Review

Cell PRESS

Caspase-3 in the central nervous system: beyond apoptosis

Marcello D'Amelio[1,2], Morgan Sheng[3] and Francesco Cecconi[1,4]

[1] Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), S. Lucia Foundation, via del Fosso di Fiorano 65, 00143 Rome, Italy
[2] University Campus Bio-Medico, Via Alvaro del Portillo 21, 00128 Rome, Italy
[3] Department of Neuroscience, Genentech Inc., South San Francisco, CA 94080, USA
[4] Dulbecco Telethon Institute, Department of Biology, University of Rome 'Tor Vergata', 00133 Rome, Italy

Caspase-3 has been identified as a key mediator of neuronal programmed cell death. This protease plays a central role in the developing nervous system and its activation is observed early in neural tube formation and persists during postnatal differentiation of the neural network. Caspase-3 activation, a crucial event of neuronal cell death program, is also a feature of many chronic neurodegenerative diseases. This traditional apoptotic function of caspase-3 is challenged by recent studies that reveal new cell death-independent roles for mitochondrial-activated caspase-3 in neurite pruning and synaptic plasticity. These findings underscore the need for further research into the mechanism of action and functions of caspase-3 that may prove useful in the development of novel pharmacological treatments for a diverse range of neurological disorders.

Introduction

Programmed cell death (PCD) is recognized as an important process in the normal development of the nervous system [1–3] and caspase-3 was identified as a key mediator of cell death in neuronal cells [3]. Caspase-3 is a member of the cysteine-aspartic acid protease (caspase) family. Like other caspases, caspase-3 exists as an inactive proenzyme that undergoes proteolytic processing at conserved aspartic residues to produce two subunits, large and small, that dimerize to form the active enzyme [4].

The evidence that caspase-3 activity is finely modulated at various levels has partly disproved the concept that caspases are merely executers of cell death [5]. Rather, in many instances, unexpected nonapoptotic functions of the entire apoptotic machinery emerged (see below and Box 1). Caspase-3 has been identified as a relevant enzyme in physiological processes that do not result in cell death and are localized in a specific neuronal compartment: the synapse. The local synaptic activation of caspase-3 in mature brains seems to be involved in many regulatory mechanisms, ranging from synaptic plasticity in normal brain functions to the synaptic pathology observed in many neurodegenerative disorders. Here, we discuss recent findings on its apoptotic and nonapoptotic neuronal functions.

Corresponding authors: D'Amelio, M. (m.damelio@hsantalucia.it); Cecconi, F. (francesco.cecconi@uniroma2.it).
Keywords: Alzheimer's disease; neuronal differentiation; synaptic plasticity; long-term potentiation; long-term depression; synaptic loss.

Mechanisms of caspase-3 activation and regulation

In mammals, two primary routes have evolved for activation of the caspase cascade: the extrinsic cell death pathway [6], which requires cell surface receptors, and the intrinsic cell death pathway [7]. The latter is characterized by mitochondrial outer membrane permeabilization (MOMP) events, in which B cell lymphoma 2 (Bcl-2) family members play an essential role. This protein family is divided into two subgroups: proapoptotic (containing members such as Bax, Bak) and antiapoptotic (including Bcl-2 and Bcl-X_L, among others); a signature of members is the presence of one or more Bcl-2 homology (BH) domains. Proapoptotic proteins, as a consequence of apoptotic stimuli, undergo conformational changes [8] and constitute oligomers, causing MOMP via the formation of pores in the mitochondrial outer membrane [9]. The activity of proapoptotic proteins, which are counteracted by antiapoptotic Bcl-2 family members [10], results in release of cytochrome *c* into the cytosol, which induces the formation of the Apoptotic protease activating factor 1 (Apaf1)-containing macromolecular complex, called apoptosome. Once the apoptosome is formed, it recruits procaspase-9 via caspase recruiting domain (CARD) interactions, involving the N-terminal CARD of Apaf1 [11]. This acts as a docking motif for procaspase-9, and the Apaf1–caspase-9 apoptosome complex then efficiently recruits and directly cleaves procaspase-3 [12].

The extrinsic and intrinsic cell death pathways converge at caspase-3, which, together with other effector caspases, orchestrates dismantling of the cell structure through cleavage of specific substrates [13]. Activation of caspase-3 is a necessary but insufficient event for execution of apoptotic cell death, and in some instances it may exert unrelated cell death roles by targeting specific substrates. Indeed, fine tuning of caspase-3 activity may prevent completion of a full apoptotic program but ensure physiological activities unrelated to cell death. Therefore, molecular controllers are required to modulate caspase activity. At least three distinct types of caspase regulator, inhibitor of apoptosis proteins (IAPs), FADD-like IL-1β-converting enzyme (FLICE)-inhibitory protein (FLIP) [14,15] and calpain [16], have been characterized. Among these, IAPs are the best studied factors in the mitochondrial route of caspase-3 activation.

Several IAPs have been identified in mammalian cells and some directly inhibit caspases, including X-linked IAP (XIAP) [17] and IAP-1 (cIAP-1) [18]. For instance, XIAP prevents cell death by directly binding to upstream caspase-9, and downstream caspase-3 and -7. The baculovirus

Box 1. Nonapoptotic roles for apoptotic molecules: just a paradox?

Besides their crucial role in initiating, mediating, controlling, and/or executing apoptosis, alternative, often paradoxically opposite, functions for several key molecules in this process are being unraveled [108]. What we describe in the text for caspase-3 activity in synaptic plasticity and degeneration is not the only alternative role of this enzyme. Terminal differentiation of vertebrate lens fiber cells [109] and erythrocytes [110], as well as the transition from spermatid to spermatozoa in *Drosophila* [47], all involve proteolytic degradation of major cellular compartments by caspase-3. The fact that a giant ubiquitin-conjugating enzyme (dBruce) protects *Drosophila* sperm nucleus from hypercondensation and degeneration suggests the possibility that other fine regulators of caspase-3 activity may protect the cell from more general destruction [111].

