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Apoptosis Review Series

Physiological and pathological roles of Apaf1 and the apoptosome

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Abstract

Different cellular pathways can lead to apoptosis. Apaf1 is the molecular core of the apoptosome, a multiproteic complex mediating the so-called mitochondrial pathway of cell death. The importance of this pathway during development has been clearly demonstrated by knocking out key genes. Also, the relevance of Apaf1 dosage during development has been recently underlined. Moreover, a growing body of evidences seems to point out a possible role of the mitochondria-dependent apoptosis in different pathologies. In particular, we discuss here some recent evidences regarding the putative role of the apoptosome in neurodegeneration and cancer.

Keywords: apoptosis - cell death - neurodegeneration - Apaf1 - apoptosome - caspases

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Introduction

Apoptosis is a form of cell death essential for tissue homeostasis and development. It is also used as a cellular response to stress or pathogens. Deregulated apoptosis can contribute to many forms of human diseases such as cancer, autoimmunity, heart disease, immunodeficiencies and neurodegenerative disorders.

Cells undergoing apoptosis show characteristic morphological features such as cell shrinkage, membrane blebbing, chromatin condensation, DNA fragmentation and breakdown into apoptotic bodies that are rapidly phagocytosed [1, 2].

Most of these typical features are the consequence of caspases activation. Caspases are a group of cysteine proteases with specificity for aspartic acid residues. To date, 14 mammalian caspases have been identified. They are expressed as inactive proenzymes and proteolitically activated to form active tetramers during apoptosis. Many caspases participate in signaling and execution of apoptosis while others are implicated in proinflammatory mechanisms [3]. Stimuli that trigger apoptosis are diverse and appear to engage the cell death machinery in a variety of ways. However, in many cases, signals generated by numerous death-promoting agents all converge, at some point, on mitochondria and trigger release of important pro-apoptotic factors from this organelle, defining the so-called mitochondrial pathway of cell death [4, 5]. The Bcl2 family (which includes pro-apoptotic and anti-apoptotic members) of proteins regulates the release of apoptogenic factors from mitochondria. The mechanism of this process is still unclear. It might involve the formation of specific pores or the rupture of the outer membrane which can be direct or mediated by the opening of the mitochondrial permeability transition pore (MPTP) followed by swelling of the matrix and subsequent disruption of the outer membrane [4, 5].

Apoptosome and Apaf1

One of the pro-apoptotic factors released from mitochondria during apoptosis is the cytochrome c. In the cytosol, the cytochrome c binds the adapter molecule Apaf1 and, in the presence of ATP/dATP, it promotes the assembly of a multiproteic complex called apoptosome which, in turn, binds and activates procaspase-9 [6] (Fig 1). The apoptosome is the executioner of the mitochondria-dependent apoptosis. Apaf1 is the core of the apoptosome. Recent evidences suggest that the apoptosome is a wheel-like particle containing seven Apaf1 monomers which, like seven spokes, radiate from a central hub [7]. Apaf1 possesses an amino-terminal CARD domain (interacting with the CARD domain of procaspase-9), a central CED-4-like domain (which contains Walkers A and B boxes for nucleotide binding) and a long carboxy-terminal domain extremely rich in WD-40 repeats. The CARD domain is located in the central hub. The cytochrome c would interact with the WD40 domain, which forms the distal part of the spoke. Normally Apaf1 has a compact autoinhibited shape in which the CARD domain binds the WD40 domain. Following its release from mitochondria, the cytochrome c displaces the CARD domain from the WD40 domain taking its place and allowing the CED-4 homology domain to bind dATP/ATP and undergo a conformational change in which Apaf1 has a more extended conformation; this is required for efficient assembly of the apoptosome and procaspase-9 binding to the exposed CARD domain [7].

The mechanism of procaspase-9 activation is not clear. Mature caspase-9 remains bound to the apoptosome where it recruits and activates executioner caspases such as caspase-3 and caspase-7 [7].

The activation of caspases requires the removal of IAPs (inhibitor of apoptotic proteins) which bind caspases. One of these, XIAP, directly interacts with the apoptosome by associating and inhibiting caspase-9 and caspase-3 [8, 9]. Another protein released from mitochondria, Smac/Diablo, promotes apoptosis by binding to IAPs and removing their inhibitory activity on caspases [10]. Omi/HtrA2 (a proapoptotic mitochodrial factor) can also induce apoptosis in a caspase-dependent manner via its ability to disrupt caspase-IAPs interaction and in a caspase-independent manner through its serine protease activity [11]. Once activated, executioner caspases cleave a wide variety of cellular substrates leading to the orderly demise of the cell.

