Apoptosis and Degenerative diseases

Corso di Biomedicina Molecolare
Genomica e Dei Sistemi Complessi

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Cell death modalities

Multiple cell death mechanisms operate in organisms

Cell death can be classified according to:

- **morphological** appearance of the lethal process (that may be apoptotic, necrotic, autophagic or associated with mitosis)

- **enzymological** criteria (with and without the involvement of nucleases or distinct classes of proteases, like caspases or cathepsins)

- **functional** aspects (programmed or accidental, physiological or pathological)
# Cell death modalities

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<tr>
<th>Cell death mode</th>
<th>Characteristic morphological aspects</th>
<th>Notes</th>
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<tr>
<td>Apoptosis (Type 1)</td>
<td>• Rounding up of the cell</td>
<td>‘Apoptosis’ is the original term introduced by Kerr et al. to define a cell death with specific morphological features.</td>
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<td>• Reduction of cellular and nuclear volume (pyknosis)</td>
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<td>• Retraction of pseudopodes</td>
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<td>• Nuclear fragmentation (karyorrhexis)</td>
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<td></td>
<td>• Little modification of cytoplasmal organelles</td>
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<td></td>
<td>• Plasma membrane blebbing</td>
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<tr>
<td>Autophagy (Type 2)</td>
<td>• Lack of chromatin condensation</td>
<td>‘Autophagic cell death’ defines cell death occurring with autophagy, though it may misleadingly suggest a form of death occurring through autophagy.</td>
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<td></td>
<td>• Massive vacuolization of the cytoplasm (double-membraned autophagic vacuoles)</td>
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<td>Necrosis (oncosis) (Type 3)</td>
<td>• Cytoplasmic swelling</td>
<td>‘Necrosis’ identifies, in a negative fashion, cell death lacking the features of apoptosis or autophagy, and usually appears as oncosis.</td>
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<tr>
<td></td>
<td>• Rupture of plasma membrane</td>
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<tr>
<td></td>
<td>• Swelling of cytoplasmal organelles</td>
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<tr>
<td></td>
<td>• Moderate chromatin condensation</td>
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<tr>
<td>Mitotic catastrophe</td>
<td>• Micronucleation</td>
<td>‘Mitotic catastrophe’ refers to a cell death occurring during or shortly after a failed mitosis.</td>
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<td>• Multinucleation</td>
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Apoptosis is an active cell death that requires intact subcellular structures and synthesis of phylogenetically conserved proteins. Apoptosis arises from the active initiation and propagation of a series of highly orchestrated specific biochemical events leading to the demise of the cell.

It can be triggered by numerous types of cellular damage and derangement: genomic instability, cell cycle checkpoint violation, loss of prosurvival signaling.
Apoptosis is characterized by a series of dramatic perturbations to the cellular architecture that contribute not only to cell death, but also prepare cells for removal by phagocytes and prevent unwanted immune responses.
The illustration shows a neuron undergoing a common form of apoptosis.

(A) The healthy neuron has a defined cell membrane and the cytoplasm and nucleus, which contains DNA, are intact.

(B) When apoptosis kicks in, the cell contorts and the DNA breaks up.

(C) In the final stage of apoptosis, the cell is broken into membrane-bound pieces. Specialized cells called macrophages or microglia remove the debris.
The cell begins to shrink following the cleavage of lamins and actin filaments in the cytoskeleton (A). The breakdown of chromatin in the nucleus often leads to nuclear condensation and in many cases the nuclei of apoptotic cells take on a "horse-shoe" like appearance (B). Cells continue to shrink (C), packaging themselves into a form that allows for their removal by macrophages. The end stages of apoptosis are often characterised by the appearance of membrane blebs (D) or blisters process. Small vesicles called apoptotic bodies are also sometimes observed (D, arrow).
During necrosis the cellular contents are released uncontrolled into the cell's environment which results in damage of surrounding cells and a strong inflammatory response in the corresponding tissue.
Removal of dead cells

Dying cells that undergo the final stages of apoptosis display phagocytotic molecules, such as phosphatidylserine, on their cell surface. Phosphatidylserine is normally found on the cytosolic surface of the plasma membrane, but is redistributed during apoptosis to the extracellular surface. These molecules mark the cell for phagocytosis by cells possessing the appropriate receptors, such as macrophages.

The removal of dying cells by phagocytes occurs in an orderly manner without eliciting an inflammatory response.
Proper development of multicellular organisms depends on the elimination of selected cells through apoptosis.

