Review

Role of Apoptosis in disease

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Abstract: Since the initial description of apoptosis, a number of different forms of cell death have been described. In this review we will focus on classic caspase-dependent apoptosis and its variations that contribute to diseases. Over fifty years of research have clarified molecular mechanisms involved in apoptotic signaling as well and shown that alterations of these pathways lead to human diseases. Indeed both reduced and increased apoptosis can result in pathology. More recently these findings have led to the development of therapeutic approaches based on regulation of apoptosis, some of which are in clinical trials or have entered medical practice.

INTRODUCTION

Since its initial description cell death has appeared as a basic biological phenomenon fundamental for development and regulation of tissue homeostasis whose alteration has important implications in pathology [1]. Indeed cell death contributes to tissue homeostasis by balancing mitosis and pathology can derive from both its increase or decrease. Since the initial description of cell death in the 1960s a number of different death mechanisms have been described and have been classified both on morphological and biochemical criteria. A recent paper published in CDD [2] by a large number of experts in the field has suggested a classification of the different types and is a good reference for the subject. This review will mostly focus on the role in different human diseases of caspase dependent apoptosis Table 1).

Caspase dependent apoptosis (Figure 1) is characterized by the activation of pathways leading to the activation of a family of proteases: caspases resulting in an ordered disruption of the cell without leakage of cellular components and induction of inflammation. Apoptosis occurs following the activation of specific pathways that result in a series of well defined morphological events. The dying cell initially shows nuclear and cytoplasmic condensation, followed by blebbing of the plasma membrane that results in release of small membrane-enclosed particles containing cellular components known as apoptotic bodies. These are rapidly identified by neighbouring cells or professional phagocytes and disposed generally without induction of inflammation or tissue scarring [3]. Apoptosis depends on activation of caspases that will then cleave a number of substrates [4] resulting in the biochemical and morphological changes typical of this form of death. All caspases are synthesized as inactive zymogens that need activation to exert their function. Full activation is achieved through cleavage of a pro-domain generally by other caspases. From a functional point of view we can distinguish two types of caspases: up-stream and down-stream caspases. Up–stream caspases are activated when more enzyme molecules are brought in close proximity and undergo conformational changes upon binding to activation complexes, this results in their cleavage and full activation [5, 6]. Once activated they will activate additional molecules of the same enzyme as well as down stream caspases. Down stream caspases on the other hand can only be activated by cleavage of the pro-domain by up-stream caspase. Two main molecular pathways lead to caspase activation and therefore to apoptosis the so-called extrinsic and intrinsic pathway.

Table 1. Diseases in which alterations of apoptosis are involved

Cancer	
Breast	[20, 28, 160, 161]
Lung	[21, 34, 162]
Kidney	[22]
Ovary and uterus	[28]
CNS	[13, 23, 25, 163-165]
Gastro-enteric trait	[24, 30-32, 166]
Head and Neck	[167]
Melanoma	[33, 35, 168-170]
Lymphomas	[18, 19]
Leukemia	[26, 171-178]
Neurological disorders	
Alzheimer	[55, 179-183]
Parkinson	[63, 184, 185]
Huntington	[186-188]
Amyotrophic Lateral Sclerosis	[189-191]
Stroke	[192-194]
Cardiovascular disorders	
Ischemia	[99, 195-198]
Heart Failure	[99, 199]
Infectious diseases	
Bacterial	[200-206]
Viral	[150, 207-214]
Autoimmune diseases	
Systemic Lupus erythematosus	[153, 215, 216]
Autoimmune lymphoproliferative syndrome	[154, 155]
Rheumatoid arthritis	[217]
Thyroiditis	[1, 217, 218]

Extrinsic apoptosis indicates a form of death induced by extracellular signals that result in the binding of ligands to specific trans-membrane receptors, collectively known as death receptors (DR) belonging to the TNF/NGF family. All death receptors function in a similar way: upon ligand binding several receptor molecules are brought together and undergo conformational changes allowing the assembly of a large multi-protein complex known as Death Initiation Signalling Complex (DISC) that leads to activation of the caspase cascade. In the FAS/CD95 signalling complex, that can be used as a prototype of this form of death, upon ligand binding FAS recruits, through a highly conserved 80 amino acid domain, known as death domain (DD), an adaptor molecule: Fas-associated protein with a DD (FADD). FADD contains another conserved protein interaction domain known as Death Effector Domain (DED) that binds to an homologous domain in caspase 8 leading to its activation. Active caspase 8 will activate additional caspase 8 molecules as well as downstream caspases such as caspase 3 [7].