Apaf1 can interact with other proteins, which regulate its function. It has been shown that Hsp70 can bind to Apaf1 and prevent recruitment of procaspase-9 to the apoptosome [12]. Hsp90 can form a cytosolic complex with Apaf1 inhibiting its oligomerization and formation of the active apoptosome [13]. Hsp27

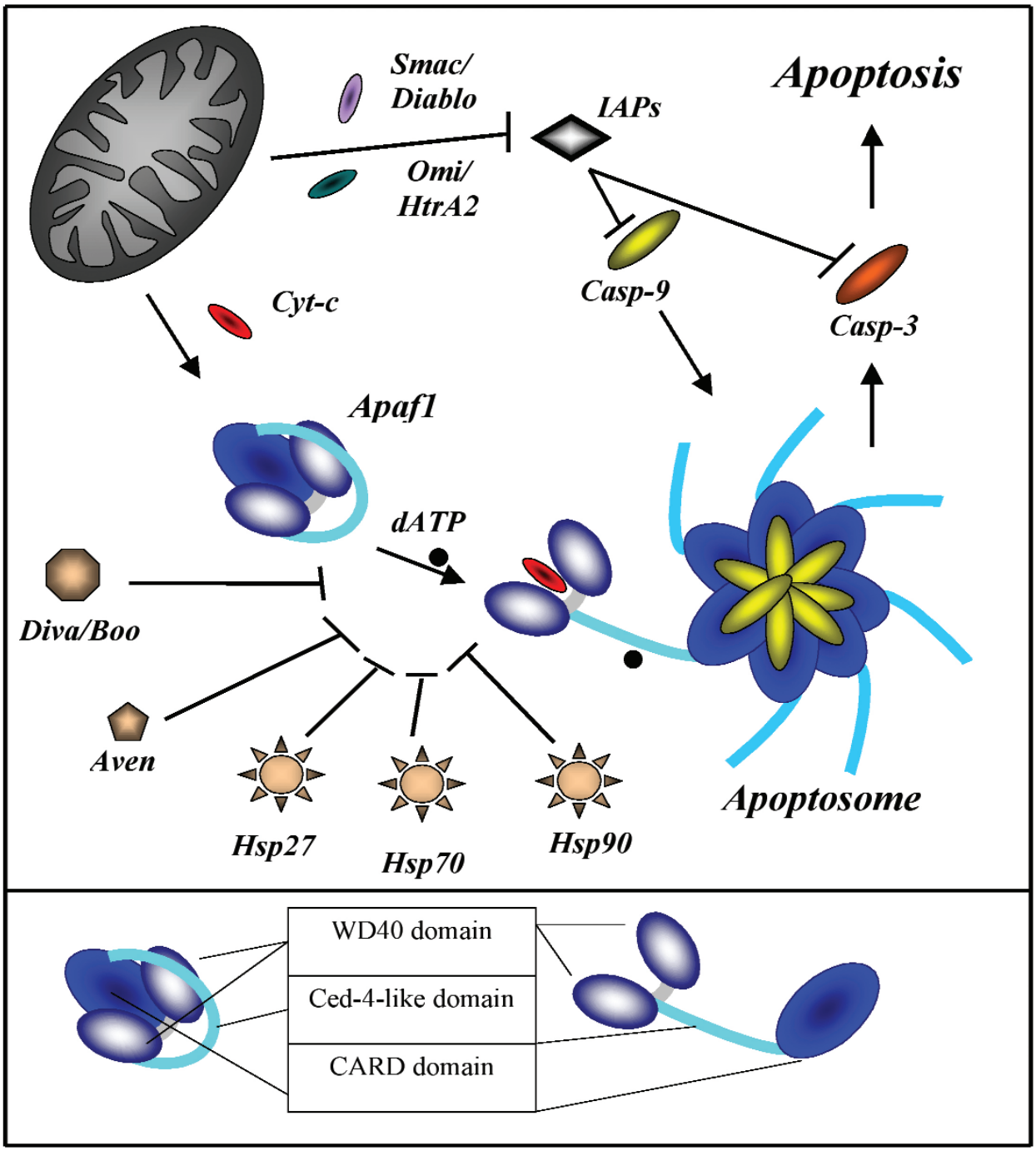

Fig. 1 The activation of the apoptosome.

binds cytochrome c preventing its interaction with Apaf1 [14]. Aven would inhibit apoptosis by binding Apaf1 and impairing its oligomerization. Diva/Boo is an antiapoptotic member of the Bcl2 family; it has been reported to be able to interact with Apaf1 but this direct interaction has been recently questioned [15]. Furthermore, some authors reported that Apaf1 can be proteolitically cleaved by caspase-3, but the significance of this processing has not been clarified yet [15].

Apaf1 roles in development

As main mediator of the mitochondria-dependent apoptosis, Apaf1 plays a crucial role in development. In fact, while no alterations were observed in heterozygous mice, Apaf1 deficient embryos die between embryonic day 16 (e16) and post-natal day 0 (pn0) [16]. Abnormal Apaf-/- embryos were found at e11.5 and later; they show alterations of the development of many organs [16].

Dramatic craniofacial alterations are clearly visible at e16.5: midline facial cleft, rostral exencephaly, cleft palate and absence of skull vault and of all vomer and ethmoidal elements. This phenotype demonstrates that apoptosis is essential in midline fusion of craniofacial structures.

