Apoptosis in the nervous system

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Neuronal apoptosis sculpts the developing brain and has a potentially important role in neurodegenerative diseases. The principal molecular components of the apoptosis programme in neurons include Apaf-1 (apoptotic protease-activating factor 1) and proteins of the Bcl-2 and caspase families. Neurotrophins regulate neuronal apoptosis through the action of critical protein kinase cascades, such as the phosphoinositide 3-kinase/Akt and mitogen-activated protein kinase pathways. Similar cell-death-signalling pathways might be activated in neurodegenerative diseases by abnormal protein structures, such as amyloid fibrils in Alzheimer's disease. Elucidation of the cell death machinery in neurons promises to provide multiple points of therapeutic intervention in neurodegenerative diseases.

Although mature neurons are among the most long-lived cell types in mammals, immature neurons die in large numbers during development. Furthermore, neuronal cell death is the cardinal feature of both acute and chronic neurodegenerative diseases. How do neurons die? This is a difficult question and we have only recently begun to understand the basic mechanisms. Like all cells, neuronal survival requires trophic support. Viktor Hamburger and Rita Levi-Montalcini described in a seminal paper that the survival of developing neurons is directly related to the availability of their innervating targets[1]. This laid the foundation for the neurotrophin hypothesis[2], which proposed that immature neurons compete for target-derived trophic factors that are in limited supply; only those neurons that are successful in establishing correct synaptic connections would obtain trophic factor support to allow their survival. The neurotrophin hypothesis predicts correctly that neuronal survival requires a positive survival signal; it did not, however, provide a concrete hypothesis as to how neurons die in the absence of trophic support.

It was assumed until recently that neurons die simply of passive starvation in the absence of trophic factors. In 1988, using cultured sympathetic neurons as a model system, Johnson and colleagues showed that inhibition of RNA and protein synthesis blocked sympathetic neuronal cell death induced by nerve growth factor (NGF) deprivation[3], providing the first tangible evidence that neurons might actually instigate their own demise. The identification of the programmed cell death genes *ced-3*, *ced-4* and *ced-9*, in the nematode *Caenorhabditis elegans* and their mammalian homologues (see review in this issue by Meier *et al.*, pages 796–801) opened a window of opportunity to examine the mechanism of neuronal cell death at the molecular level[4]. It was soon discovered that vertebrate neuronal cell death induced by trophic factor deprivation requires the participation of cysteine proteases, later termed caspases, which are the mammalian homologues of the *C. elegans* cell death gene product CED-3 (ref. 5). This was the first functional evidence that trophic factor deprivation activates a cellular suicide programme in vertebrate neurons. What are the critical components of this neuronal suicide programme? How is it activated by lack of trophic support during development and by pathological conditions in neurodegenerative diseases? These questions have been studied intensively during the past decade and are the subject of this review.

Key molecules in neuronal apoptosis

Mammalian apoptosis is regulated by the Bcl-2 family of proteins, the adaptor protein Apaf-1 (for apoptotic protease-activating factor 1) and the cysteine protease caspase family, which are homologues of the *C. elegans* cell-death gene products CED-9, CED-4 and CED-3, respectively (see review in this issue by Hengartner, pages 770–776). Neurons share the same basic apoptosis programme with all other cell types. However, different types of neurons, and neurons at different developmental stages, express different combinations of Bcl-2 and caspase family members, which is one way of providing the specificity of regulation.

The role of the Bcl-2 family in neuronal cell death

The Bcl-2 family of proteins has a crucial role in intracellular apoptotic signal transduction. This gene family includes both anti-apoptotic and pro-apoptotic proteins that contain one or more Bcl-2 homology (BH) domains[6]. The major anti-apoptotic members of the Bcl-2 family, Bcl-2 and Bcl-x_L, are localized to the mitochondrial outer membrane and to the endoplasmic reticulum and perinuclear membrane. Garcia *et al.*[7] showed that Bcl-2 can support the survival of sympathetic neurons in the absence of NGF, providing the first functional evidence that the overexpression of Bcl-2 can override the death signal induced by the withdrawal of a trophic factor. Subsequently, transgenic mice expressing Bcl-2 in the nervous system were found to be protected against neuronal cell death during development[8], as were neuronal injury models such as middle cerebral artery occlusion and facial nerve axotomy[9,10]. These results suggest that the suppression of apoptosis might protect neurons against insults ranging from trophic factor deprivation to pathological stimuli.

The expression of Bcl-2 is high in the central nervous system during development and is downregulated after birth, whereas the expression of Bcl-2 in the peripheral nervous system is maintained throughout life[6]. Although the development of the nervous system in Bcl-2-knockout mice is normal, there is a subsequent loss of motor, sensory and sympathetic neurons after birth[11,12], suggesting that Bcl-2 is crucial for the maintenance of neuronal survival. Bcl-x_L is