Another example of paradoxical effects of apoptotic molecules involves Apaf1. First, Apaf1 migrates to the nucleus and regulates cell cycle arrest on DNA damage in cell culture systems [112], although the mechanisms involved are still unknown. Second, and more surprisingly, Apaf1 plays clear pro-survival roles by allowing centrosome formation and cytoskeleton activities such as cell migration, mitotic spindle formation, and maintenance of the nuclear morphology in neural progenitors [113]. The double action of Apaf1 may indeed only sound paradoxical, because the Apaf1 ortholog CED4 in *Caenorhabditis elegans* exerts both pro- and antiapoptotic functions by means of two different isoforms [112].

IAP repeat 2 (BIR2) domain of XIAP directly inhibits the active sites of caspase-3 and caspase-7, whereas the BIR3 domain blocks caspase-9 activity [19]. Thus, XIAP inhibits caspase-mediated cellular destruction. It was more recently demonstrated that XIAP has E3 ligase activity [20], and it has been proven that the antiapoptotic role of XIAP depends on its ability to ubiquitinate active caspase-3 and target it for degradation by the proteasome.

Apoptotic roles for caspase-3 in brain development

During normal central nervous system (CNS) development, caspase-3-mediated apoptosis plays an essential role in sculpting and remodeling of neuronal networks of the mature CNS. It is estimated that approximately half of the neurons produced during development are eliminated by apoptosis during CNS maturation [21–25]. Neurodevelopmental apoptosis is mainly regulated by the Bcl-2 family proteins, by the adaptor protein Apaf1, and by the caspase family through molecular mechanisms in both the mitochondrial and the death receptor pathways. According to the timing of apoptosis, it is possible to identify two different types of cell death in neurodevelopment: proliferative cell death, involving neuronal precursor cells (NPCs), and neurotrophic-related cell death, involving post-mitotic neurons.

The major role in proliferative cell death seems to be carried out by morphogenetic signals such as bone morphogenetic proteins (BMPs), Wingless-type proteins (Wnts), fibroblast growth factors (FGFs), and Sonic hedgehog (Shh) [25,26], as confirmed by the evidence that NPCs undergo apoptosis by apoptosome formation on withdrawal of morphogens. By contrast, the absence of neurotrophins such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin 3 (Nt3) and Nt4/5 and the consequent lack of stimulation of their receptors induce apoptosis in post-mitotic neurons (Figure 1) [25]. Although each type of cell death is regulated by different stimuli and involves different pro- and antiapoptotic molecules clustered in developmental-stage-specific pathways, the role of caspase-3 in apoptosis remains essential during all stages of CNS development.

The key role of neuronal apoptosis and the crucial involvement of caspase-3 in CNS development is underscored by the severe abnormalities of CNS architecture and neuronal circuits observed in animal models in which the neural cell death program is impaired. In *Drosophila*, mutations of the initiator caspase Dronc and the executioner caspase Drice prevent most embryonic PCD [26–29].

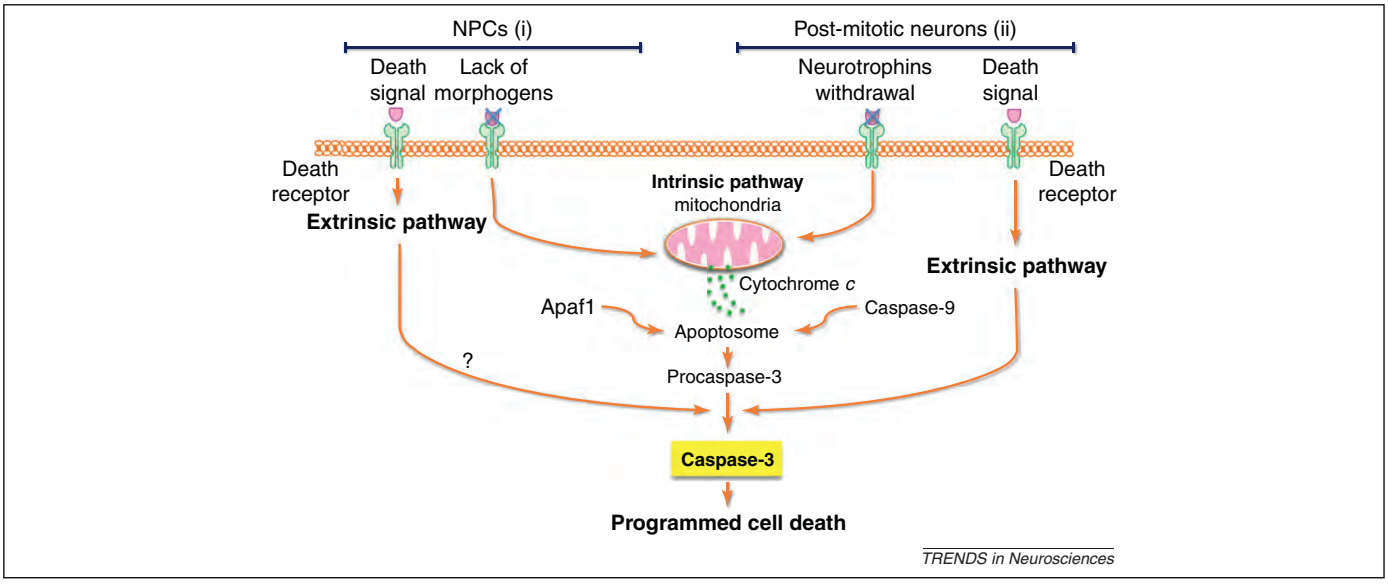

Figure 1. Caspase-3 and the regulation of programmed cell death (PCD). Caspase-3 regulates PCD in neural precursor cells (NPCs) and post-mitotic neurons [21–25]. (i) NPCs undergo apoptosis by apoptosome formation on the withdrawal of morphogens, or potentially on signaling via death receptors [25,26]. (ii) By contrast, the absence of neurotrophins and the consequent lack of stimulation of its receptor induce PCD in post-mitotic neurons [25]. The action of the extrinsic death receptor pathway has been described in post-mitotic neurons; by contrast, this role has not clearly been elucidated in NPCs. The lack of morphogens in NPCs or neurotrophin withdrawal in post-mitotic neurons results in activation of the intrinsic cell death pathway. Activation of this pathways results in induction of cytochrome *c* release from mitochondria, which together with caspase-9 and Apaf1 constitutes the apoptosome [11]. The apoptosome is a catalytic multiprotein platform responsible for converting procaspase-3 into active caspase-3 [12], which subsequently orchestrates the dismantling of cell structure.