The intrinsic pathway is activated in response to a number of stressing conditions including DNA damage, oxidative stress and many others. In all cases this multiple forms of stress converge on the mitochondria and determine mitochondrial outer membrane permeabilization (MOMP) this in turn results in dissipation of the mitochondrial membrane potential and therefore in cessation of ATP production as well as release of a number of proteins that contribute to caspase activation. At least two molecular mechanisms (not mutually exclusive) have been proposed to explain how different signals converge at the mitochondria resulting in MOMP. One involves the pore forming ability of some of the BCL-2 family proteins in the outer mitochondrial membrane [8] and the other is the result of the opening in the inner membrane of the permeability transition pore complex (PTPC), that

would require the Adenine Nucleotide Transporter (ANT) and the Voltage Dependent Anion Channel (VDAC) [9, 10]. The Bcl-2 family proteins are essential regulators of this type of apoptosis and are all characterized by the presence of at least one Bcl-2 Homology (BH) domain. From a functional point of view they can be classified in anti-apoptotic members containing three or four BH domains (such as Bcl-2, Bcl-xl, Bcl-w, Mcl-1) and pro-apoptotic members with two or three BH domains (such as Bax, Bak, Bcl-xs, Bok) or with just one (such as Bad, Bik, Bid, Bim, Noxa, Puma). Pro-apoptotic members of the family mediate apoptosis by disrupting membrane integrity either directly forming pores or by binding to mitochondrial channel proteins such as VDAC or ANT, while anti-apoptotic members would prevent apoptosis by interfering with pro-apoptotic member aggregation. The different apoptotic signals are sensed by BH3 only proteins that are induced or activated and migrate to the mitochondria where they bind the pro-survival members of the family removing their block or to the pro-apoptotic members promoting their aggregation [11].

In any case once MOMP occurs a number of proteins are released from the mitochondria, these include Cytochrome C (CYTC), apoptosis-inducing factor (AIF), endonuclease G (endo G), Direct IAP-binding protein with low PI (DIABLO, also known as SMAC) and others. Once CYTC is released it binds to APAF-1 inducing the formation of a large complex, known as the apoptosome, that recruits caspase 9. In the apoptosome, caspase 9 is activated and cleaved and will activate additional molecules of caspase 9 as well as down-stream caspases such as caspase 3. Due to its lethality the system is subject to a number of controls as an example the cytoplasm contains a class of proteins known as Inhibitors of Apoptosis IAPS that bind and inactivate caspases. Upon MOMP the mitochondria also releases proteins such as DIABLO/SMAC that bind to IAPS removing their inhibition and allowing apoptosis to occur.

The intrinsic and extrinsic pathways are not completely independent: in some cells in fact activation of caspase 8 results in activation of the mitochondrial pathway. In this case caspase 8 among other things cleaves a BH3 only protein BID generating a truncated fragment known as truncated BID (tBID) that can permeabilize the mitochondrion resulting in MOMP [12].

