediated caspase cleavage	121, 122
FAK	Focal adhesion molecule	↑ Outgrowth	123
GATA-1	Erythropoetic transcription factor	Regulate erythropoiesis	15
GLUR 1 & 4	AMPA glutamate receptors	↓ Glutamate responsiveness	124
Lamin B Actin, Vimentin, Gelsolin	Cytoskeletal/structural proteins	↑ Outgrowth/cell spreading	102
MSTI	Mammalian sterile twenty like kinase	Important for skeletal muscle differentiation	125, 126
PARP	Poly ADP ribose polymerase	↓ Energy consumption	102, 127, 100
Plectin	Cross-linking protein		128
PLA ₂	Phospholipase A ₂	↓ Production of reactive oxygen species	19
p21 ^{CIP1/WAF1}	cdk2 inhibitor	↑ Cylin dependent kinase 2 activity	129
p35	Pan caspase inhibitor	↑ Caspase inhibition	130
RasGAP	Ras GTPase activating protein	N' terminal cleavage blocks caspase 9 induced cell death	131
Wee1	Inhibitory kinase of cdc2 & cdk2	↑ Cell cycle progression	101

Table 2

Caspase substrates whose cleavage results in cell death

	Name	Effect of cleavage	Reference
Bax, Bad, Bcl, Bcl _{XL}	Bcl2 family members	Proapoptotic fragments formed or loss of anti-apoptotic activity	132, 133
c140	Replication factor C	Required for DNA replication	101
DFF45	DNA fragmentation factor	Cleavage results in massive DNA damage	101
IAPs	Inhibitors of apoptosis	Increased vulnerability to apoptosis	134
NFκBp65	Nuclear factor kappa B p65 subunit	Proapoptotic fragment formed	135
Parkin	Mutated in some forms of Parkinson's disease; a ubiquitin ligase	Cleavage enhances cell death presumably by enhancing protein aggregation	110, 111