Apoptosis is needed to remove the interdigital cells of the limbs by e15.5, for the sculpting of the digits. Apaf1-/- embryos, at this stage, show poorly shaped digits with persistence of interdigital webs confirming that Apaf1 is a key component for this process. At e16.5, however, all mutants have a normal development of limbs and show a form of cell death caspase-independent, TUNEL (terminal-deoxynucleotidyl-mediated dUTP nick end labeling) negative and without overall cell condensation. An alternative Apaf1 and caspase-independent pathways, obviously, can mediate the removal of these cells causing cell death with a non-apoptotic morphotype [17]. This form of cell death (paraptosis) resembles the death morphotype caused by AIF [18]. This suggests that an AIF-dependent pathway could cause cell death in absence of Apaf1.

Apaf1 mutant embryos also show an abnormal eye development in particular of retina, lens and eye vascular system. At e12.5, the retina of the mutant is already thicker than the retina of the wild-type animal. At e14.5, the hyperplasic retina occupies most of the optic cup. This is a consequence of the lack of Apaf-dependent cell death involved in the regulation of the retina cell number. The lens of Apaf1-/- embryos is smaller and mis-polarized. It is conceivable that defective apoptosis of lens ephitelium (which is a significant component of lens development) induces alterations in shape and size of the lens primordium (related to the presence of supernumerary cells in the epithelium) that would lead to incorrect interactions with the environment and thus to incorrect polarization. Furthermore, Apaf1 regulates the number of hyaloid capillaries already in prenatal phase since in Apaf1 mutant embryos, by e14. 5, vascular endothelial cells seem to obliterate completely the optic cup.

The analysis of the Apaf1-/- phenotype suggests that Apaf1 can be involved also in apoptosis which contributes to morphogenetic remodeling of inner ear in mice.

Cell death of early stem cells in the brain is essential for a normal development of the nervous system. From e12.5 onward, the brain of Apaf1 mutants shows important morphological distortions. When apoptosis is prevented, like in Apaf1 knockouts, a huge brain overgrowth due to an enlargement of proliferative zones is observed as a bilateral protrusion of the forebrain or an exencephalic brain mass. This brain hyperplasia is particularly intense in the diencephalon and midbrain and exerts a pressure on telencephalic vesicles, which become misfolded and reduced in size. The obliteration of the lumen of the neural tube could have as a consequence a certain degree of hydrocephalia. The enlarged brain, compounded by the defect of skull and facial midline cleft and by the hydrocephalia has, as a final result, the exencephaly found in Apaf1 mutants by e16.5. Grossly, two general populations die during neuronal development, neuronal precursors and postmitotic neurons. It has been found that Apaf1-/- supernumerary cells express proneural markers. Apaf1 is therefore necessary for apoptosis in neural progenitor cells whose apoptosis plays a role in cortical expansion. Critical for death of neuronal precursors are even caspase-9 and -3, as shown by mice lacking any of these two proteins.

Apaf1 dosage effect

It has recently been found that an autosomal recessive mutation affecting the neural tube development, fog (forebrain overgrowth), is a hypomorphic allele of Apaf1 which results in reduced levels of the normal Apaf1 transcript [19].

The absence of alterations in Apaf1+/- mice could lead to the hypothesis that only the total deficiency of Apaf1 is able to affect the development and to produce an alterated phenotype. The fog mutation offers an important tool to study the effects of Apaf1 dosage in vivo. In fact, fog heterozygous, fog homozygous, and Apaf1-/fog produce intermediate levels of Apaf1

if compared with Apaf1+/+ (100%), Apaf1+/- (50%) and Apaf1-/- (0%) and they also show intermediate phenotypes including forehead bumps. These features demonstrate that not only the absence of Apaf1 but even downregulation of the protein (below 50%) is deleterious for the development causing milder effects compatible with life and with possible links to human diseases [20].

Role of Apaf1 in cancer

Apaf1 as a tumor suppresor gene

Apoptosis is one of the mechanisms by which organisms may keep tumour growth in check. Tumour suppressor genes (TSGs) promote cell cycle arrest or induce apoptosis in response to several cellular stresses (DNA damage, hypoxia, oncogenes activation, etc.). A growing body of evidences has put in relationship the apoptosome-dependent cell death with the tumor suppressor pathways of p53 and pRB (Retinoblastoma protein) (Fig 2).