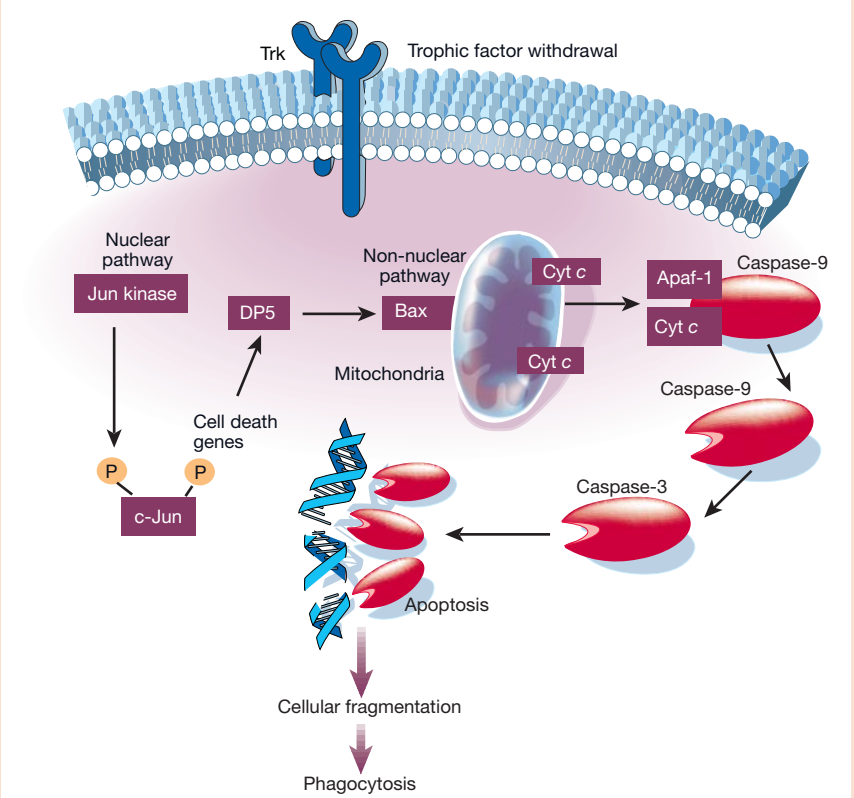

Figure 1 Activation of apoptosis in sympathetic neurons by trophic factor withdrawal. Trophic factor withdrawal induces JNK activation and the phosphorylation of c-Jun, which in turn induces the expression of DP5/Hrk, a 'BH3-domain only' member of the Bcl-2 family. DP5 might activate Bax, causing mitochondrial damage, which results in the release of cytochrome *c*. Formation of the cytochrome *c*/Apaf-1/caspase-9 complex induces the activation of caspase-9. Activated caspase-9 in turn activates caspase-3, resulting in apoptosis. A lack of trophic factor signalling also induces a non-nuclear competence-to-die pathway that facilitates the formation of the cytochrome *c*/Apaf-1/caspase-9 complex, resulting in caspase-9 activation.

expressed in developing brain; but unlike Bcl-2 expression, Bcl-x_L expression continues to increase into adult life[13]. Bcl-x_L-null mice die around embryonic day 13 with massive cell death in the developing nervous system[14]. Cell death occurs primarily in immature neurons that have not established synaptic connections. Thus, Bcl-x_L might be critical for the survival of immature neurons before they establish synaptic connections with their targets.

Bcl-2 and Bcl-x_L act by inhibiting pro-apoptotic members of the Bcl-2 family through heterodimerization[6]. Bax is a pro-apoptotic member of the Bcl-2 family that is widely expressed in the nervous system[15]. In Bax-deficient mice, superior cervical ganglia and facial nuclei display increased neuron number. Furthermore, neonatal sympathetic neurons and facial motor neurons from Bax-deficient mice are more resistant to cell death induced by NGF deprivation and axotomy, respectively. Thus, the activation of Bax might be a crucial event for neuronal cell death induced by trophic factor withdrawal as well as injury.

Apaf-1 and caspases in neuronal cell death

Apaf-1 is a mammalian homologue of the *C. elegans* cell-death gene product CED-4 and transmits apoptotic signals from mitochondrial damage to activate caspases. Apaf-1 forms a complex with mitochondrial-released cytochrome *c* and caspase-9 to mediate the activation of pro-caspase-9 (see Fig. 1)[16]. Activated caspase-9 in turn cleaves and activates caspase-3. Apaf-1-null mice die during late embryonic development, exhibiting reduced apoptosis in the brain with a marked enlargement of the periventricular proliferative zone[17]. Thus, Apaf-1 is indispensable in the apoptosis of neuronal progenitor cells.

The ability of caspase inhibitors to block neuronal cell death induced by trophic factor deprivation and other cytotoxic conditions has provided indisputable evidence for a crucial role of caspases in neuronal cell death[18]. But it has been more challenging to determine the role of specific caspases because mammals have at least 14 different caspases. Like other cell types, neurons can express several of them simultaneously. This has ruled out the simplistic model that neuronal cell death is regulated by neuron-specific caspases. Instead, biochemical and genetic analysis of caspase-mutant mice suggest that caspases are organized into parallel and sometimes overlapping pathways that are specialized to respond to different stimuli. Caspases are expressed as catalytically inactive proenzymes composed of an amino-terminal pro-domain, a large subunit and a small subunit. Caspases can be classified on the basis of the sequence motifs in their pro-domains. Caspases with the death-effector domain, which include caspase-8 and caspase-10, are activated by interacting with the intracellular domains of death receptors such as the CD95 (Apo-1/Fas) and tumour necrosis factor (TNF) receptors. Caspases with caspase-activating recruitment domains (CARDs), which include caspase-1, -2, -4, -5, -9, -11 and -12, are most probably activated through an intracellular activating complex exemplified by the cytochrome *c*/Apaf-1/caspase-9 complex[19]. Whereas caspases with short pro-domains, such as caspase-3, might be activated by most, if not all, caspase pathways, recent data indicate that some caspases, such as caspase-11 and caspase-12, are activated only under pathological conditions[20,21]. This offers the prospect of being able to inhibit pathological cell death therapeutically without disturbing developmental and homeostatic apoptosis (see review in this issue by Nicholson, pages 810–816).