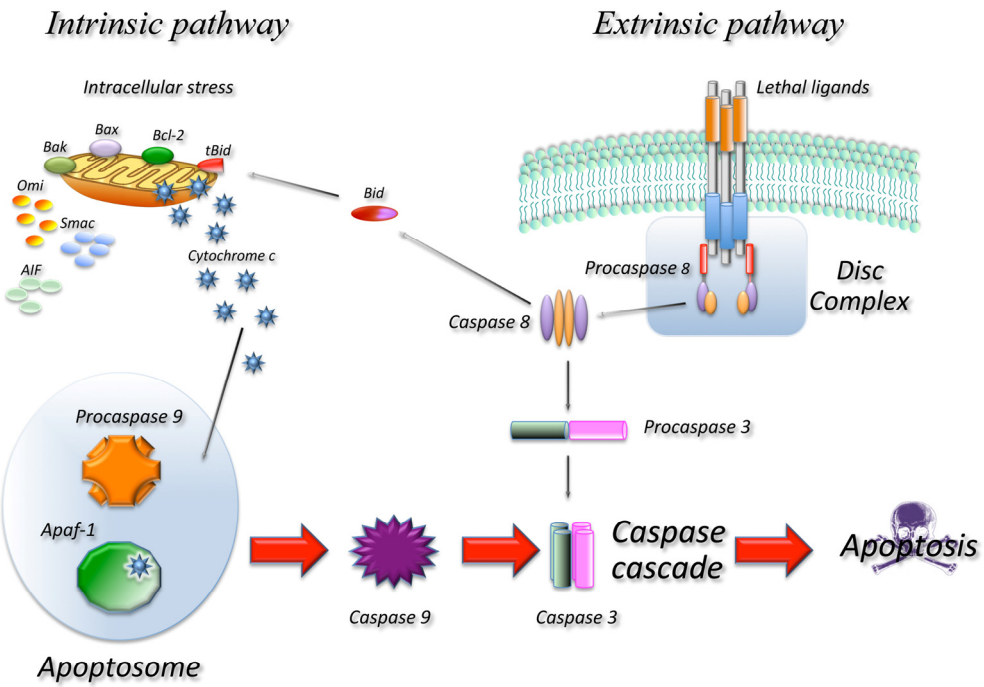

Figure 1. Schematic representation of the main molecular pathways leading to apoptosis. In the extrinsic pathway upon ligand binding to specific receptors the DISC complex is formed and caspase 8 activated. In the intrinsic pathway release of cyt c from the mitochondria result in the formation of the apoptosome and activation of caspase 9. Caspase 8 and 9 then activate downstream caspases such as caspase 3 resulting in cell death. The two pathways are connected through the cleavage of the BH3 only protein BID.

Cancer

Based on its role in maintaining tissue homeostasis it is not surprising that alterations of apoptosis play an important role in cancer development. Moreover defects in the apoptotic pathways are responsible for resistance to therapy and new therapeutic approaches attempting to re-activate these pathways bypassing the block are currently being studied [13-17]. Alteration of many proteins involved in both intrinsic and extrinsic signaling pathways have been described and are in part summarized below. It is clear that alterations of up-stream regulators of these pathways are the most common alterations found in cancer cells, as an example p53 that can induce cell death in response to a number of different stress stimuli, is the most frequently mutated gene in human cancers. A description of these defects however is beyond the scope of this review.

BCL-2 Family proteins alterations. Bcl-2 was initially isolated and characterized in a subset of B-cell lymphomas. These tumours carry a typical translocation t(14;18) that involves Bcl-2 gene and results in its over-expression. Thus it appeared that this gene played a role in cancer even before his function in regulating apoptosis was clearly defined. Since those early days a bulk of data have proven the role of Bcl-2 alterations in development of cancer including a number of animal models. In fact transgenic mice carrying bcl-2 overexpression are actually susceptible to develop different forms of lymphoproliferative disorders [18].
Bcl-2 has been found overexpressed not only in B-cell lymphomas, but also in a variety of other cancers such as: Hodgkin lymphoma where it seems to be associated with worse overall survival [19]; breast cancer where it correlates with tumor aggressiveness, reduced survival and resistance to endocrine therapy [20]; non-small and small cell lung carcinoma (moreover squamous) [21], renal cell carcinoma [22]. A novel polymorphism of the BCL-2 promoter (-938C>A) associates with increased aggressiveness and worse prognosis in glioblastoma multiform [23], chronic lymphocytic leukemia, as well as with a better survival and outcome in breast and ovarian cancers.