The tumor suppressor p53 induces cell cycle arrest or apoptosis, especially when mitogenic oncogenes are overactivated; it acts mainly through trascriptional regulation of target genes, such as p21WAF1/CIP1 for cell cycle arrest and Bax and Noxa for apoptosis [21]. Although Bax is functionally downstream of p53 in apoptotic pathways, it cannot substitute for all p53's proapoptotic activities, suggesting that p53 acts through other mediators in addition to Bax. In contrast, both Apaf1 and caspase-9 are essential for p53-dependent cell death and appear to act as tumor suppressor [22]. Soengas and coworkers analysed p53-null, Apaf1-null and caspase-9-null MEFs, by overexpressing c-myc and by using apoptotic stimuli which mimic conditions in developing tumors; they showed that Apaf1 or caspase-9 inactivation substitutes for p53 loss in promoting the oncogenic transformation of myc-expressing cells [23].

pRB has also a key role as cell growth regulator and it acts mainly by modulating transcription regulators such as members the E2Fs family; these factors could have both a role in promoting cell death and proliferation and are tissue-specific. During development, the disruption of pRB leads to aberrant S-phase entry in differentiating neuroendocrine cells, but apoptosis is also induced to recover the ectopic cell proliferation. Homozygous mutation of pRB, indeed, induces embryonic death (e13.5-14.5) accompanied by incomplete differentiation, and both proliferation and apoptosis in the peripheral and central nervous system (PNS and CNS), skeletal muscles and lens. In pRB-/- embryos, the apoptotic phenomenon is E2F/p53-mediated in CNS and lens and it is E2F/p53-independent in skeletal muscle and PNS. pRB-/-/Apaf1-/- embryos did not show apoptosis in CNS and lens, (which are the same subset of tissues in which death is E2F/p53-dependent), while apoptosis level was unchanged in skeletal muscle and PNS [24]. These data suggest that, in mouse development, Apaf1 is a specific effector of cell death mediated by p53 and E2F when pRB is not functional. On the other hand, in tumorigenesis due to pRB loss, abnormally proliferating cells are frequently allowed to survive because of the accumulation of mutations which prevent apoptosis such as in absence of p53: in fact, genetic analysis revealed that pRB+/-/p53-/- individuals have higher tumors incidence compared with pRB+/- mice.

Although it has not been directly demonstrated that Apaf1 could act as general tumor suppressor gene, more and more evidences point out a role for Apaf1 in tissue-specific oncogenesis, since it has been often found mutated in cancer or correlated to the function of other tumor suppressor genes such as E2Fs and p53.

Evidences of Apaf1 dysfunction in cancer

In the last years, works have been done to determine whether Apaf1 or apoptosome functions were abolished in cancer cells. Results are variable, depending on the analysed cell line.

Kimura et al. found that the region containing the Apaf1 locus (in humans, locus 12q23) was linked with pancreatic ductal adenocarcinoma (PDAC) at a frequency of 60% of tested samples; further analysis of pancreatic cell lines showed that 16 out of the 19 samples displayed allelic loss within the same region [25]. Bala et al. found that the Apaf1 containing region was deleted or an aberrant protein was produced in many cell lines [26]; Frame-shift mutations in the Apaf1 gene were detected at low frequencies