The two major caspases involved in neuronal cell death are caspase-3 and caspase-9, in which the latter activates the former (Fig. 1). Both caspase-3-null[22] and caspase-9-null[23] mice show severe and similar defects in developmental neuronal cell death. Ectopic cell masses appear in the cerebral cortex, hippocampus and striatum of the caspase-3-null and caspase-9-null mice with marked expansion of the periventricular zone, a phenotype very similar to that of Apaf-1-null mice[17]. The prominent neuronal apoptosis defects of Apaf-1-null, caspase-3-null and caspase-9-null mice (Table 1) suggest that this pathway is important in regulating neuronal cell death in the developing brain.

Neurotrophins: a matter of life and death

Staying alive with neurotrophins

As mentioned above, the survival of developing immature neurons depends on the availability of neurotrophic factors. What do these

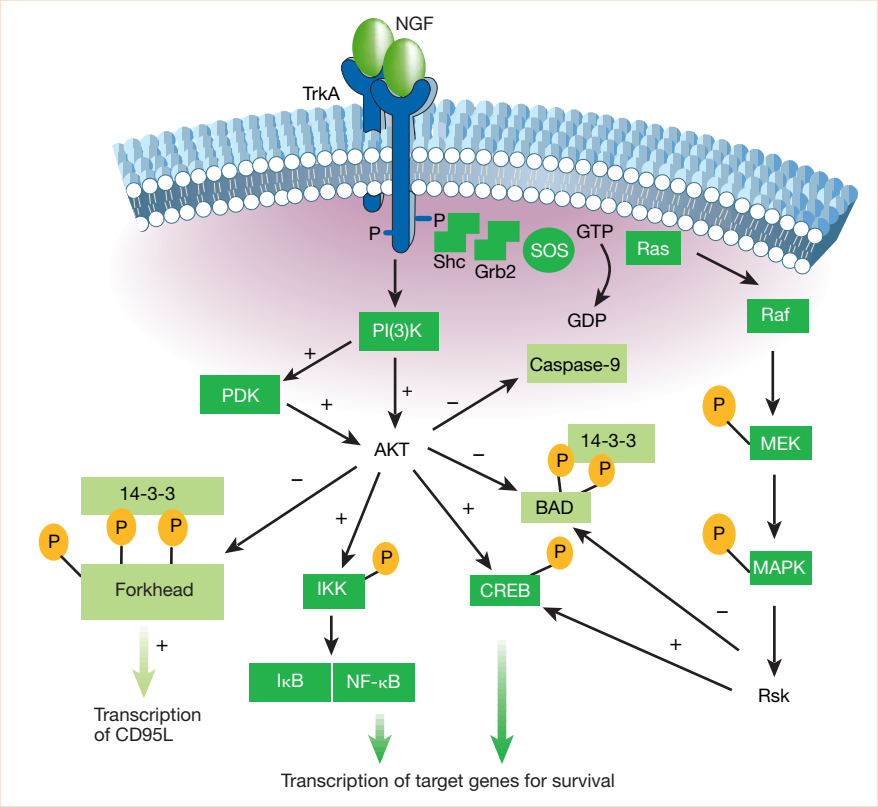

Figure 2 Neuronal survival pathways induced by the binding of NGF to its receptor TrkA. NGF induces the autophosphorylation of TrkA which provides docking sites for signal transduction molecules such as phospholipase Cγ, phosphoinositide 3-kinase (PI(3)K) and the adaptor protein Shc. Activated PI(3)K induces the activation of Akt through 3′-phosphorylated phosphatidylinositol as well as phosphoinositide-dependent kinase (PDK), which in turn phosphorylates and activates Akt. The phosphorylation of CREB and IKK stimulates the transcription of pro-survival factors; whereas the phosphorylation of Bad, Forkhead and caspase-9 inhibits the pro-apoptotic pathway. In a parallel pathway, the interaction of Shc–Grb2 and SOS activates the Ras–Raf–MEK–ERK pathway, resulting in the activation of Rsk. Bad and CREB are also the targets of Rsk that might act synergistically with Akt to activate the survival pathway.

survival factors do? Neurotrophins generally activate and ligate the Trk receptors (TrkA, TrkB and TrkC), which are cell-surface receptors with intrinsic tyrosine kinase activity. They can autophosphorylate[24]; for instance, after the binding of NGF to TrkA, the receptor phosphorylates several tyrosine residues within its own cytoplasmic tail. These phosphotyrosines in turn serve as docking sites for other molecules such as phospholipase Cγ, phosphoinositide 3-kinase (PI(3)K)[25] and adaptor proteins such as Shc, and these signal transduction molecules coordinate neuronal survival (Fig. 2).

PI(3)K–Akt pathway. A central role of the PI(3)K pathway in neuronal survival was first suggested by the observation that PI(3)K inhibitors block the survival effect of NGF[26]. PI(3)K enzymes are normally present in cytosol and can be activated directly by recruitment to an activated Trk receptor, or indirectly through activated Ras. Active PI(3)K enzymes catalyse the formation of the lipid 3′-phosphorylated phosphoinositides, which regulate the localization and activity of a key component in cell survival, the Ser/Thr kinase Akt (ref. 27).