In the last years a variety of alterations of the different Bcl-2 family members have been described and have proven the importance of this group of proteins in cancer development. Bax and Bak mutations [24] have been described in colon and gastric carcinomas. Unexpectedly however neither BAX single KO or BAX/BAK double KO show increased tumour formation suggesting that compensatory mechanisms can allow apoptosis in these cells. Interestingly however Bax/pARF double KO mice exhibit an increased variety of tumours (sarcomas and carcinomas). Furthermore Bax deletion accelerates growth of brain tumours in a p53 mutant mice model [25], and of mammary tumours in a SV40 large T antigen transgenic mouse model. Various BH3 protein alterations have also been implicated in cancer development, as an example Bid-deficient mice are prone to develop a form of chronic myelomonocytic leukemia [26], as well as diffuse large B-cell lymphoma.

The possibility to target Bcl-2 family members proteins to induce apoptosis in cancer cells has been studied for many years now and particular attention has been given to BH3 only proteins in designing drugs that would mimic their pro-apoptotic functions. Some of these are currently being tested in phase I/II clinical trials [27-29]. Antisense oligonucleotides targeting Bcl-2 have also been developed and in one case have reached a phase III clinical trial in patients with chronic lymphocytic leukemia.

Apoptosome defects. Apaf1 inactivation can substitute for p53 defects in promoting transformation of myc-expressing cells, moreover it is frequently silenced or inactivated in human cancers. Indeed melanomas, leukemias, glioblastomas, and cervical carcinomas have been shown to down-regulate Apaf1 by epigenetic mechanisms. In addition in some cancers a defective Cyt-C dependent caspase 9 activation in the presence of normal or elevated Apaf1 levels has been reported but the underlying molecular mechanism is still elusive and the existence of an unidentified apoptosome inhibitor has been suggested. Sequestration of Apaf1 into lipid rafts has also been demonstrated in Burkitt lymphoma cells providing another molecular mechanism for apoptosome dysfunction.

Death receptor pathway defects. Death receptor pathways alterations have profound implications in cancer and in particular in the mechanism leading to the ability of tumours to avoid immune response. In a simplistic way one can imagine a scenario in which reduced expression of death receptors protects cells from the immune system and increased surface (or soluble form) expression of death ligands allows killing of reactive cells. Indeed CD95 null mice are prone to accumulate abnormal T-cells, with splenomegaly and lymphoadenopathy and CD95 is lost or mutated in several human cancers. In fact CD95 is lost in hepatocarcinomas [30], present in less than 5% of invasive esophageal cancer cells in 79% of patients [31] where it correlates with depth of invasion and nodes metastasis, mutated in adult T cell leukemia, down-regulated in colon cancer [32], ovarian, cervical and endometrial cancers, melanoma (where lymphocyte

infiltration of the invasive layer correlates with prognosis) [33] and in more than 90% of lung cancers [34]. In support of the above mentioned model, down-regulation of Fas-L leads to decreased tumor volume and increased lymphocyte infiltration. Over-expression of FAS-L has been reported in hepatocarcinomas, esophageal cancers [31], breast cancers, melanomas [35], astrocytomas , metastatic colon cancers, gastric cancers and in more than 60% of sarcomas (reaching 95% in metastatic ones). The soluble form of Fas-L (sFas-L), was also found in peripheral blood of cancer patients, where it possibly exerts an immunosuppressive effect [36, 37].

Defects of the CD95 signaling pathway can also be a consequence of alterations of other components of the DISC. FADD mutations were reported in non-small-cell lung cancer [38] and complete loss was described in a subset of diffuse large B-cell lymphoma. However FADD defects have also been shown to have a pro-oncogenic function, probably due to a role of this protein different from apoptosis. As an example phosphorylated-FADD in lung adenocarcinomas correlates with poor survival [39], and its overexpression associates with poor prognosis in oral squamous cell carcinomas.