In addition, flies with mutations of the apoptosome Apaf1 homolog (Dark/Hac-1/Dapaf-1) display a phenotype similar to that of Dronc or Drice mutants, proving that the mitochondrial (intrinsic) pathway is the main route for PCD in the *Drosophila* embryo [30,31].

Gene-targeted or gene trap knockout mice have provided major insights into the importance of caspase-3 and its upstream regulators in CNS development. In fact, caspase-3-deficient embryos show prominent protrusion of brain tissue associated with skull defects and ectopic cell masses between the cerebral cortex, the hippocampus, and the striatum [3]. However, the phenotype of caspase-3-deficient mice greatly depends on the genetic background, as demonstrated by the minimal macroscopic brain pathology observed in caspase-3-deficient C57BL/6J mice [32]. Severe neural phenotypes are also found in mice deficient for the apoptosome components Apaf1 or caspase-9, which are required for the mitochondrial caspase-activating route [33,34].

Caspase-3 in postnatal brain sculpture

Apoptosis of post-mitotic neurons (before synaptogenesis) may be relevant to the elimination of ectopically located neurons [21–25,35]. Recent data on the temporal and spatial distribution of apoptosis introduce the concept that neuronal death in a specific brain region is important for late compartmentalization of the same area and for its wiring [36].

The cerebellar cortex is a well-studied example of how postnatal and selective apoptosis of Purkinje cells (PCs) contributes to the formation of cortical structures that are functionally and morphologically organized [36,37]. The importance of postnatal PCD in the cerebellum was demonstrated in a study of postnatal cerebellar development in *Bax* knockout mice. Ablation of the proapoptotic gene *Bax*, which appears to be critical for post-mitotic neuronal PCD [38], led to a selective reduction in PCD [39]. Moreover, a subpopulation of PCs was misplaced by the end of the major period of cerebellar histogenesis (approx. 15 days after birth). These results suggest that PCD might play an important role in removing misplaced neurons in the postnatal period.

Nonapoptotic roles for caspase-3 activity in the CNS

Even though caspase-3 is a pivotal protein in apoptosis execution, completely new avenues of research were opened when recent reports indicated a possible nonapoptotic role for this enzyme in neuronal differentiation and development. It is interesting to note that inhibition of caspase-3 by z-DEVD-fmk (an irreversible cell-permeable inhibitor with some selectivity for caspase-3 and -7) on the one hand did not affect apoptosis during differentiation, but on the other hand altered the expression of key proteins involved in differentiation. Changes in the expression profile of proteins such as nestin (a neuronal intermediate filament protein implicated in axon growth), glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP) (GFAP and MBP are two proteins involved in the differentiation of astrocytes and oligodendrocytes, respectively) or β-III tubulin delayed differentiation, as demonstrated by reduced extension of neurites protruding from neurosphere bodies [40]. Although the exact mechanism by which caspase-3 activity promotes neuronal differentiation remains to be elucidated, it may depend on cleavage-dependent activation of specific substrates leading to activation of signaling pathways. This has been demonstrated during caspase-mediated differentiation of skeletal muscle myoblasts [41] and epithelial cell types [42], and in growth cone guidance, in which caspase-3 has been identified as a potential target of p38 signaling for responding to the chemotropic molecules netrin-1 and lysophosphatidic acid [43].

Other nonapoptotic roles for caspase-3 have also been reported. For instance, caspase-3 activity is critical for the post-translational mechanism that regulates the self-renewal machinery of embryonic stem cells [44]. In particular, it has been demonstrated that caspase-3 acts on Nanog (a transcription factor critically involved in self-renewal of undifferentiated embryonic stem cells [45]) by specific proteolytic cleavage that induces its destabilization and degradation, thereby promoting stem cell differentiation. Caspase-3 is also required for the refinement of neuronal circuits during development, which involves selective elimination of axons, dendrites, and synaptic connections, without necessarily the death of parental neurons (discussed further below). One of the issues regarding these more recently described roles is how the function of caspase-3 can be confined. Specifically, how is caspase-3 activated locally (i.e. at specific spatially defined cellular compartments rather than throughout the entire neuron) to promote the dismantling of specific neuronal structures that are destined to be eliminated in a genetically defined program of neurodevelopment? Such a constraint could be imposed by at least three different cellular strategies: (i) local caspase activation via dynamic interactions with specific endogenous inhibitors [46]; (ii) the creation of subcellular barriers to caspase translocation within the cell via cytoskeletal or organellar limitations such as a perinuclear shield [47]; and (iii) destruction of the structure that needs to be removed (i.e. a dendritic spine; see below) and extracellular release of active caspase-3, an event that would impair its translocation to the rest of the cytosol and execution of its full proapoptotic roles (including activation of lethal nucleases). Although the first two processes have been experimentally demonstrated, the latter possibility remains speculative.

Upstream regulation of caspase-3 activity is more fully understood. For example, mitochondria are critical for caspase-3 activation, as demonstrated by the neurological phenotype of mice deficient in genes involved in caspase-3 maturation and activation. Both *Apaf1*- and *caspase-9*-deficient mice display misrouted axons, impaired synaptic formation, and defects in the maturation of olfactory sensory neurons (OSNs), confirming a role for Apaf1- and caspase-9-mediated signaling during the establishment of olfactory neuronal networks [48]. It has been demonstrated that Apaf1 and caspase-9 signaling regulates OSN development and participates in the establishment of axonal wiring in the olfactory system by modulating the amount of functional Sema7A (a member of the semaphorin family of guidance proteins) in the axons of OSNs during development [49].