Akt has three cellular isoforms, of which c-Akt3/RAC-PKγ is the major species expressed in neurons[28]. In addition to a centrally located kinase domain, Akt contains a pleckstrin homology domain at its N-terminus, which mediates its interaction with proteins and phospholipids. After the binding of lipid, Akt is translocated from the cytoplasm to the inner surface of the plasma membrane, which brings the kinase into close proximity with its activators. The kinases that phosphorylate and activate Akt, the 3-phosphoinositol-dependent protein kinases are — as their name suggests — themselves regulated by phospholipids. Thus, the lipid products generated by PI(3)K enzymes control the activity of Akt by regulating its location and activation.

Active Akt protein supports the survival of neurons in the absence of trophic factors, whereas a dominant-negative mutant of Akt inhibits neuronal survival even in the presence of survival factors[28]. These results establish an essential role for Akt in neuronal survival. How does Akt act?

Akt in action. Akt targets several key proteins to keep cells alive, including apoptosis regulators and transcription factors (Fig. 2). For example, Bad is a pro-apoptotic member of the Bcl-2 family, which in its unphosphorylated form can bind to Bcl-x_L and thus block cell survival[29]. But the activation of Akt induces the phosphorylation of Bad and promotes its interaction with the chaperone protein 14-3-3, which sequesters Bad in the cytoplasm and inhibits Bad's pro-apoptotic activity[30]. Akt has been shown to affect, directly or indirectly, three transcription factor families: Forkhead, cAMP-response-element-binding protein (CREB) and NF-κB, all of which are involved in regulating cell survival, and whereas the phosphorylation of Forkhead family members by Akt negatively regulates death-promoting signals[31], the phosphorylation of CREB and IκB kinase (IKK) stimulates survival pathways[32–34]. It is clear that Akt is a potent kinase that keeps neurons alive in various ways, and that additional targets of Akt will no doubt be identified.

Mitogen-activated protein (MAP) kinase pathway. But there is more to neurotrophins than only the activation of PI(3)K and Akt: they also stimulate docking of the adaptor protein Shc to activated Trk receptors. This triggers the activation of the small GTP-binding protein Ras and the downstream MAP kinase cascade, which includes the subsequent sequential phosphorylation and activation of the kinases Raf, MAP kinase/ERK kinase (MEK) and extracellular signal-regulated protein kinase (ERK)[35] (Fig. 2). The effect of the MAP kinase pathway on survival is mediated at least partly by activation of the pp90 ribosomal S6 kinase (RSK) family members. Like Akt, RSK phosphorylates Bad, and both kinases might act synergistically in inhibiting Bad's pro-apoptotic activity. The effect of RSKs on neuronal survival is not limited to the phosphorylation of Bad; RSKs are also potent activators of the CREB transcription factor. Because CREB is known to activate transcription of *bcl-2*, it can stimulate cell survival directly. Thus, although there is a divergence in the survival pathways downstream of the neurotrophin receptors, both the PI(3)K–Akt and MAP kinase pathways converge on the same set of proteins, Bad and CREB, to inhibit the apoptosis programme.

It is noteworthy that neurotrophins are not the only factors that promote neuronal survival: electrical stimulation and depolarization at high KCl concentration have long been known to inhibit neuronal cell

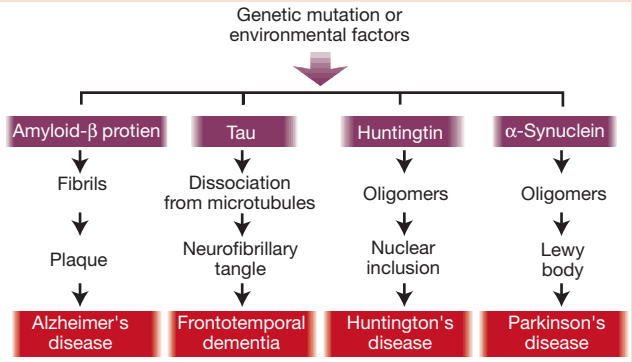

Figure 3 Abnormal protein structures and the pathogenesis of neurodegenerative disease. Normal proteins might become pathogenic when subjected to genetic mutations or environmental factors that promote the formation of abnormal structures in specific neuronal subpopulations.

death[36]. Recent studies indicate that membrane depolarization also activates neuronal survival pathways; whether or not these are the same as those activated by the neurotrophins is unresolved[37,38].

Dying without neurotrophins

Although it is clear that neurotrophins and membrane depolarization activate signal transduction pathways that suppress apoptosis, it is less clear what triggers the activation of apoptosis in the absence of survival signals. It is possible that neurotrophins simply suppress a default apoptosis programme. However, a number of processes need to happen before cultured immature sympathetic neurons are committed to die (Fig. 1).

The removal of NGF results in a decrease in MAP kinase and PI(3)K activities, followed by a series of early metabolic changes including the increased production of reactive oxygen species, decreased glucose uptake and decreased RNA and protein synthesis. In some cells, the removal of NGF results in a slow and sustained increase in c-Jun amino-terminal kinase (JNK) and p38 MAP kinase activities[39]; in other cells, c-Jun, one of the downstream targets of JNK, is induced and phosphorylated[40,41]. The activation of JNK itself might be necessary, but not sufficient, to induce neuronal apoptosis.