As a tadpole becomes a frog it deletes its tail cells. Human embryos are also thought to use apoptosis to remove webbing between digits.

<table>
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<th>Apoptotic Cells in the adult.</th>
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<td>The cells usually commit suicide for the greater good of the body.</td>
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<td>The following is NOT a complete list of such cells, but shows only a few notable examples</td>
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</table>

| Eye: The lens of the eye, which forms during embryonic development, consists of apoptotic cells that have replaced their innards with the clear protein crystallin. |
| Intestines: Cells composing the small finger-like projections of the intestinal wall (called "villi") arise at the base of the "finger" and, over several days, travel to the tip. There they eventually die and are sloughed off to be replaced by new cells. |
| Skin: Skin cells begin life in the deepest layers and then migrate to the surface in layers, undergoing apoptosis along the way. The resulting dead cell layer forms the protective outer skin layer, called the epidermis. |
| Thymus: T Lymphocytes (or T-cells) are white blood cells that are critical components of the immune system; these immune cells mature in the thymus gland (located in the upper chest area just below the neck). T-cells that would be ineffective or that would attack the body's own tissues commit suicide before they have the chance to enter the blood stream. |
| Uterus: The cells of the uterine wall die and are sloughed off during menstruation. This action is accomplished by apoptosis. |
| Other: Cells that become infected by a virus or that sustain irreparable genetic mutations often commit suicide. The failure of a genetically altered cell to commit suicide can contribute to the development of cancer. |
Apoptotic Pathways

Apoptotic programmed cell death pathways are activated by a diverse array of cell extrinsic and intrinsic signals, most of which are ultimately coupled to the activation of effector caspases.
The caspase family can be subdivided into initiators, which are able to auto-activate and initiate the proteolytic processing of other caspases, and effectors, which are activated by other caspase molecules.

The effector caspases cleave the vast majority of substrates during apoptosis.
Caspases

Mammals:

Initiator Caspases
- Casp-9
  - CARD
- Casp-2
  - CARD
- Casp-8
  - DED
- Casp-10
  - DED

Effector Caspases
- Casp-3
- Casp-7
- Casp-6

Activating Complex
- ~p20
- ~p10

- Apoptosome
- PIDDosome
- DISC
- DISC

[Diagram showing the structures and interactions of caspases, including CARD and DED domains, with arrows indicating activation processes.]
Caspases coordinate demolition of key cellular structures and organelles

**Effector caspases** (such as caspase-3, -6 and -7 in mammals) orchestrate the dismantling of diverse cell structures through cleavage of specific substrates. Collectively, these proteolytic events produce the phenotypic changes to the cell that are characteristic of apoptosis.

- Cleavage of ICAD releases CAD, which can then catalyse inter-nucleosomal DNA cleavage.
- Proteolysis of proteins at focal adhesion sites and cell–cell adhesion sites allows cell detachment and retraction.
- Caspases also cleave the Golgi-stacking protein GRASP65 and other Golgi proteins, causing fragmentation of the Golgi apparatus.
- Important cellular functions such as translation are disrupted through caspase-mediated proteolysis of multiple translation initiation factors (eIFs).
- Caspase activity is required for the exposure of phosphatidylserine (PS) and other phagocytic signals on the cell surface.
Two main pathways of caspase-mediated cell death have been described in mammals

The “intrinsic” pathway, involves mitochondria, and is triggered and controlled by members of the Bcl-2 protein family.

The “extrinsic” pathway is mediated by death receptors, a subgroup of the tumor necrosis factor (TNF) receptor superfamily.
Apoptotic Pathways

**Mitochondrial:** activates caspase-9 on a scaffold formed by Apaf-1 in response to cytochrome c released from damaged mitochondria. This pathway, also termed ‘intrinsic’, is primarily regulated by the Bcl-2 family.
The Mitochondrial Apoptotic Pathway

Apoptosome: “wheel of death”
Cytochrome c released from damaged mitochondria

Inter-nucleosomal DNA cleavage
Death receptor (extrinsic) pathway:
apoptosis does not require cytochrome c release and it is controlled by death receptor signaling.
The Death Receptor Apoptotic Pathway

FADD: Fas-associated death domain protein
DISC: death-inducing signaling complex

Figure 9-31a The Biology of Cancer (© Garland Science 2007)
A model of Fas-mediated signaling, caspase activation, and the induction of a death signal

Once TRADD is recruited to TNF-R1, it functions as an adapter protein to recruit several signalling proteins, including TRAF2. TRAF2 seems to be essential for the recruitment of the IKK complex.
Following TRADD binding, three pathways can be initiated

Activation of NF-κB

Activation of the MAPK pathways

Induction of death signaling
The apoptotic response depends on the balance of pro-apoptotic and anti-apoptotic signals.
The activation of NF-κB

The receptor-interacting protein (RIP) is recruited via the death domain to the TNF–Receptor-complex upon ligand binding and can interact with NF-kB essential modulator I KKγ.