Caspase-3 in synaptic plasticity

N-Methyl-D-aspartic acid (NMDA) receptor (NMDAR)-dependent long-term depression (LTD) involves 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA) receptor (AMPAR) internalization from the post-synaptic membrane [50]. LTD is associated with shrinkage and loss of dendritic spines [51] and thus may represent a functional correlate of synapse elimination. Interestingly, LTD is most robustly inducible in the first 2–3 weeks of rodent life [52], corresponding to the period of maximal synapse elimination.

It has recently been demonstrated that the mitochondrial pathway of apoptosis is necessary for NMDAR-dependent LTD and AMPAR internalization [52,53]. Specifically, release of cytochrome *c* from mitochondria, activation of the apoptosome, activation of caspase-3, and cleavage of the protein kinase Akt are all required for LTD induction (Figure 2a), as demonstrated by the observation that LTD is abolished in knockout mice lacking caspase-3, or BAD and Bax (regulators of MOMP and hence cytochrome *c* release) [52,53]. Moreover, LTD-like stimulation of cultured hippocampal or cortical neurons is associated with rapid but transient and modest activation of caspase-3 in dendrites, as well as in cell bodies, without causing neuronal death [52,53]. LTD is believed to show some degree of synapse specificity, so these findings imply that caspase-3 activation serves a localized nonapoptotic function in the vicinity of synapses during LTD. Indeed, activated caspases can be detected in dendrites, axons, and pre- and post-synaptic compartments of nonapoptotic neurons [1,54,55]. The role of caspase-3 in LTD is one of the most convincing examples of a non-death role of the intrinsic pathway of apoptosis in neurons.

Neurophysiological roles for caspase-3 have been demonstrated by a collection of *in vivo* experimental observations. For example, intracerebroventricular injection of the caspase-3/7a-selective inhibitor z-DEVD-fmk led to a reduction in avoidance reactions in some blocks of behavioral trials during active avoidance learning in adult rats [56].