Paradoxically, although protein and RNA synthesis are significantly reduced in the early stages of sympathetic neuronal cell death, death cannot occur in the presence of inhibitors of RNA and protein synthesis, indicating that the continued synthesis of certain pro-apoptotic molecules is required. In view of this, it is interesting to note that DP5 (also known as Hrk), a 'BH3-domain only' pro-apoptotic member of the Bcl-2 family, is induced in NGF-deprived sympathetic neurons[42]. Perhaps it is the synthesis of DP5-related proteins that is vital to the execution of the cell death programme. DP5 could be required to help Bax move from its location in the cytosol to the mitochondria, after which Bax can induce the release of cytochrome *c* (ref. 43) (Fig. 1).

As in other cell types, the release of cytochrome *c* from mitochondria induces the activation of caspases in sympathetic neurons. The addition of a pan-caspase inhibitor, but not NGF, rescues sympathetic neurons even after mitochondrial damage and the release of cytochrome *c*[44]. Thus, these neurons are not committed to die until caspases are fully activated. This indicates that the point of no return is at, or downstream of, caspase activation, and suggests that the inhibition of caspase activity might be sufficient to block neuronal cell death under certain pathological conditions.

Neurotrophins: a double-edged sword?

The neurotrophin hypothesis predicts that neurotrophins function as survival signals to suppress the death programme. However, the interaction of neurotrophins with the neurotrophin receptor p75NTR can induce cell death under certain conditions, suggesting that neurotrophins might act as death ligands in a cell-context-dependent manner. The p75 neurotrophin receptor (p75NTR) is a member of the TNF receptor superfamily that can bind all neurotrophins[45]. Its intracellular domain contains a region that bears similarity with the 'death domain', which mediates protein–protein interactions and is present in other members of the TNF family. p75NTR was originally thought to cooperate with Trks to modulate the response to neurotrophins. However, p75NTR might have an additional role in orchestrating neuronal cell death. Barde and colleagues found that application of antibodies that block the binding of NGF to p75NTR inhibited the death of chick retinal ganglion cells that express p75NTR but not trkA[46], indicating that the interaction of NGF with p75NTR cells promotes cell death in this system. Death-inducing activity of various neurotrophins has now been documented for different neuronal cell types[47,48]. p75NTR-dependent cell death seems to be inhibited by Trk signalling[49]. In view of these opposing roles of neurotrophins, they might be more appropriately referred to as 'neuromodulators' that function to adjust neuronal cell number and regulate differentiation.

Pathological apoptosis in the adult brain

Physiological apoptosis in the developing brain and pathological apoptosis in the adult brain share similar molecular mechanisms in the effector phase. But there are key differences in the mechanisms by which apoptosis is triggered. Whereas trophic factor withdrawal has a prominent role in apoptosis during development, there is little evidence to implicate trophic factor withdrawal as a primary pathogenic mechanism in adult neurodegenerative disorders. Rather, toxic insults resulting from biochemical or genetic accidents might trigger neurodegenerative diseases by co-opting apoptotic signalling pathways, for example through free-radical generation or caspase activation. An emerging theme in adult neurodegenerative disorders is the toxicity of abnormal protein structures or aggregates, which might be important in the pathogenesis of Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis (Fig. 3).

Cell death due to ischaemia

Ischaemic injury-induced neuronal cell death has traditionally been characterized as necrosis, in which cells and their organelles swell and rupture. However, morphological and biochemical evidence of apoptosis have now been well documented in experimental animal models of ischaemic brain injury. Apoptotic neurons are more easily

Table 1 Neuronal phenotypes of caspase-knockout mice

Caspase	Development	Neuronal apoptotic phenotype	References
Caspase-1	Normal	Resistant to ischaemic brain injury and a decrease in incidence and severity of experimental autoimmune encephalomyelitis	98
Caspase-2	Normal	Hippocampal neurons from caspase-2-null mice are resistant to amyloid-β(1–42) toxicity	66
Caspase-3	Perinatally lethal (depend on genetic background)	Lack of apoptosis in neuroepithelial progenitor cells during development	22
Caspase-9	Embryonic lethal (a small percentage of caspase-9-knockout can survive to adult)	Lack of apoptosis in neuroepithelial progenitor cells during development	23,99
Caspase-11	Normal	Resistant to apoptosis induced by ischaemic brain injury	20
Caspase-12	Normal	Cortical neurons are resistant to amyloid-β toxicity	21

detected early after the onset of an ischaemic insult, in the penumbra where the insult is less severe and during reperfusion[50,51]. It is possible that only neurons that maintain a minimum level of metabolic activity can undergo apoptosis, which is consistent with it being a cellular suicide programme.

Mitochondria might be important in transmitting apoptotic signals during ischaemia to induce caspase activation. There is strong evidence of caspase-3 activity in ischaemic brain[52], which might be mediated by caspase-11 — a caspase that is specifically induced by ischaemic injury[20]. Moreover, caspase inhibitors significantly attenuate ischaemic neuronal injury.

Although there is strong evidence for apoptosis in ischaemic brain injury, not all cells die by apoptosis. Among cells with typical apoptotic features, there are clearly cells with a swollen morphology and highly vacuolated features[53]; thus, the death of a significant number of neurons in ischaemic brain is likely to occur through a non-caspase-mediated mechanism.