This results in the recruitment of IKKα and IKKβ, which in turn phosphorylate inhibitors of kB (IkB), leading to IkB degradation and

**NF-kB activation**
Endoplasmic Reticulum stress can lead to apoptosis
The ER stress response

Apoptosis, as a last resort, to eliminate infected cells and maintain homeostasis
ER stress mediated apoptosis
There is an intensive cross talk between the extrinsic and intrinsic pathway, which enhances an apoptotic signal originally initiated by either pathway.

Figure 9.32  The Biology of Cancer (© Garland Science 2007)
The BCL-2 family

B-cell lymphoma-2 (BCL-2)-family proteins have a crucial role in the regulation of apoptosis through their ability to regulate mitochondrial cytochrome c release.

The Bcl-2 family includes antiapoptotic as well as proapoptotic proteins, and can be divided into three main subfamilies that contain between one and four ‘BCL-2 Homology regions’ (BH).
Members of the ‘BH3-only’ subgroup appear to serve as upstream sensors that respond to specific death signals.

The BH3-only protein BID is cleaved by caspase-8 during extrinsic apoptosis signalling and serves to engage a mitochondrial amplification loop in certain cell types. BAD is switched on and off by its phosphorylation in response to growth/survival factors, whilst NOXA and PUMA are regulated at the transcriptional level by p53 in response to DNA damage. Antiapoptotic Bcl-2 family members serve mainly to sequester BH3-only molecules, thereby preventing Bax/Bak activation and release of apoptogenic factors from mitochondria.

In response to damage or derangement, activators (BIM, BID) activate effectors (BAX, BAK), causing mitochondrial permeabilization and commitment to death.

Antiapoptotic proteins sequester activators to prevent their contacting effectors, and sensitizers act as selective antagonists of antiapoptotic proteins.
Implications for human disease

In every human being about a hundred thousand cells are produced every second by mitosis, and a similar number die by apoptosis.

It is therefore of crucial importance that the balance between cell death and proliferation is tightly regulated.

Consequently, the violation of cellular homeostasis may play a primary or secondary role in various human diseases, with essentially too little or too much apoptosis leading to proliferative or degenerative diseases, respectively.
Normal Tissue

Diseases of Disordered Cell Death

New Cells
Cell Death

New Cells
Cell Death
New Cells
Cell Death

Homeostasis

Neurodegeneration
Immunodeficiency
Infertility

Cancer
Autoimmunity
The number of people with neurodegenerative disorders is rapidly increasing as the average lifespan gets longer
Neurodegenerative diseases are defined by the progressive **loss** of specific neuronal cell populations.
A - In Alzheimer’s disease, neurons in the hippocampus and certain regions of the cerebral cortex degenerate.

B - Huntington’s disease involves the death of neurons in the striatum, which control body movements.

In Parkinson’s disease, dopamine neurons in the substantia nigra undergo apoptosis.
NEURODEGENERATIVE DISEASES

A wide variety of them are characterized by the accumulation of intracellular or extracellular protein aggregates.

Once formed, aggregates tend to be resistant to degradation. Cells have adapted mechanisms to avoid the accumulation of incorrectly folded proteins. These mechanisms are molecular chaperones that fold proteins correctly and the ubiquitin proteasome degradation system that degrades misfolded or unwanted proteins.

In neurodegenerative disorders, these cellular disposal mechanisms become ineffective and, as a result, misfolded proteins accumulate and become toxic for distinctive sets of neurons.