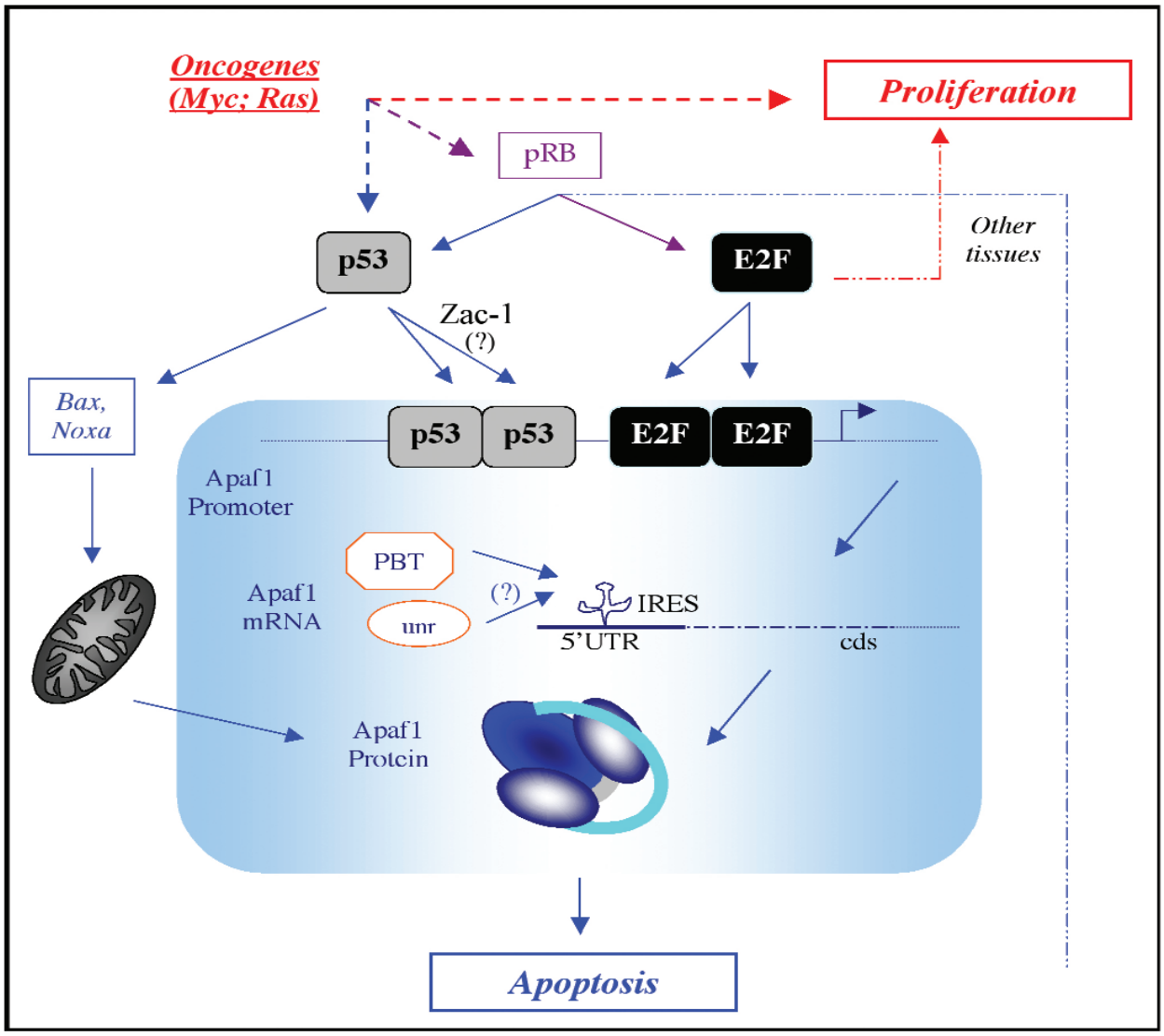

Fig. 2 Tissue-specific Apaf1 regulation by different regulators of cell cycle and apoptosis.

(13-15%) in gastrointestinal cancer (microsatellite mutator phenotype) [27].

Soengas and colleagues, by *in vivo* and *in vitro* experiments, showed that metastatic melanomas often lose Apaf1. Loss of Apaf1 confers chemoresistant properties and unability to execute a typical apoptotic program in response to p53 activation. Interestingly, the allelic loss of Apaf1 could be recovered in the melanoma cell lines treated with a methylation inhibitor. This suggests a possible transcriptional silencing of the Apaf1 gene due to methylation [28].

Also in ovarian cancer cells, mitochondria-dependent apoptosis was found defective and was correlated with high chemoresistance; nevertheless, this dysfunctional apoptosis was not attributed directly neither to reduced Apaf1 protein level nor to mutated isoforms: Wolf et al. demonstrated a diminished apoptosome activity with equal level of Apaf1 and other caspases, suggesting the presence of a putative Apaf1 inhibitor molecule [29], while Liu et al. found diminished level of caspase-9 [30]. A similar jam in apoptosome-dependent cell death was also found in leukemic cell lines, but it was not clear which molecules were involved in this dysfunction.

The Apaf1 role in apoptosis of developing nervous system could lead to consider a possible role of Apaf1

mutations in embryonic tumors of CNS, a heterogeneous group of tumors whose histogenesis and molecular markers are poorly known. Although specific analyses have not been performed, 12q mutation are not common in pediatric embryonic brain tumors, such as medulloblastomas [31] or primitive neuroectodermal tumors (PNETs), atypical teratoid/rhabdoid tumors (AT/RTs) and malignant gliomas [32]. Apaf1 also seemed to be expressed and functionally active in neuroblastoma tumor cell lines which overexpress c-myc and have LOH (loss of heterozygosis) in 1p36 (the caspase-9 locus) [33]; in fact, despite of emizygous caspase-9 level, those lines could undergo apoptosome-mediated apoptosis. However, a small percentage of the analysed neuroblastoma lines showed high resistance to apoptotic stimuli (staurosporine and radiation) which correlated with an epigenetic silencing of the caspase-8 gene that gives rise to a low cytochrome c release; noteworthy, neuroblastomas likewise melanomas are originated by neural crest progenitor cells and share the same epigenetic regulation mechanism for proapoptotic genes (Apaf1 and caspase-8 respectively).

Apaf1 regulation: importance of Apaf1 dosage in cancer

The implication of a trascriptional regulation of the APAF1 promoter in allelic loss in melanomas and the dosage effect of components of the apoptosome in other kinds of cancer, make of the Apaf1 regulation an important topic to be explored (Fig 2).

Many groups confirmed that p53 controls the Apaf1 promoter. Fortin et al, by using DNA microarrays analysis of cortical neurons undergoing p53-dependent apoptosis (induced by camptothecin, a DNA damaging agent), noticed an upregulation of the Apaf1 mRNA. Moreover, they identified two p53 consensus-binding sequences in the Apaf1 promoter and demonstrated a p53-DNA binding activity at both of them [34].

Moroni et al, confirmed the direct targeting of p53 to Apaf1 promoter; furthermore, the authors demonstrated the direct control of Apaf1 also by E2Fs transcription factors family. Since E2F could also induce p53, it was also showed in p53 null contest, that Apaf1 activation by p53 or by E2F are two independent processes [35].