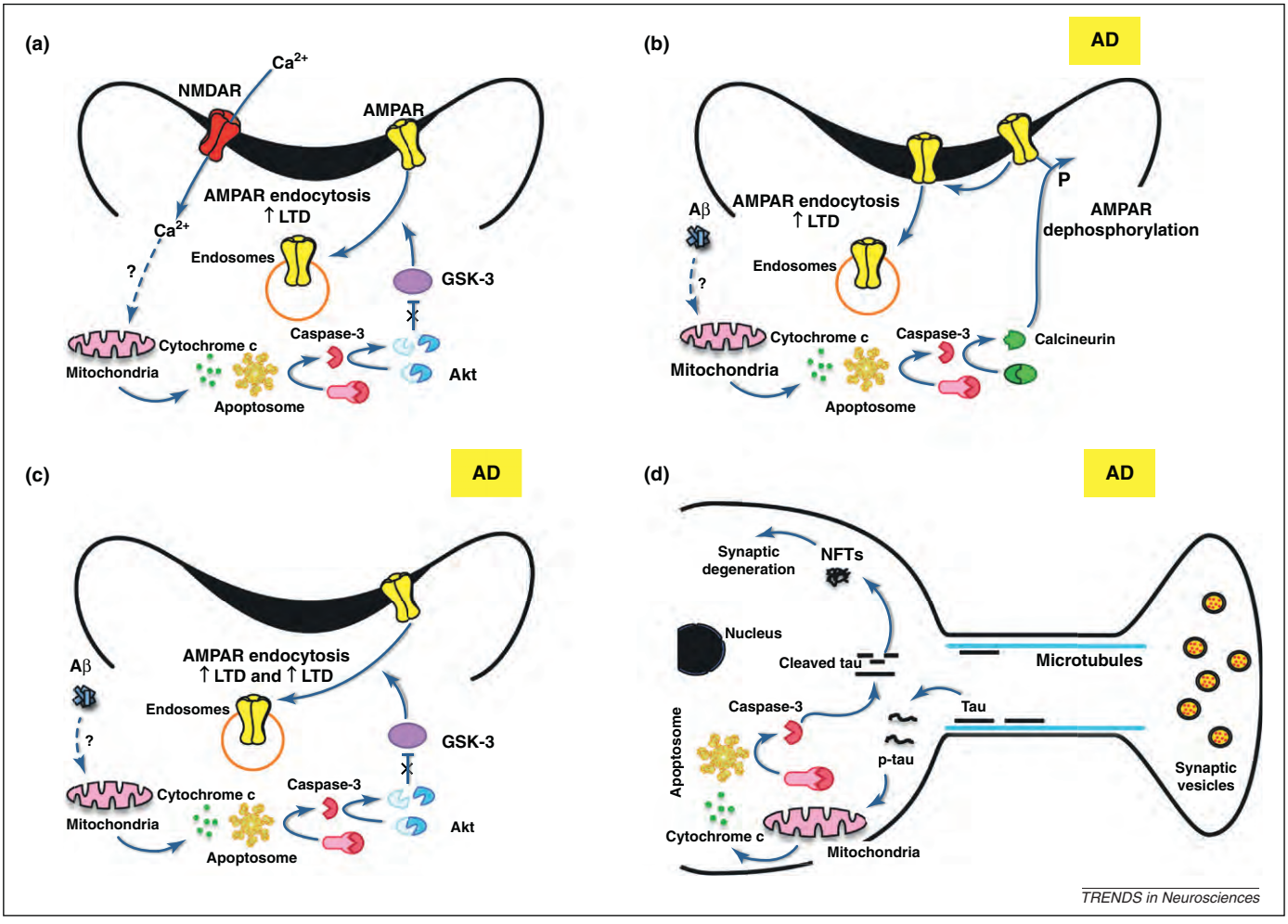

Figure 2. Nonapoptotic roles of caspase-3: from health to disease. **(a)** Under physiological conditions, chemical induction of long-term depression (LTD) in cultured hippocampal neurons requires mitochondrial-activated caspase-3 [52]. LTD-like stimulation leads to activation of the calcium ion-permeable N-Methyl-D-aspartic acid (NMDA) receptor (NMDAR). A series of downstream intermediate signaling steps, including activation of protein phosphatases, causes the release of cytochrome *c* from mitochondria and activation of the apoptosome and caspase-3. Caspase-3-dependent cleavage of the protein kinase Akt removes tonic inhibition of glycogen synthase kinase 3 (GSK-3) activity, which is required for 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA) receptor (AMPAR) endocytosis and induction of LTD [99]. **(b)** In amyloid precursor protein (APP) mutant mice, a model of Alzheimer's disease (AD), amyloid β (Aβ) causes activation of the phosphatase calcineurin via a signaling pathway involving caspase-3. Calcineurin dephosphorylates AMPARs, promoting their internalization and induction of LTD [96]. **(c)** In hippocampal slices from wild-type mice, treatment with the toxic Aβ peptide causes caspase-3-dependent cleavage of the protein kinase Akt and removes tonic inhibition of GSK-3 activity, which is required for AMPAR endocytosis, LTD induction, and long-term potentiation (LTP) inhibition [97]. **(d)** Accumulation of free cytosolic tau activates caspase-3 [94]. Activated caspase-3 cleaves tau, which becomes more prone to fibrillar formation. Truncated tau, including caspase-cleaved tau, rapidly aggregates and forms neurofibrillary tangles (NFTs) [94]. It should be noted that neurons survive in all of the cases presented in (b)–(d), despite the presence of caspase-3 activation. Such activation is an early event promoting the pathology underlying the disease, rather than a terminal event killing the cells.

Furthermore, when z-DEVD-fmk was applied to the cerebellar vermis, it stimulated extinction of an acoustic startle reaction [56]. Similarly, it has been demonstrated that injection of Ac-DEVD-CHO (a caspase-3 inhibitor) in the auditory forebrain of zebra finch resulted in impaired long-term habituation to a song [46].

Nonapoptotic roles for caspase-3 in neurodegeneration

Mitochondrial dysfunction leads to a number of deleterious consequences for neurons, including impaired calcium buffering, generation of reactive oxygen species (ROS), activation of nitric oxide synthase, and activation of the mitochondrial permeability transition. Persistent mitochondrial dysfunction leads to catastrophic effects on mitochondrial energetic balance and contributes to neuron death through release of cytochrome *c* and other proapoptotic factors into the cytosol, and ultimately to caspase-3 activation [57–62].

Although neurodegenerative diseases have widely disparate genetic etiologies and are characterized by disease-specific profiles of adult-onset neuronal loss, mitochondrial dysfunction may be considered a final common pathway that leads to progressive neuronal death within areas of the cerebral cortex, hippocampus, basal ganglia, cerebellum, and brain stem [63–66]. In addition to the bulk of evidence derived from post mortem studies [67–70], the role of PCD in neurodegenerative diseases has been extensively studied using both *ex vivo* and *in vivo* approaches [71–74]. By contrast, in this section we focus on the pathological role of caspase-3 in both the onset and the progression of neurodegenerative disorders in the absence of PCD (Figure 2b–d and Figure 3).

Neurodegenerative diseases such as Huntington's disease (HD), Alzheimer's disease (AD), Parkinson's disease (PD), frontotemporal lobar degeneration (FTLD), and motor neuron disease all share a striking common pathological feature: the aggregation and deposition of abnormal proteins, to which neurons are particularly vulnerable [75]. In neurodegenerative diseases, caspase-3 activity may contribute to chronic neurodegeneration via an apoptotic program by eliminating neurons that have accumulated toxic aggregates over time. Another possibility is that caspase-3 activity may contribute to neurodegeneration by generating toxic fragments from several specific proteins linked to neurodegenerative diseases. Alternatively, caspase-3 may disable antiapoptotic functions of proteins, thus protecting neurons from apoptotic death.

Huntington's disease

A seminal study supporting such an alternative and nonapoptotic role of caspase-3 in neurodegeneration was carried out ~15 years ago and revealed that caspase-3 is implicated in proteolytic cleavage of huntingtin [76,77]. This finding led to the development of a model connecting caspase-3 cleavage of huntingtin to amplification of caspase activity in a type of vicious cycle, known as the toxic fragment hypothesis (Figure 3) [78]. This model postulates that proteolytic cleavage of huntingtin liberates toxic fragments containing the expanded polyglutamine tract, and that accumulation of these fragments leads to activation of additional caspases, and eventually apoptotic cell death. Direct support for a role of caspases in HD pathology is provided by the evidence that caspase inhibitors abrogate huntingtin cleavage, thereby reducing its toxicity [79]. It