This results in a clinical manifestation of disease
Many signals can initiate or ‘trigger’ apoptosis in neurons

- lack of neurotrophic factor support
- overactivation of glutamate receptors
- oxidative stress
- metabolic stress
- toxins
- genetic and environmental factors

<table>
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<th>Factors that may modulate apoptosis in neurodegenerative disorders</th>
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<td>Disorder</td>
<td>Genetic factors</td>
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<tr>
<td>Alzheimer’s$^{47-51}$</td>
<td>APP, presenilin mutations, ApoE</td>
</tr>
<tr>
<td>Parkinson’s</td>
<td>α-synuclein, parkin mutations</td>
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<tr>
<td>Huntington’s$^{51,62,66}$</td>
<td>Poly-CAG expansions in huntingtin</td>
</tr>
<tr>
<td>ALS$^{57}$</td>
<td>Cu/Zn-SOD mutations</td>
</tr>
<tr>
<td>Stroke$^{63,84}$</td>
<td>Cadasil mutations</td>
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</table>
Roles for altered synaptic signalling in neurodegenerative disorders

Overactivation of glutamate receptors under conditions of reduced energy availability or increased oxidative stress (from reactive oxygen species, ROS) results in $\text{Ca}^{2+}$ influx into postsynaptic regions of dendrites.

$\text{Ca}^{2+}$ entering the cytoplasm through plasma membrane channels and endoplasmic reticulum (ER) channels induces apoptotic cascades that involve Par-4, pro-apoptotic Bax and Bad, and/or p53.

These factors act on mitochondria to induce $\text{Ca}^{2+}$ influx, oxidative stress, opening of permeability transition pores (PTP) and release of cytochrome $c$.

This results in caspase activation and execution of the cell death process.
Anti-apoptotic signalling pathways are also concentrated in synaptic compartments.

For example, activation of receptors (R) for neurotrophic factors (NTF) in axon terminals stimulates kinase cascades and transcription factors and increased production of survival-promoting proteins such as Bcl-2, Bcl-xL and inhibitor of apoptosis proteins (IAPs) (which inhibit caspases).
Increasing evidence demonstrates that oxidative stress causes damage to cell function with aging and is involved in a number of age-related disorders including atherosclerosis, arthritis, and neurodegenerative disorders (amyotrophic lateral sclerosis, Parkinson disease, Huntington disease, and Alzheimer disease).
This imbalance can occur as a result of increased free radical production or a decrease in antioxidant defenses.
Subsequent events in the neurons are glutamate-induced neurotoxicity and increased cytosolic Ca\(^{2+}\) levels, resulting in activation of Ca\(^{2+}\)-dependent enzymes. These enzymes produce ROS/RNS, which oxidatively modify nucleic acid, lipid, sugar, and protein, leading to nuclear damage, mitochondrial damage, proteasome inhibition, and ER stress.
Alzheimer’s disease (AD) is the most common type of dementia in advanced age. Recent statistics in the United States regarding AD are alarming: 5.3 million people have AD and it is currently the sixth leading cause of death.
AD is characterized by progressive impairment of cognition and emotional disturbances that are strongly correlated with synaptic degeneration and death of neurons in Limbic Structures, such as the hippocampus and the amygdala, and associated regions of the cerebral cortex.
Degenerating neurons show aggregates of hyperphosphorylated tau protein. A defining feature of Alzheimer’s disease is accumulation of amyloid plaques formed by aggregates of amyloid-β peptide, a 40-42 aminoacid fragment generated by proteolytic processing of the amyloid precursor protein (APP).

Brain tissue section from the hippocampus of a patient who died with Alzheimer’s disease.
The amyloid precursor protein (APP) is a transmembrane protein located predominantly in the endoplasmic reticulum (ER). APP can be proteolytically processed in two main ways.
Cleavage of APP within the Aβ sequence by α-secretase releases a secreted form of APP (sAPPα) from the cell surface. Secreted APPα activates a putative receptor (R) linked to cyclic-GMP production and activation of cGMP-dependent protein kinase (PKG). PKG can then promote opening of K+ channels, resulting in membrane hyperpolarization, and can also activate the transcription factor NF-κB; these effects of sAPPα are believed to mediate its neuron survival promoting properties.

A second pathway of APP processing involves cleavages of Aβ by β-secretase and γ-secretase. This releases Aβ from cells, which, under suitable conditions (high concentration, oxidizing environment), begins to self-aggregate. Aβ induces membrane lipid peroxidation (MLP), which impairs the function of membrane ion-motive ATPases (Na+ and Ca2+ pumps) and glucose transporters. Neurons are thus vulnerable to apoptosis.
Presenilin-1 (PS-1) is an integral membrane protein located primarily in the endoplasmic reticulum (ER). Presenilins together with Nicastrin, Aph1 and Pen2 create a $\gamma$-secretase complex that is responsible for the cleavage of APP and other integral membrane proteins. Most familial Alzheimer-inducing mutations have been identified in the Presenilin genes.