Finally, Kannan et al, reported a DNA microarray/Northern Blot analysis of genes expression pattern after p53 activation; while in a mouse myeloid leukemic cell line (LTR6) Apaf1 resulted to be activated, the same did not happen in human lung cancer cells (H1299); moreover, the two system shared only 15% of common up-regulated genes which suggests that the p53 regulated genes would be cell type specific [36]. A further analysis performed by Rozenfeld-Granot and coworkers [37] confirmed a physical and functional interaction between p53 and Apaf1 promoter. Moreover, the same authors demonstrated that Zac-1, in human contest, could enhance this activation. Zac-1 is a transcription factor that was previously shown to inhibit cell proliferation through induction of G1-arrest and apoptosis. Zac-1 recognition sites were found in Apaf1 promoter, but Zac-1 had no effect if used alone (by itself). Zac-1 is also transcriptionally upregulated by p53 activation.

An additional post-transcriptional regulation of Apaf1 could be also considered since the 5'UTR contains an internal ribosome entry site (IRES). The initiation of translation of Apaf1 mRNA might occur by IRES even if it is not certain since the Apaf1 IRES is approximately 70% fold less efficient than the canonical scanning mechanism and it differs from IRES found in other mRNAs (c-Myc and FGF-2) [38]. Moreover, the lack of activity of Apaf1 IRES in certain cell types, and cell type-specific function of c-myc IRES, would suggest that cellular IRESs function in a cell specific fashion; this is probably due to differential expression of trans-acting factor that are required for each IRES function. Recently two specific RNA binding protein, polypyrimidine tract binding protein (PBT) and upstream of N-ras (unr), have been found to bind specifically to Apaf1 IRES and to stimulate its activity both in vitro and in vivo [39].

Importance of apoptosome components in cancer

Generally, chemotherapy could act by two different, not-mutually exclusive, mechanisms: direct differentiation induction and selective destruction of the malignant cells. However, therapy-resistance makes sometimes difficult to erase completely cancer cells.

From evidences described above, the apoptosome components (but even other proapoptotic molecules

such as SMAC/DIABLO) [40] seem to be really important in the chemo-/radio-induced cell death. Moreover, it has been often found that: a) in cellular cancer model, Apaf1/caspase-9 transfection or co-transfection increase the sensitiveness to chemical/physical apoptotic stimuli [41]; b) cisplatin treatment of human embryonic carcinoma induces the transcription of Apaf1, caspase-8 and TNFR1, three pro-apoptotic genes [42]. Therefore, the control of the normal level of apoptotic components by transcriptional/posttranscriptional mechanism could be considered a target for pharmaceutical modulation of cell death in cancer [43].

Neurodegenerations and apoptosis

While the role of apoptosis (and Apaf1) has been fully demonstrated in brain development, its involvement in neurodegenerative diseases is much less clear. In this kind of disorders two major events contribute to neurodegeneration: cell loss and loss of neuronal connectivity.

To date, data on this topic are confusing and even contradictory. In particular, no relevant results are available regarding the specific involvement of Apaf1 in neurodegenerations. However, data correlating caspases with neurodegenerations could suggest a possible role for Apaf1 since Apaf1 is activated downstream of caspase-8 and it can be needed for the activation of caspase-9 and of executioner caspases (Fig 3).

Key proteins in neurodegenerations can be substrates of caspases

Within the past few years, work by several laboratories has indicated that inappropriate activation of apoptotic pathways and increased rate of apoptosis are contributing events in neurodegenerative diseases. In particular, it has been shown that caspases are able to cleave important molecules implicated in some neurodegenerations; for example amyloid-β precursor protein (APP), presenilins (PS1 , PS2), tau, huntingtin (Htt) and androgen receptor [44-50].

The neurodegenerative pathology most widely studied for its apoptotic component is Alzheimer's disease (AD). Caspase-3 is involved in APP cleavage and it is interesting to notice that APP cleavage by caspase-3 occurs also in vivo in hippocampal neurons (whose loss is a prominent feature of AD) following acute brain injury. In addition, it has been found that cleavage of APP by caspases results in elevated neurotoxic amyloid-β (Aβ) peptide formation. Furthermore, APP mutations associated with an inherited form of AD creates a site for caspase-6 cleavage and subsequent Aβ peptides release [47].

Caspases, thus, could play a dual role: in the proteolytic processing of APP with the resulting increase of neurotoxic Aβ peptide formation, as well as in the ultimate apoptotic death of neurons in AD. In other words, if caspases do attack APP to release Aβ peptide, then there might be a vicious cycle in which cytotoxic Aβ peptide induces a neuronal stress with progressive caspases activation, exacerbated APP proteolysis and, finally, cell death (Fig 3). It should be mentioned that data from other authors contradict this finding; some evidences, in fact, indicate that APP is cleaved by caspases but its cleavage decreases Aβ peptide secretion by cells [51].