Neuronal cell death in Alzheimer's disease

The relative contribution of apoptosis to neuronal loss in Alzheimer's disease is difficult to assess because of the chronic nature of the disease process, so that at any one time only a limited number of apoptotic neurons can be detected. Some neurons exhibit morphological features of apoptosis, but many degenerating neurons do not show evidence of apoptosis, suggesting that apoptosis might not be the only mechanism of degeneration in Alzheimer's disease[54,55].

The proximal cause of neurodegeneration in Alzheimer's disease is an actively debated issue that has become focused on several proteins implicated by genetics (Box 1). A central role for amyloid-β protein is supported by the effects of genetic mutations that cause familial Alzheimer's disease[56], all of which predispose to amyloid deposition, and by the observation that amyloid-β can be neurotoxic *in vitro* and *in vivo*[57,58]. The toxicity of abnormal structural forms of amyloid-β provides a unifying theme with other age-related neurodegenerative disorders characterized by the appearance of pathological protein structures, such as Parkinson's disease, Huntington's disease, frontotemporal dementia and amyotrophic lateral sclerosis (Fig. 3). The mechanism of amyloid-β neurotoxicity and its precise cellular locus of action are unsettled, but it has been shown that amyloid-β can induce oxidative stress and elevate intracellular Ca^{2+} concentration[59,60] (Fig. 4). Amyloid-β might induce apoptosis[61] by interacting with neuronal receptors, including the receptor for advanced glycation endproducts (RAGE), which can mediate free-radical production[62], the p75 neurotrophin receptor, which can induce neuronal cell death[63], and the amyloid precursor protein, which can also induce neuronal cell death[64]. These various amyloid-β–receptor interactions might activate several different cell-death-signalling pathways (Fig. 4). For example, amyloid-β can activate a set of immediate early genes similar to those induced by trophic factor withdrawal[65], and can activate caspases. Furthermore, neurons deficient in caspase-2 and caspase-12 have decreased vulnerability to amyloid-β toxicity[21,66], suggesting that selective caspase inhibition might be a potential therapeutic approach in Alzheimer's disease.

The identification of mutations in the presenilin genes as a major cause of early-onset familial Alzheimer's disease has provided a new approach to understanding the mechanism of neuronal cell death in Alzheimer's disease[67–69]. Presenilin mutations increase the production of a 42-residue form of amyloid-β, the major constituent of plaques in the Alzheimer's disease brain[70]. Several recent studies

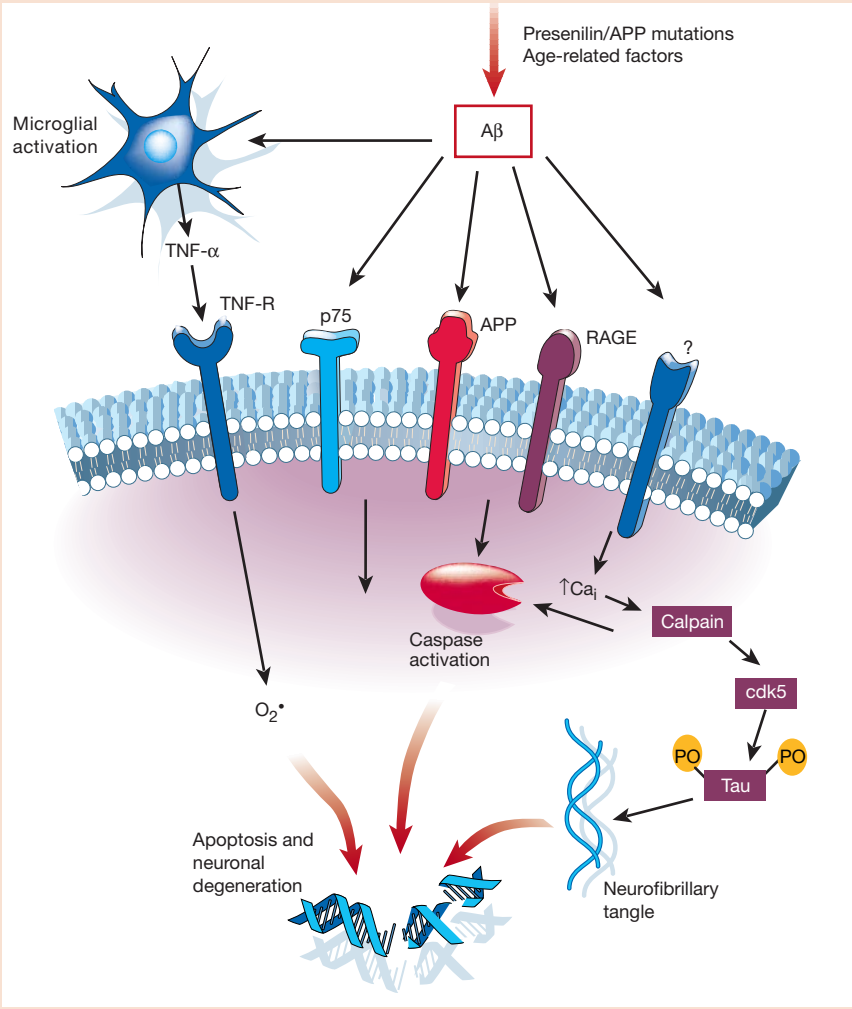

Figure 4 Cellular pathways of amyloid-β protein neurotoxicity in Alzheimer's disease. Aggregated forms of amyloid-β interact with several different neuronal cell-surface receptors and with microglia, triggering signal transduction cascades that result in caspase activation, free-radical generation and Ca^{2+} influx. An increased intracellular concentration of Ca^{2+} (Ca_i) might activate calpain proteases which can, in turn, activate caspases and the tau protein kinase Cdk5.