Mutations in PS-1 perturb ER Ca$^{2+}$ homeostasis, resulting in increased release of Ca$^{2+}$. The enhanced Ca$^{2+}$ release triggers further Ca$^{2+}$ influx through Ca$^{2+}$ release channels in the plasma membrane, and this altered Ca$^{2+}$ homeostasis makes neurons vulnerable to apoptosis and excitotoxicity, and alters APP processing in a manner that increases A$\beta$ production.
As a microtubule associated protein, tau plays an essential role in maintaining microtubule stability. Aberrant phosphorylation and proteolysis of tau in AD results in impaired functions of tau.

**NFTs** consist of paired helical filaments (PHF) resulting from the hyper-phosphorylation of the microtubule-binding protein tau.

As a microtubule associated protein, tau plays an essential role in maintaining microtubule stability.

In AD neuronal tau is aberrantly phosphorylated and proteolyzed resulting in an impairment of the normal functions of tau.
The deposition of Aβ is an early event in the pathogenesis of AD that precedes the formation of NFTs and collectively is referred to as the ‘‘beta-amyloid hypothesis’’
A series of studies showed that APP can also be cleaved by *caspases* in cells and in human brain tissue.

It was reported that processing of APP at position 720 by caspases increases the rate of Aβ secretion. This observation has not been substantiated however. In fact accumulating evidence indicates that caspase-mediated cleavage of APP does not contribute to Aβ production. Cleavage of APP by caspases at position 720 removes an internalization motif (Y739ENP) in the intracellular tail of the protein that has been shown to be required for Aβ secretion. APP cleavage by caspases would therefore diminish rather than increase Aβ secretion.

*Brain Research Bulletin 80 (2009) 251–267*
If the cleavage of APP by caspases does not increase Aβ production, does it nevertheless participate somehow in the apoptotic process?

Processing of APP at position 720 by caspases produces a cytoplasmic fragment, called C31, which favors cell death.
The implication of APP cleavage at position 720 has been assessed in vivo. Using in vivo mice model with the D[720]A caspase-resistant model, it was found that the mice, despite similar soluble Aβ accumulation and plaque formation compared to the mice expressing the transgene encoding APP with the D720 cleavage site, did not develop the synaptotoxicity phenotype.

Mice prone to Alzheimer-like neurodegeneration that express a caspase-resistant APP mutant (D720A) are protected from synaptotoxicity.

This study supports the notion that the formation of senile plaques is not a triggering event for the decreased neuron functionality observed in this disease.
Recent evidence suggests that caspase activation and cleavage of tau may link plaques and tangles in AD.
More recently, transgenic mice studies have provide additional evidence that caspases play a proximal role in promoting tangle formation in AD.
To understand the contribution of caspases in disease progression, a triple transgenic Alzheimer’s mouse model (3xTg-AD) overexpressing the anti-apoptotic protein Bcl-2 was generated.

Overexpression of Bcl-2 blocked caspase activation (caspase-9 and caspase-3) and the cleavage of tau leading to its accumulation within neurons.
They also investigated whether a similar effect occurred with the APP, a substrate for caspase-3-mediated cleavage. Further, despite the fact the protein levels of APP were significantly higher in 3xTg-AD/Bcl-2-OE mice versus 3xTg-AD mice, there was no evidence for extracellular plaques in these mice. Overexpression of Bcl-2 attenuated the processing of APP and tau and reduced the number of NFTs and extracellular deposits of Ab associated with these animals. These results suggest a significant role for caspase-like proteolytic activity in the processing of APP and production of Aβ.

Despite the high protein levels of tau, there was little evidence for fibrillary tangle formation in 3xTg-AD mice overexpressing Bcl-2, suggesting that the caspase-cleavage of tau is a critical step leading to NFT formation.
Active Caspase-6 and Caspase-6-Cleaved Tau in Neuropil Threads, Neuritic Plaques, and Neurofibrillary Tangles of Alzheimer’s Disease

Caspase cleavage of tau: Linking amyloid and neurofibrillary tangles in Alzheimer’s disease


Departments of *Cell and Molecular Biology and Biochemistry and Molecular Pharmacology, Cell Death Regulation Laboratory, Division of Endocrinology, Metabolism, and Molecular Medicine, Department of Medicine, and Cognitive Neurology and Alzheimer’s Disease Center, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611

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Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology

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Caspase cleavage of the amyloid precursor protein modulates amyloid β-protein toxicity

Daniel C. Lu*, Salvador Soriano*, Dale E. Bredesen† and Edward H. Koo*
The role of caspases in promoting the pathology associated with AD

**Step 1:** activation of caspases

**Step 2:** c-terminal cleavage of APP may facilitate the production of Aβ. This in turn can create a vicious feed-forward cycle of Aβ production and caspase-3 activation.