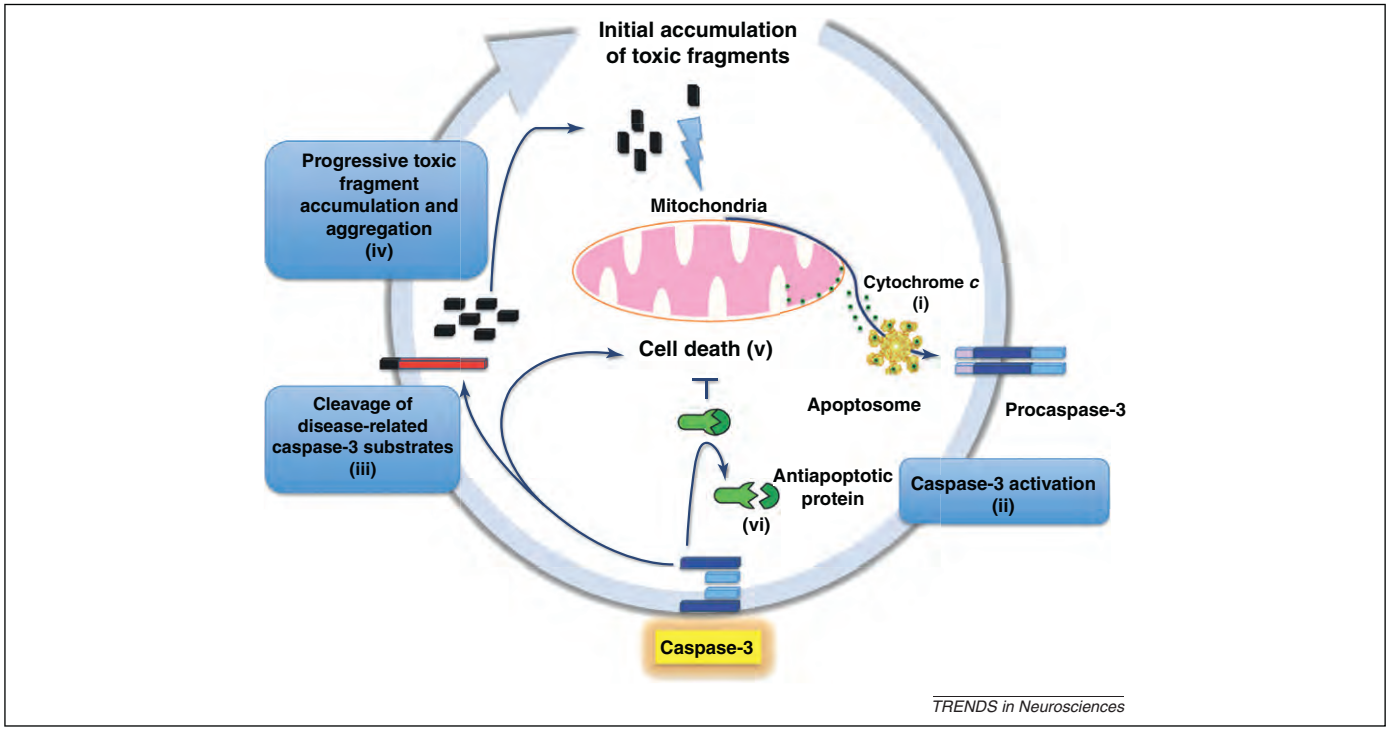

Figure 3. The caspase-3 toxic hypothesis. According to this hypothesis, originally proposed by Wellington and Hayden [78], genetic or sporadic etiologies might alter mitochondrial function, triggering (i) cytochrome *c* release, apoptosome assembly, and (ii) sublethal caspase-3 activation [56–61]. Caspase-3 might then (iii) cleave target proteins, including huntingtin [76,77], amyloid precursor protein [81], and presenilins [84,85]. (iv) Accumulation of toxic fragments generated by caspase-3 can lead to further mitochondrial dysfunction, caspase activation, and ultimately to (v) neuronal death. (vi) Alternatively, caspase-3 might disable antiapoptotic functions, such as presenilin activity, contributing to amplification of its own death function by removing inhibitory mechanisms [90,91].

has also been demonstrated that neurons overexpressing a mutant form of huntingtin that is resistant to caspase-3 proteolysis can escape apoptotic death [79].

Alzheimer's disease

Four proteins have been identified as crucial etiological factors in early-onset AD: amyloid precursor protein (APP), presenilin-1 (PS-1), presenilin-2 (PS-2) and apolipoprotein E. Several caspase-3 cleavage sites have been mapped for three of these proteins. APP is a single-pass transmembrane protein expressed at high levels in the brain. It can be cleaved directly by α-secretase and then by γ-secretase, a process that does not generate the amyloid β (Aβ) peptide (i.e. the non-amyloidogenic pathway). In addition, it can be reinternalized via clathrin-coated pits into another endosomal compartment that contains the proteases β- and γ-secretase [80]. This results in the production of Aβ40 or Aβ42, accumulation of which is a key neuropathological event in AD (i.e. the amyloidogenic pathway) [80]. In addition to proteolytic cleavage by α-, β-, and γ-secretases, APP is also a substrate for caspase-3 cleavage, as confirmed by the presence of three cleavage sites within the protein: two in the extracellular domain and one within the C-terminal intracellular tail [81]. Several studies demonstrated that caspase-mediated cleavage of APP alters its normal proteolytic processing to favor the amyloidogenic pathway. This could potentially occur via dissociation of key protein interaction domains contained within the C-terminal tail of APP [82] or production of a second unrelated C-terminal peptide, the C-31 fragment, a potent inducer of apoptosis [83]. These findings suggest a model of caspase-3-mediated amplification of the toxic fragments of APP very similar to the one proposed for huntingtin in HD (Figure 3).

Presenilins are a family of related multi-pass transmembrane proteins that function as part of the γ-secretase intramembrane protease complex. AD-associated mutations in PS-1 enhance γ-secretase activity and result in increased production of the neurotoxic Aβ42 peptide. PS-1 and PS-2 are also cleaved by caspase-3 [84,85]. One proposed model is that caspase-mediated cleavage of PS may generate toxic fragments, such as the PS-2 loop peptide, perturbing intracellular calcium homeostasis and accelerating apoptosis and/or pathological production of the Aβ peptide [86,87]. Another model is based on evidence that expression of the endoproteolytic C-terminal fragments of PS-2 protects cells from apoptotic death [88,89], suggesting that these fragments may act as dominant negative inhibitors of the proapoptotic effect of full-length PS expression. Caspase effects on PS (i.e. cleavage within the C-terminal fragment produced by the normal endoproteolytic pathway) might sensitize cells to apoptotic stimuli [90,91].