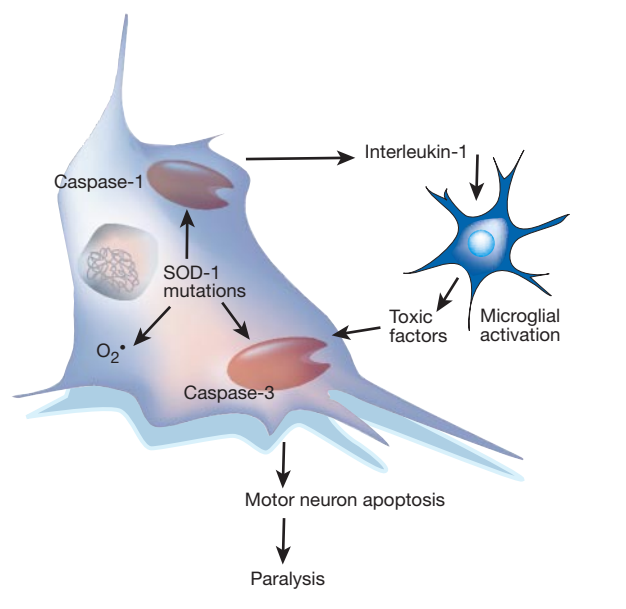

Figure 5 SOD-1 mutations activate cell death pathways in familial amyotrophic lateral sclerosis. SOD-1 mutations can activate caspase-1 and caspase-3, and might increase free-radical generation, leading to motor neuron apoptosis. The activation of caspase-1 leads to interleukin-1 production, which can induce a local microglial inflammatory response and increase the number of neurons affected.

suggest that presenilins might be λ-secretases, proteases that participate in the generation of amyloid-β, although this remains to be established definitively[71,72]. Presenilin mutations can also increase neuronal vulnerability to apoptosis[73]. But it remains to be determined whether these mechanisms contribute to neuronal cell death in Alzheimer's disease.

Activation of microglial cells is a prominent feature of the inflammatory response in the brain in Alzheimer's disease that is likely to contribute to neuronal cell death. Microglial activation is associated with amyloid plaques and can be induced experimentally by amyloid-β[74]. Amyloid-β-induced microglial activation results in the secretion of TNF-α and other toxic factors that can induce neuronal apoptosis[75]. Similar microglial-based mechanisms have been implicated in other neurodegerative disorders. For example, a fibril-forming peptide derived from the prion protein induces neuronal apoptosis through microgilial activation and the generation of reactive oxygen species[76]. Microglial activation also has a central role in neuronal cell death associated with viral infections of the central nervous system. Microglia and macrophages are the predominant cell types infected by HIV in the brain[77], and induce the apoptosis of neurons and astrocytes in AIDS[78]. Thus, pathological neuronal cell death might be a direct consequence of toxic insults such as amyloid-β, or an indirect consequence of a complex interaction between neurons, microglia and toxic factors.

Death from expanded polyglutamine repeats

The adult-onset neurodegenerative diseases caused by proteins with expanded polyglutamine tracts are characterized by a selective loss of specific neuronal subpopulations. Proteins with polyglutamine repeats can aggregate *in vitro* and form amyloid-like fibrils similar to the amyloid-β fibrils in Alzheimer's disease[79]. Such aggregates are also observed in the brains of patients with Huntington's disease, spinocerebellar ataxia types 1 and 3, and dentatorubral–pallidoluysian atrophy[80]. Ubiquitinated derivatives of the mutant proteins can be found in large intranuclear inclusions, and in Huntington's disease the number of inclusions is correlated with the length of the polyglutamine tract[81]. Ineffective clearance of polyglutamine expansion proteins by the ubiquitin–proteasome pathway might contribute to the formation of intranuclear inclusions[82]. Transgenic mice that express expanded polyglutamine-containing mutants of huntingtin and ataxin 1 also develop inclusions that appear at about the same time as the neurological deficits[83]. Despite the correlation of the appearance of neuronal inclusions with disease in patients and transgenic mouse models, several studies have questioned their pathological significance[84,85]. Moreover, the inhibition of inclusion formation can increase neuronal apoptosis *in vitro*, indicating that the formation of inclusions might be neuroprotective.

But there is evidence that expression of expanded polyglutamine tracts can result in the formation of small aggregates that do induce apoptosis[86]. Apoptosis is mediated by the recruitment of the adaptor protein FADD (for Fas-associated death domain protein) and caspase-8, resulting in the activation of a caspase cascade. Furthermore, caspases might have a role in generating highly toxic fragments of

Box 1

Molecules implicated in the pathogenesis of Alzheimer's disease

Amyloid-β protein[56,57]

1. This is the main component of senile plaques and cerebrovascular amyloid deposits.
2. All known genetic mutations that cause Alzheimer's disease predispose to amyloid deposition.
3. Individuals with trisomy 21, who carry an additional copy of the amyloid precursor protein gene, develop early-onset Alzheimer's disease.
4. Amyloid-β can be neurotoxic.