Caspases are activated in brains of patients with neurodegenerations

Studies on post-mortem tissues have demonstrated a high number of neurons containing activated caspases in patients' brains compared with age-matched controls who died of other causes. In particular cleaved caspase-3, -6 and –9 were found in AD (47, 53-55), caspase-1 and -3 in amyotrophic lateral sclerosis (ALS) [56, 57], caspase-1 and –8 in Huntington disease (HD) [58], caspase-3, -8 and –9 in Parkinson's disease [59, 60] and caspase-1 and -3 in ischemia [61]. Caspase-cleaved APP colocalizes with senile plaques in human Alzheimer's brains, consistent with the hypothesis that caspase-generated APP contributes to plaque formation. But, whether or not caspases increase Aβ production in AD, the evidence that caspases are activated in Alzheimer's brains is the presence of cleaved fodrin and actin. Moreover, many of the stained neurons for cleaved fodrin also contained Alzheimer's characteristic neurofibrillary tangles [51, 52].

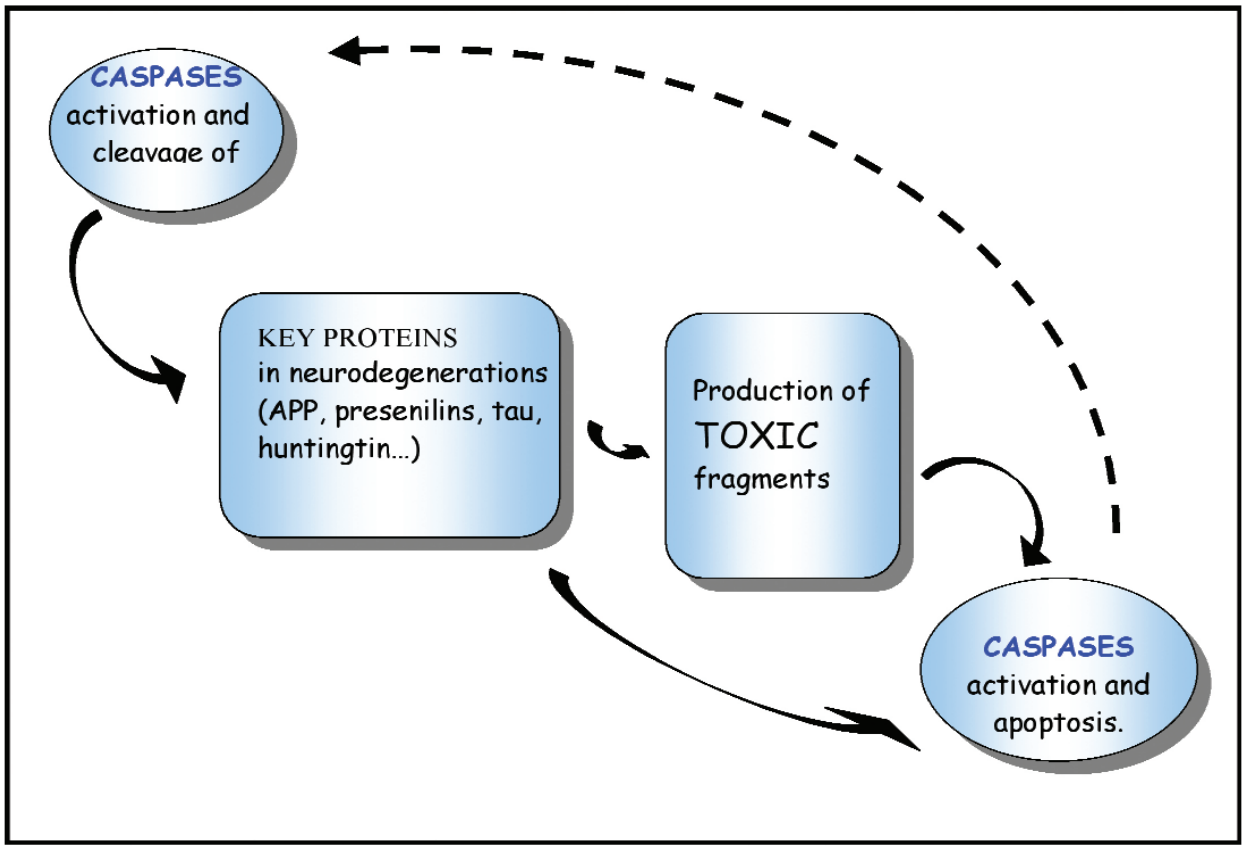

Fig. 3 Possible dual role of caspases in neurodegenerations: in the upstream events leading to cleavage of key factors and in the final steps of cell death.