**Step 3:** Conversely, caspase-3 cleavage of tau may promote tau aggregation and PHF formation thereby linking Aβ to NFTs.

**Step 4:** Aβ deposition and plaque formation.

**Step 5:** Ultimately, the activation of caspases and cleavage of critical cellular proteins may disrupt axonal and dendritic transport processes leading to cell death and neurodegeneration.
Can targeted inhibition of this class of proteases provide an effective means to treat this disease?
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<th>Drug Target</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td>Z-VAD-fmk, pan caspase inhibitor</td>
<td>Extensively used in vitro with a proven efficacy in preventing apoptosis. Previous studies using Z-VAD-fmk in animal models of diseases have proven to be successful and efficacious</td>
<td>Transport into the CNS, bioavailability, and selectivity for specific caspases could be problematic. Toxicity as a result of the ketone group has been reported.</td>
</tr>
<tr>
<td>Q-VD-OPh, small molecule inhibitor of caspases</td>
<td>Improvements over Z-VAD-fmk include potency, stability, and cell permeability. Low toxicity and systemically actively as compared to Z-VAD-fmk</td>
<td>Limited number of studies available compared to Z-VAD-fmk. Selection between “good” versus “bad” apoptosis. Cost prohibitive for long-term study (3-24 months)</td>
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<tr>
<td>Bcl-2 agonists</td>
<td>Represents a critical convergence point in apoptotic pathway. High-affinity small molecule antagonists have already been synthesized for treatment of cancer</td>
<td>Presently, no selective agonist drugs are available for testing. Potential for widespread tumor formation is possible following inhibition of apoptosis.</td>
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</table>
Besides approaches to activate or increase the expression of Bcl-2, another potential drug of interest that already has an impressive history is minocycline. It is a tetracycline that has been demonstrated to be effective in the treatment of Huntington’s disease, Parkinson’s disease as well as in multiple sclerosis.

The advantage of minocycline is that it is orally available, crosses the blood brain barrier, and appears to be safe in humans. Neuroprotection by minocycline appears to be afforded by its ability to directly inhibit the release of cytochrome c and prevent the activation of caspase-3. Minocycline has been tested in an animal model of AD and was shown to slow neuronal cell death. Minocycline may be a good candidate as a therapeutic agent for AD but additional studies are needed.
Parkinson’s Disease

Parkinson's disease (PD) is the second-most common chronic neurological disease of aging.

The cardinal neuropathological features of PD include progressive loss of dopaminergic terminals in the striatum with subsequent death of neurons in the substantia nigra.

Its symptoms are slowness of movements, tremor, rigidity.
Dopamine levels in a normal and a Parkinson’s affected neuron
Although subject to intensive research, the etiology of PD is still enigmatic and the treatment is basically symptomatic.

Many factors are speculated to operate in the mechanism of cell death of the nigrostriatal dopaminergic neurons in PD, including oxidative stress and cytotoxicity of reactive oxygen spices (ROS), disturbances of intracellular calcium homeostasis, exogenous and endogenous toxins, and mitochondrial dysfunction.
Presence of cytoplasmic, \( \alpha \)-synuclein positive inclusions called **Lewy bodies**
Defective clearance of modified, toxic proteins via the ubiquitin proteasome system appears to play a key role in cell death and protein aggregation.

Aggregates tend to be resistant to degradation.

Cells have adapted mechanisms to avoid the accumulation of incorrectly folded proteins:

• **Molecular chaperones that fold proteins correctly**

• **Ubiquitin proteasome degradation system that degrades misfolded or unwanted proteins**
- **α-Synuclein** is a protein abundantly expressed in presynaptic terminals of vertebrates. One of its normal functions is to regulate dopamine transporter activities. Different α-synuclein missense mutations (A30P and A53T) are associated with rare, autosomal dominant, early-onset PD and have been shown to form fibrils.