A number of evidence highlight the importance of the Trail receptor pathway in tumour onset and development. The importance of Trail as a tumour suppressor is supported by a number of results in different animal models including the onset of spontaneous haematopoietic malignancies in Trail KO mice [40]. As described for CD95 also in the case of Trail, defects mostly allow tumour cells to escape immune control. Trail receptors 1 and 2 map to chromosome 8p21-22 frequently lost in tumours and mutations of trail receptors have been described in up to 20% of various human tumours, including breast cancer, head and neck cancers, and non-Hodgkin lymphomas.

The possibility to develop cancer therapies based on the activation of death receptors has been attractive since their discovery, however toxicity of therapies targeting TNF and CD95 have greatly reduced the initial enthusiasm. The finding that recombinant Trail preferentially induced apoptosis in cancer cells while sparing normal cells supported by its low or absent toxicity in vivo [40, 41] have attracted growing interest on the possibility to exploit this pathway for cancer therapy. Despite a large investigative effort however the reason for this selective activity is poorly understood.

Altered caspase activity. Caspases are the final effectors of both extrinsic and intrinsic apoptosis, therefore it is expected that interfering with their function impairs these pathway leading to a survival advantage for cancer cells and indeed caspase alterations are not rare in a variety of tumours. These can be due to mutations, promoter methylation, alternative splicing, and post-translational modifications. Some of these defects are loss of functions, but in other cases mutated caspases act as dominant negatives preventing activation also of the wild type protein [42].

Altered caspase function can also be a consequence of modified expression of their specific inhibitors, as an example cFLIPs that competes with caspase 8 for FADD binding, thus preventing its activation, is often elevated in tumours, while its down-regulation can sensitize tumour cells to therapy. Among caspase inhibitors an important role is played by IAPs. Indeed alterations of IAPs are found in a variety of human cancers and are associated with poor prognosis and resistance to therapy. In some cases however loss of IAPs correlates with tumour progression complicating the issue and suggesting that the role of IAPs has to be carefully evaluated based on cell context. While initially described as caspase inhibitors now IAPs have been recognized to regulate a multitude of other cellular functions including regulation of the immune response cell migration, mitosis and proliferation [43]. As many of these processes are often modified in cancer it is clear how alteration of IAPs can play a role in tumorigenesis not only as a consequence of altered apoptosis. In fact probably the most important pathway regulated by IAPs that contributes to cancer development is the NF-kB signaling pathway. XIAP, cIAP1 and cIAP2 have been shown to regulate this pathway and as a consequence inflammation, immunity and cell survival. Moreover cIAPs protect from TNF killing. In addition, recent findings show a role for IAPs in metastatization as a XIAP/survivin complex would trigger NF-kB pathway leading to activation of cell motility kinases [43]. This however is still a controversial issue and other studies show a suppressive effect of IAPs on cell mobility.

In any case due to their involvement in cancer progression and to their ability to suppress apoptosis IAPs have become an attractive therapeutical target, leading to the development of IAP inhibitors, some of which are based on natural inhibitors such as Smac/DIABLO [43, 44]. These drugs appear to be able to directly kill cancer cells or at least sensitize them to other killing agents while sparing normal cells. A number of these compounds are currently entering

clinical trials and their efficacy will be evaluated in the next few years.

Neurological disorders

From a physiological point of view apoptosis plays a key role in central nervous development [45, 46], while in adult brain it is involved in the pathogenesis of a number of diseases including neurodegenerative diseases and acute injury (i.e. stroke).

Neurodegenerative diseases

Alzheimer's disease (AD) is the seventh leading cause of all deaths in the United States, it is a progressive neurodegenerative disorder characterized by accumulation of amyloid-β β peptides in extracellular senile plaques intra-cellular neurofibrillary tangles (NFTs) formation resulting from hyper-phosphorylated microtubule-associated protein tau resulting in loss of neurons and consequent progressive dementia [47-49].
Neuronal apoptosis plays an important role in AD pathogenesis and caspases seem to be involved also in some of the upstream pathological events. Exposure of cultured hippocampal neurons to β results in caspase 3 activation and apoptosis [50]. Aβ is generated following sequential cleavage of the amyloid precursor protein (APP), and caspase 3 is considered the predominant caspase involved in APP cleavage [51, 52]. Tau protein is also a substrate for caspase 3; cleavage of tau at its C-terminus would promote tau hyper-phosphorylation and accumulation of NFTs. Moreover, β-induced caspase 3 activation causes abnormal processing of the tau protein in models of AD [53]. APP is also cleaved by caspase 6 in vivo [54], moreover the N-terminal APP fragment is a ligand for death receptor 6 (DR6 also known as TNFRSF21) activation of which triggers caspase 6 dependent axonal degeneration [55].