Caspase-3 also cleaves Golgi-localized γ-ear-containing ADP Ribosylation Factors (ARF) binding protein (GGA3) [92], an adaptor protein involved in trafficking of β-secretase 1 (BACE-1) to the lysosome for degradation. Caspase-3-induced depletion of GGA3 impairs the degradation of BACE-1 and enhances β-secretase activity, thereby causing an increase in Aβ peptide production [92]. Caspase-3 might also play a role in Frontotemporal lobar degeneration (FTLD). TAR DNA-binding protein-43 (TDP-43), a major disease protein in FTLD with ubiquitin-positive inclusions (FTLD-U), has been identified as a substrate of caspase-3 [93], although it remains to be determined how this cleavage contributes to neurodegeneration.

Collectively, these are only a few examples supporting the hypothesis that caspase-dependent cleavage of proteins linked to neurodegeneration may contribute to a vicious cycle in neurons (Figure 3), in which caspase activation lowers the cellular stress threshold, compounding mitochondrial dysfunction and leading to further caspase activation, and ultimately to neuronal death. Cleavage of these proteins may thus promote both onset of the disease and execution of the final step of apoptosis.

Accumulating evidence indicates that disruption of connectivity within neural circuits in key brain regions, loss of synapses, and impairment of synaptic plasticity precede the death of neurons [94,95]. Neurodegenerative diseases such as AD do not develop in a few weeks, but over many years prior to the appearance of clinical signs and symptoms. For instance, studies in the Tg2576 mouse model, in which the human *APP* gene harboring the Swedish mutation associated with familial AD is expressed, have demonstrated that apoptosome-dependent caspase-3 activation in hippocampal dendritic spines correlates with the onset of memory decline and dendritic spine loss [96]. Activation of caspase-3 is not associated with neuronal death; conversely, active caspase-3 is localized specifically at synapses, where it activates the phosphatase calcineurin. Activation of calcineurin results in dephosphorylation of GluA1 AMPAR subunit, causing removal of AMPAR from synaptic sites. AMPAR removal leads to progressive spine shrinkage and ultimately to loss of dendritic spines (Figure 2b). Dendritic spine loss represents one of the best pathological correlates of AD.

The crucial role of caspase-3 in synaptic plasticity was also shown in an independent study in which Aβ inhibited long-term potentiation (LTP) through activation of caspase-3, which in turn cleaved Akt [97], disabling its function. This caspase-3-dependent Akt cleavage removes tonic inhibition of glycogen synthase kinase-3 (GSK-3). The subsequent increase in GSK-3 interferes with synaptic plasticity (Figure 2c) [98], besides promoting tau phosphorylation and neurofibrillary tangle formation [99]. Thus, it seems possible that persistent caspase-3 activation would be sufficient to cleave the tau protein, promoting tau aggregation (Figure 2D), as elegantly demonstrated by a multiphoton imaging study [94].

Collectively, these findings suggest that caspase-3 may be involved in neurofibrillary tangle formation and in degenerative synapse loss (Figure 4). Importantly, it has been demonstrated that treatment with the caspase inhibitor z-DEVD-fmk improves early synaptic failure in Tg2576 mice, as assessed by contextual fear conditioning tasks [96]. However, it is important to note that these results were observed in a prodromal stage of AD-like disease (i.e. in young and amyloid-plaque-free mice); whether similar improvements are possible at later stages of the disease process remain to be assessed.

Finally, it is interesting to note that selective enrichment of caspase-3 has been observed in the post-synaptic compartment in post mortem brain from early AD patients