- **Parkin** is a 465 amino acid protein which is a component of a multiprotein E3 ubiquitin ligase complex which in turn is part of the ubiquitin-proteasome system that mediates the targeting of substrate proteins for proteasomal degradation. Mutations in this gene are known to cause a familial form of Parkinson disease known as autosomal recessive juvenile Parkinson disease.
Fas-associated factor 1

- Caspase activation can be triggered by activation of death receptors such as Fas located on the cell surface of target cells.

- FAF1 is a Fas-binding protein, whose role in apoptotic cell death is not well understood. Interestingly, the human FAF1 gene has been localized to chromosome 1p32 at the PARK 10 locus, a locus that has been associated with late-onset Parkinson's disease.

- Furthermore, in vitro evidence suggests that FAF1 can initiate or enhance Fas-mediated apoptotic cell death.
R. Betarbet et al. in this study examined the role of this novel protein, FAF1, in PD pathogenesis. They demonstrate here that FAF1 expression level is specifically upregulated in PD and in Alzheimer's disease.
Stressors, specific to PD, including oxidative stress and increased A53T mutant human α-synuclein expression could significantly increase the expression levels of endogenous FAF1 indicating that endogenous FAF1 levels can be modulated by exogenous insults.

These studies clearly demonstrated that FAF1 overexpression induced cell death. In addition, FAF1 overexpression could markedly aggravate the toxic effects of oxidative stress and proteasomal inhibition as suggested by increased cell death in response to these treatments.

FAF1 expression potentiates caspase 3 activation and apoptotic cell death.

- FAF1 levels were significantly increased in PD samples as compared to control tissue.

- FAF1 upregulation is specific to neurons with α-synuclein-positive inclusions.
FAF1 is associated with PD pathology and may have a role in PD pathogenesis

Further studies will be required to better understand mechanisms of FAF1-induced cell death and neurodegeneration.
Huntington’s Disease

HD is an autosomal dominant inherited neurodegenerative disease characterized by progressive degeneration of GABAergic medium-sized spiny neurons in the caudate nucleus and putamen.

Clinically, HD is characterized by the mid-life onset of progressive chorea, cognitive decline, and psychiatric disturbance.

The causative gene in the disease is located on chromosome 4p and encodes an abnormal CAG triplet repeat expansion resulting in aberrant huntingtin.
CAG trinucleotide expansions

The specific mechanisms linking the abnormally expanded huntingtin with pathology of the disease are not well clarified, however, excitotoxicity, metabolic impairment and oxidative stress have been suggested as contributing factors in the processes leading to neuronal death.
How does mutant huntingtin promote selective degeneration of striatal neurons? Although this is not known, activation of an apoptotic programme is implicated.

Huntingtin possesses three caspase sites specific for different caspases. Cleavage by caspases induces aggregation but this aggregation is more pronounced with the mutated form of Huntingtin.

In HD, cleavage of mutant htt would release N-terminal fragments with the potential for increased toxicity and accumulation caused by the presence of the expanded polyglutamine tract. Furthermore, accumulation of expanded htt fragments in neurons may lead to corticostriatal dysfunction as an early event in the pathogenesis of HD.
The normal form of Huntingtin inhibits caspase-9 but this is deficient with poly-glutamine bearing Huntingtin mutants. Both normal and mutant Huntingtins can prevent activation of caspase-3 but the normal forms does it more efficiently. Huntingtin can bind HIP1 and blocks its pro-apoptotic function. Poly-glutamine bearing Huntingtin mutants bind HIP1 less well than the normal form.
Wild-type htt is predominantly cytoplasmic and probably functions in vesicle transport, cytoskeletal anchoring, clathrin-mediated endocytosis, neuronal transport or postsynaptic signalling. Htt facilitates dynein-mediated vesicle motility.

Htt may be transported into the nucleus and have a role in transcriptional regulation.
The molecular chaperones promote the folding of newly synthesized huntingtin (htt) into a native structure. Chaperones can facilitate the recognition of abnormal proteins, promoting either their refolding, or ubiquitination and subsequent degradation by the proteasome. The HD mutation induces conformational changes and is likely to cause the abnormal folding of htt, which, if not corrected by chaperones, leads to the accumulation of misfolded htt in the cytoplasm. Ultimately, toxicity might be elicited by mutant full-length htt or by cleaved N-terminal fragments, which may form soluble monomers, oligomers or large insoluble aggregates.