The potential benefit of inhibiting the intrinsic apoptotic pathway has been suggested through the use of a triple transgenic AD mouse model wherein overexpression of the anti-apoptotic Bcl-2 gene blocked activation of caspases 9 and 3; in these conditions, the degree of caspase cleavage of tau was limited, the formation of plaques and tangles was inhibited, and memory retention was improved [56, 57].

Parkinson's disease (PD) is considered the 2nd most common chronic neurodegenerative disorder after AD, it is associated with movement disorders, tremors, and rigidity and is characterized by a specific loss of dopaminergic neurons of the substantia nigra. This degeneration leads to the formation of fibrillar cytoplasmic inclusions known as Lewy bodies. A preponderant role of the aberrant activation of intrinsic and extrinsic apoptotic pathways in PD pathogenesis has been suggested. The involvement of caspases 1 and 3 in apoptotic cell death has been proved using PD animal models [58]. PD has been linked to mutations in several genes such as parkin [59], DJ-1, and a gene codifying for a mitochondrial kinase, (PTEN)-induced kinase 1 (PINK1) [60]. PINK1 function is related to the inhibition of mitochondria-dependent apoptosis [61]. In human and mouse neurons deleted for PINK1 Bax translocation to the mitochondria and cytochrome c release to the cytoplasm occur earlier than in control cells. Furthermore loss of PINK1 results in elevated levels of caspase activation (caspases 3 and 9) [61]. Gene-expression profiling studies performed on material from patients affected by PD confirmed down-regulation of PINK1 as well as other anti-apoptotic proteins such as Bcl-2 but also found evidence for the involvement of the extrinsic pathway. Indeed death receptors such as FAS, TNFRSF10B and TNFRSF21 were up-regulated in PD-affected neurons [62, 63].

Huntington's disease (HD) is a disorder characterized by a degenerative process, which affects medium spiny striatal and cortical neurons. HD is an autosomal dominant disease caused by a mutation in the gene encoding the huntingtin protein (htt); this mutation is responsible for abnormal expansion of a trinucleotide CAG repeat encoding polyglutamine tract expansion in the N terminus of htt [64]. The expanded polyglutamine alters protein folding, leading to generation of aggregates in neurons that seem to be crucial for the neurodegenerative process [65, 66]. Mutant htt is cleaved by different proteases including caspases [67] and accumulation of caspase cleaved fragments is an early pathological finding in brains of HD patients [68]. Moreover transgenic mice models have demonstrated that caspase 6 cleavage of mutant htt is required for the development of the characteristic behavioral and neuro-pathological symptoms. In addition activation of caspase 6, is observed before the onset of motor abnormalities in HD brains, suggesting that these activation could be used as an early marker of the disease [69].

An additional molecular mechanism involves htt-interacting protein 1 (HIP-1) that binds a polypeptide named Hippi (HIP-1 protein interactor) forming a complex that can activate caspase 8. The free cellular HIP-1 concentration is increased when htt is mutated (HD), this would favor the pro-apoptotic Hippi-Hip complex formation [70, 71].