Tau[100]

1. Hyperphosphorylated tau is the main component of neurofibrillary tangles.
2. Mutations in tau cause frontotemporal dementia with Parkinsonism associated with chromosome 17 (FTDP-17), suggesting that aberrant forms of tau can give rise to neurodegeneration. However, tau mutations have not been found in cases of Alzheimer's disease.
3. The number and distribution of neurofibrillary tangles are correlated with the degree of dementia in Alzheimer's disease.
4. Activation of protein kinase cdk5 might contribute to both tau phosphorylation and neuronal apoptosis[101].

Presenilins[67–69]

1. Mutations in presenilin 1 and 2 are a major cause of early-onset familial Alzheimer's disease.
2. Presenilin mutations increase production of the 42-residue form of amyloid-β, which has a high propensity for forming amyloid fibrils.
3. Presenilins are required for amyloid-β production and might be γ-secretases.
4. Presenilin mutations increase neuronal vulnerability to apoptosis.

Apolipoprotein E[57]

1. Inheritance of the ε4 allele is the most common known genetic risk factor for Alzheimer's disease after the age of 60.
2. The ε4 allele promotes the polymerization of amyloid-β into plaque-forming fibrils.
3. The ε4 allele might impair neuronal regeneration or promote oxidative stress.

proteins with expanded polyglutamine tracts. Support for this idea comes from studies on a transgenic model of Huntington's disease. A dominant-negative mutant of caspase-1, or the intracerebroventricular administration of a broad-spectrum caspase inhibitor, delays the onset and progression of pathology and prevents the appearance of a huntingtin cleavage product[87].

A central issue is the relative contribution of neuronal apoptosis to neurological deficits in polyglutamine expansion diseases and other age-related neurodegenerative disorders. Early-stage Huntington's disease patients develop characteristic motor deficits without evidence of striatal atrophy; striatal atrophy becomes prominent in later stages of the disease[88]. Similarly, a transgenic mouse model of Huntington's disease exhibits motor symptoms in the absence of striatal atrophy or neuronal apoptosis[89]. Furthermore, in a conditional huntingtin transgenic mouse, neuronal intranuclear inclusions and neurological deficits could be reversed by turning off expression of the mutant transgene[90]. Thus, neuronal dysfunction, rather than cell death, might be responsible for early neurological deficits.

Mutations in superoxide dismutase and amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive motor disease characterized by the degeneration of motor neurons in the spinal cord and brain, leading to paralysis. A major leap in understanding the disease mechanism came from the identification of mutations in the gene encoding superoxide dismutase (SOD-1) in familial ALS[91]. Transgenic mice that express mutant forms of SOD-1 show progressive motor neuron degeneration that is similar in many respects to that in the human disease[92]. In contrast, mice deficient in or overexpressing wild-type SOD-1 do not develop motor neuron disease. These findings suggest that mutant SOD-1 is somehow toxic to neurons. Although the mechanism of toxicity is not yet clearly established, it has been shown that mutant SOD-1 can form intraneuronal aggregates and induce oxidative stress, which is reminiscent of pathogenic mechanisms in Alzheimer's disease and polyglutamine repeat diseases[92] (Fig. 5).

A role for apoptosis in familial ALS is suggested by the pro-apoptotic activity of mutant SOD-1 in cultured neural cell lines[93,94], and the neuroprotective effect of overexpressing Bcl-2 in mutant SOD-1-transgenic mice[95]. Moreover, activated caspase-1 and caspase-3 can be detected in spinal cords of ALS patients and mutant SOD-1-transgenic mice[94,96]. Importantly, the inhibition of caspase-1 activity delays disease progression in SOD-1-transgenic mice[96,97]. Caspase-1 might predispose to neuronal cell death in two ways: by increasing production of the pro-inflammatory cytokine interleukin-1 and by directly activating caspase-3 (Fig. 5). All the evidence suggests that caspase activation may be an essential component of the pathology of ALS, and offers the possibility that early treatment with inhibitors targeted to specific caspases might arrest motor neuron apoptosis.

Conclusion

During the past decade there have been major advances in our understanding of the fundamental mechanisms of neuronal cell death. We now know that the key components of the apoptosis programme in neurons, like that of other cell types, are Apaf-1 and proteins in the Bcl-2 and caspase families. The regulation of apoptosis through interactions of Bcl-2 family members and caspase cascades has a major role in sculpting the developing brain. We are now beginning to understand how neurotrophins suppress apoptosis by regulating critical protein kinase cascades, such as the PI(3)K–Akt and MAP kinase pathways. Furthermore, not only are caspases important in regulating neuronal cell death during development, they might also mediate cell death in human neurodegenerative diseases. These exciting developments suggest that the targeted inhibition of apoptosis might be effective in the treatment of various neurodegenerative diseases.

Naturally, many fundamental questions remain to be answered. We do not yet understand exactly how a toxic stimulus, be it trophic factor deprivation, ischaemic injury, amyloid-β peptide, mutant huntingtin or mutant SOD, triggers the activation of the apoptosis programme in neurons. This signal transduction process might be brief, as in trophic factor deprivation and ischaemic injury, or prolonged, as in neurons that express disease-causing mutant proteins. It will be important to define the molecular point of no return, when neurons become irreversibly committed to die. Obviously neuronal dysfunction might be initiated before neuronal degeneration, and from a therapeutic point of view a central question is whether the inhibition of neuronal cell death will result in healthy, normally functioning neurons. The answers to these questions and the design of rational therapeutic approaches will require a detailed understanding of how neurons survive and die in the brain. □

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