In the cytoplasm, mutant forms of htt may impair the ubiquitin–proteasome system (UPS), leading to the accumulation of more proteins that are misfolded. These toxic proteins might also impair normal vesicle transport and clathrin-mediated endocytosis. Also, the presence of mutant htt could activate proapoptotic proteins directly or indirectly by mitochondrial damage, leading to greater cellular toxicity and other deleterious effects.
The Mitogen-Activated Protein Kinase (MAPK) family, is involved in the survival, proliferation and differentiation of nervous cells. Some of the MAPKs promote the differentiation towards the neuron lineage, others towards the glial one.

The MAPKs are also involved in apoptosis and may, therefore, play a role in neurodegeneration.

This dual role of MAPKs may make it possible to design alternative and/or synergistic approaches to the treatment of degenerative diseases, either by using specific inhibitors of the MAPKs involved in apoptosis, or by increasing the activation of the MAPKs involved in neuronal survival and differentiation. The increased activation of pro-differentiative MAPKs can lead to the replacement of damaged neurons by undifferentiated progenitors and the slowing down of the disease’s progression.
Mitogen-activated protein kinases (MAPKs) are a family of serine-threonine kinases. The MAPK family includes four groups:

- extracellular signal regulated kinase (ERK),
- c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK),
- P38,
- ERK5

MAPKs are activated in response to a wide range of extracellular stimuli including growth factors, hormones, cytokines and others more related to stress responses (i.e. UV radiation, X-rays, heat and osmotic shock), and they are involved in many different cellular processes such as embryogenesis, proliferation, differentiation, transformation and apoptosis.
MAPK activation occurs through a cascade of three kinases which, after stimulation, are sequentially phosphorylated.
MAPKs and Alzheimer’s Disease

The observation of a colocalization between MAPKs and Aβ-amyloid or hyperphosphorylated tau deposits suggests that these proteins may play a role in AD pathogenesis, and many studies have confirmed that MAPKs can be differently modulated by Aβ-amyloid administration.

Furthermore, transgenic mice overexpressing mutated amyloid precursor proteins show, compared with wild type controls, increased JNK/SAPK and p38 activity in the cortex at both 7 and 12 months of age, in concomitance with the increase in amyloid deposition, tau phosphorylation, and loss of synaptophysin, an index of synaptic integrity.

MAPKs are also involved in tau hyperphosphorylation as observed in both transgenic models of AD and in brain samples obtained from AD patients. All three of the MAPKs participate in tau hyperphosphorylation in AD although they act at different stages of the disease: active ERK1/2 appear at the early stages while p38 and JNK/SAPK act at the later stages. A sequential activation of ERK1/2, JNK/SAPK and p38 in susceptible neurons has, therefore, been related to disease progression and severity.
MAPKs and Huntington Disease

- Many studies have shown the involvement of MAPKs and, in particular, of JNK/SAPK and p38 in different models of HD.

- The expression of mutated huntingtin with 48 or 89 polyglutamine repeats stimulates JNK/SAPK activity and induces apoptotic cell death in HN33 cells, an immortalized rat hippocampal cell line, while the expression of normal huntingtin with 16 polyglutamine repeats has no toxic effects.

- In addition, the JNK/SAPK activation precedes apoptotic cell death while the co-expression of the dominant negative mutant form of SEK1 (an upstream JNK/SAPK activator) nearly completely blocks the activation of JNK/SAPK and neuronal apoptosis mediated by mutant huntingtin.

- On the other hand, ERK1/2 are thought to have a protective role, since increased levels of active ERK1/2 protect against cellular death.
Increasing evidence indicates that MAPKs are involved in dopaminergic neuronal degeneration in PD, as has been observed in both in vitro and in vivo models obtained with exposure to toxic substances such as MPTP.

In particular, the role of JNK/SAPK and p38 pathways seem to be important in PD neurodegeneration, while ERK1/2 is more often related to a neuroprotective action even if its role is not very clear.

Moreover parkin is normally involved in ubiquitination and degradation of p38 and, therefore, mutations in parkin determine an alteration of p38 turnover which suggests that there may be a correlation between p38 and PD pathogenesis.
Current therapies are focused on counteracting the degenerative events by acting on the molecular mechanisms involved in cellular death, or by the exogenous administration of pro-survival factors.

The presence in many areas of both the peripheral and central nervous systems of niches of neural progenitors which can differentiate, under specific conditions, into neurons or glial cells opens up new therapeutic perspectives.