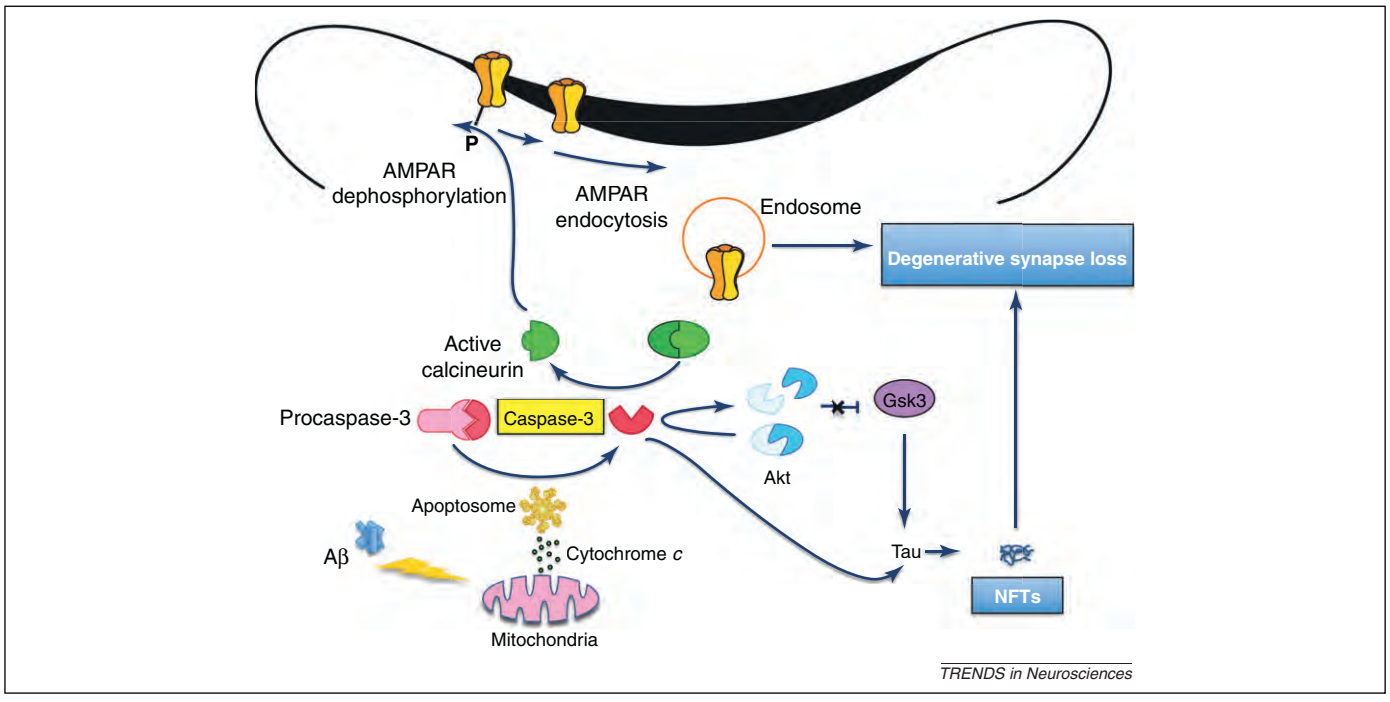

Figure 4. Proposed model for amyloid β (Aβ)-mediated synapse loss. In dendrites, accumulation of Aβ leads to mitochondrial stress, cytochrome *c* release, apoptosome assembly, and caspase-3 activation [96]. Caspase-3 activates calcineurin, which dephosphorylates AMPARs, leading to their subsequent removal from the post-synaptic density (PSD) and causing dendritic spine degeneration [96]. Caspase-3 is also implicated in tau truncation and activation of GSK-3 [52,94,97]. Tau truncation, together with glycogen synthase kinase-3 (GSK-3)-dependent tau phosphorylation, promotes neurofibrillary tangles (NFTs), contributing to synaptic degeneration [98].

[100,101]. Such findings are in line with the above-mentioned experimental observations of a potential role for caspase-3 in the pathogenesis of AD.

Emerging role of caspase-3 in PD

Several studies have suggested that an apoptotic cascade also occurs in PD. In addition to the bulk of evidence derived from post mortem studies [102], numerous *ex vivo* and *in vivo* approaches have revealed how pharmacological or genetic manipulation of apoptosis-related molecules may interfere with the pathogenetic processes leading to PD [71,103,104]. As discussed for other chronic neurodegenerative diseases, caspase-3 might play a crucial non-apoptotic role in PD onset and/or progression.

In the same vein, it has been demonstrated that caspase-3 cleaves synphilin-1 [105], a novel α-synuclein cellular partner whose main functions remain elusive [106]. The functional relevance of this cleavage is puzzling, because caspase-3 in this case seems to confer pro-survival capacities to its target. Indeed, caspase-3 cleavage of synphilin-1 generates a synphilin-1-derived product with antiapoptotic properties. Here, cleavage of a disease-related protein leads to a gain of function, as demonstrated by the evidence that the synphilin-1-induced antiapoptotic phenotype is reduced when synphilin-1 is made resistant to caspase-3 proteolysis [105].

In addition to induction of neuronal apoptosis, many neurodegenerative diseases are characterized by neuroinflammation and the presence of activated microglia. Furthermore, it has recently been revealed that caspase-3 signaling plays a novel role in the control of microglia and brain inflammation in individuals with PD and AD [107]. A strong increase in cytoplasmic expression of both active caspase-3 and active caspase-8 in microglia of the ventral mesencephalon in PD subjects and in microglia in the frontal cortex of AD subjects has been observed compared to controls. Such findings suggest an important cell death-independent role of caspase-3 in inflammation of the CNS. This hypothesis was supported by findings showing that inhibition of caspase-3 (and caspase-8 and -7) effectively blocks microglia activation.

Box 2. Outstanding questions

As reviewed here, converging lines of evidence suggest a crucial role for caspase-3 in both physiological and pathological conditions in the CNS. Moreover, fine tuning of caspase activity is critical for limiting its proteolytic effect to localized sites to avoid dismantling of the entire neuron. The following critical questions need to be addressed to improve our understanding of how caspase-3 activity is controlled and how this fine control is gradually lost in pathological conditions, leading to neuronal death.

- Why is caspase-3 activated only in the synaptic compartment in some cases?
- In physiological conditions, caspase-3 is important for LTD [52]. What are the physiological stimuli that trigger caspase-3 activity?
- Is caspase-3 regulatory (nonapoptotic) activity present during development?
- Does specific caspase-3 inhibition provide therapeutic benefits for AD patients? Experimental evidence [96] with a caspase-3/7 inhibitor suggests that such pharmacotherapy may be beneficial in delaying the symptoms of AD. However, a cautionary note needs to be sounded about chronic therapeutics based on caspase inhibition. In fact, as discussed, a basal level of caspase activation is important for normal synaptic LTD [52]. Moreover, caspase inhibition in wild-type animals leads to an inverse effect on memory formation, confirming a physiological role for caspase-3 in hippocampal function [96]. Thus, the potential consequences and side effects of long-term caspase-3 inhibition need to be explored further.

Concluding remarks

The temporal and spatial activation of caspase-3 substrates seems to be involved in many regulatory mechanisms, ranging from physiological neuronal death during neurulation to physiological nonapoptotic functions during early post-natal life. Similarly, during adult life, caspase-3 is involved in specific functions at the synaptic level and an increase in synaptic caspase-3 activity may lead to progressive dismantling of neuronal circuits in key brain regions that mediate memory function. By contrast, strong or uncontrolled activation of caspase-3 might lead to activation of the full apoptotic cascade and cell death, as in neurodegenerative diseases. This dual role of caspase-3 effectively links neuronal apoptosis with neuronal function and helps to maintain a homeostatic state, promoting brain functioning. Although many questions remain concerning the molecular control of these nonapoptotic functions and the detailed cellular mechanisms of how this control is lost in pathological conditions (Box 2), the recent studies outlined here underscore the importance of further investigations into the diverse functions of caspase-3 in the nervous system.

Disclosure statement

M.S. is an employee of Genentech Inc., a member of the Roche Group.

Acknowledgments

M.D.A. is financially supported by Projects for Research of National Interest (PRIN) and by a grant from the Alzheimer's Association (NIRG-11-204588). F.C. is supported by the Telethon Foundation, the Italian Ministry of Health, and the Italian Ministry of Research, through Ricerca Finalizzata, Ricerca Corrente, PRIN, and Fondo per gli Investimenti della Ricerca di Base (FIRB) funds. We are grateful to Drs Virve Cavallucci and Annalisa Nobili for helpful discussions.

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