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by muscle

atrophy, paralysis, and, death due to progressive loss of motor neurons [72]. About 10% of cases are familial as a result of mutations in the copper-zinc superoxide dismutase (SOD1) gene [73], whereas the majority of them are sporadic. SOD1 catalyzes conversion of the superoxide anion to hydrogen peroxide, however the mechanism by which SOD1 mutations cause ALS is still not completely understood. Several alterations have been identified in ALS and are thought to play a role in motor neuron death, including: neurofilament abnormalities, aggregate formation, oxidative stress, and inflammatory processes [74]. Mice overexpressing a human mutant SOD1 develop neuron degeneration, and have been used as a model [75, 76]. These mice show increased p38 activity that determines increased NO production that in turn results in increased FasL expression and activation of the extrinsic pathway [77]. In addition mutated SOD would localize to the mitochondria and directly trigger CYTC release and therefore neuronal death [78].

Motor neurons degeneration in ALS is also accompanied by inflammation, but the exact mechanism triggering inflammatory response remains unclear. Meissner et al have recently reported that mutant SOD1 induces IL-1 beta and promotes caspase 1 activation resulting in neuro-inflammation that would contribute to the pathogenesis [79]. Finally caspase 1 would act as a chronic activator of caspase 3 contributing to neuronal loss [80].

Acute CNS insults

Stroke is the leading cause of acquired adult disability in USA [81]. Ischemic injury is caused by the loss of blood flow to the brain, usually as a consequence of an embolism. The decrease in perfusion determines both apoptotic and necrotic neuronal cell death in the affected region (core) due to energy depletion [82, 83]. Around this area of tissues that is irreversibly lost there is an area of partially damaged tissue known as penumbra that triggers local inflammation. Several evidences suggest that inflammation is a crucial event in the progression of ischemic brain damage. Cyclooxygenase-2 (COX-2) induction and prostaglandin E2 elevation have been reported to occur after cerebral ischemic insult [84]. Takadera et al showed that prostaglandin E2 directly induced apoptosis in hippocampal neurons through the activation of caspase 3 [85], suggesting that a direct effect of prostaglandin E2 on hippocampal neurons was mediated by activation of the EP2 receptors. The deletion of EP3 receptors is known to ameliorate stroke injury in experimental stroke models [86], and recently it has been demonstrated that EP3 receptors are involved in the enhancement of inflammatory and apoptotic response in the ischemic cortex [87].

An important role in ischemic brain damage seems to be played also by activation of the receptor pathway. TNF deletion in mice protects the brain from ischemic damage [88]. Fas and FasL levels seem to be increased during brain ischemia [89], and interfering with the Fas signaling pathway using a blocking anti-FasL antibody markedly reduces death of neurons and improves functional recovery in animal models of stroke and spinal injury. Moreover chronic extrinsic cervical spinal cord compression leads to Fas-mediated apoptosis of neurons [90].

C-Jun N-terminal protein kinase (JNK) signaling pathway is known to be activated in response to stress and ischemia. JNK activation precedes inflammation and apoptosis in neuronal cells [91]. Indeed, JNK is involved in the regulation of several pro-apoptotic proteins such as Bim and inhibition of JNK activity attenuated Bax translocation in ischemic neurons [92].

The involvement of caspases in mediating ischemic neuronal cell death has been also demonstrated using caspase 1 and 3 KO mice. Cortical neurons from caspase 3 -/- mice subjected to oxygen-glucose deprivation, were more resistant to cell death [93], while caspase 1 deletion in mice led to reduced production of IL-1 beta [94]. Consistently the reduction of IL-1 beta function, using specific antagonists, results in neuroprotective effects in stroke animal models [95].
Benchoua et al. proposed activation of different and specific pathways in the core or in the penumbra area of the brain infarction. In particular, after cerebral infarction, in neurons of the core area the first apoptotic events are mediated by ligand binding to specific death receptors leading to caspase 8 activation. In the penumbral area, where mitochondria provide residual energy supply, neuronal death is instead induced through the mitochondrial pathway [96].

The reperfusion of ischemic tissue usually improves clinical outcome of patients, but in others it may amplify brain damage due to the ischemia-reperfusion injury. Reactive oxygen species (ROS) levels are immediately increased after a vessel occlusion is cleared, and are considered the main mediators of reperfusion injury [97] resulting in the release of cytochrome C [98].

Heart diseases

Adult cardiomyocites are post-mitotic cells, therefore this tissue has limited response capability to damage. In