Evidence of caspase activation and other apoptotic features in *in vivo* and *in vitro* models of neurodegenerations

The best way to define the specific role of each caspase or apoptotic molecule would be utilizing cell culture (primary neuronal cultures and cell lines) or animal models of the disease. Of course each model mimic various aspects of the disease, but none of them completely recapitulates the human pathology. For AD, Aβ-induced death is used as an *in vitro* model. Cultured neurons treated with Aβ peptides undergo death with apoptotic features, such as nuclear fragmentation and DNA condensation [62-64]. Furthermore, intracellular production of peptides induces activation of caspase-3 and neuronal apoptosis [65, 66]. In cultured primary human neurons, intracellular Aβ peptide 1-42 induces apoptosis by acting through p53 and Bax [67]. Very recently Kusiak and colleagues demonstrated that treatment of a neuroblastoma cell line with Aβ 17-42 leads to apoptosis; it activates caspase-8, -3 and PARP cleavage, but not caspase-9 [68]. It has been even shown that Aβ induces cytochrome c release from isolated mitochondria [69].

At least 7 different caspases (caspase-1, -2, -3, -6, -8, -9, -12) have been implicated in regulating neuronal cell death in response to Aβ exposure in vitro, in animal models and in AD brain itself [70]. Neuronal cultures lacking caspase-2 and –12, for example, resist Aβ-induced cell death [71, 72]. Furthermore, the dominant-negative inhibition of FADD has shown an involvement of the death receptor pathway in cell death caused by Aβ [64, 73]. Neurons engineered to produce the mutant form of presenilin-2 (presenilins genes are mutated in some inherited forms of AD) were more sensitive to undergo apoptosis triggered by Aβ than normal cells [74].

Studies showed that also in Huntington disease (HD), caspase-dependent apoptosis is activated in

affected neurons. In culture, polyglutamine repeats induce neuronal cell death by activating initiator (caspase-8) and effector caspases [49, 75, 76]. Mutant huntingtin (Htt) expressed in striatal cells induces apoptosis and caspase inhibition promotes neuronal survival [75, 77].

As we already said, Htt is a substrate of caspase-3 and -6 and truncated Htt fragments are toxic *in vitro*. Caspase inhibitors abrogate Htt cleavage in cells and protect them from apoptotic stress. Neurons expressing a caspase resistant Htt with an expanded polyglutamine tract are less susceptible to apoptosis and aggregate formation. Thus caspase cleavage of Htt can be a crucial step in aggregate formation and neurotoxicity in HD [78].

Very interestingly Nicholson and coworkers showed how the binding to Htt of a protein which normally associated with Htt (Hip-1), is reduced by polyglutamine expansion. Then, free Hip-1 is available to bind the polypeptide named Hippi (Hip-1 protein interactor) and the heterodimer Hip-1-Hippi can recruit procaspase-8 activating the "extrinsic" apoptotic pathway [79].

Recently, Zhu and colleagues demonstrated that the inhibition of cytochrome c release delays ALS progression in mice; this evidence strongly suggests an involvement of the apoptosome and, thereby, of Apaf1 in ALS disease [80]. In mouse model of ALS, dominant negative of caspase-1 delays disease and caspase-3 activation showed some promising slowing of neural death and prolonging life [44]. Moreover, redistribution of Bax, cleaved Bid, release of *cytochrome c* and activation of caspase-9 were found during neurodegeneration in spinal cords of transgenic mutant SOD1 mice. In the same study the authors also demonstrated that a dominant negative mutant of caspase-1 attenuates Bid-cleavage and the following apoptotic features, while caspase-8 seems to be activated only at the end–stage of the disease [81].

In Parkinson's disease, the involvement of both extrinsic and intrinsic apoptotic pathways was shown but the major mechanism of neuronal death seems to be the mitochondrial apoptotic pathway. An Apaf-1 dominant negative inhibitor can prevent nigrostriatal degeneration in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model of Parkinson's disease and caspase inhibitors, also, attenuate MPTP toxicity in primary cell cultures [59, 60, 82].

Acute traumatic or ischemic injury to mature brain induces cytochrome c-dependent cleavage of caspase-9 followed by activation of caspase-3. In addition, marked increases in Apaf-1 and caspase-3 mRNA and protein expression were detected [83].

Loss of connectivity and caspases

Caspase-dependent apoptosis could, therefore, be involved in cell loss. But what's about the loss of connectivity? Loss of connectivity occurs before cell death and seems to initiate degenerative processes in the nervous system. It has been shown that in AD, HD and Parkinson disease the pathogenesis can be independent on neural loss, at least in early stages, and that many of these diseases could begin with a nerve-terminal degeneration or "synaptosis". Neurons may even be able to survive this damage for a while. Ultimately though, as nerve cells lose more and more of their terminals, they will die because of the absence of trophic factors produced by close neurons.

Mice overexpressing APP show deficits in synaptic activity but minimal cell death [84]. Transgenic mice overexpressing mutated huntingtin show motor alterations prior to evidence of cell death [85]. Mice expressing exon 1 of the human huntingtin gene with an expanded GAC/polyglutamine repeat exhibit a significant decrease in striatal volume without any detectable neural loss [86].

Furthermore, some data suggest that nerve terminals are the prime target for caspase activation; positive staining for caspases activated by Aβ are found in the dendrites, and in particular in the synapses. In addition, Aβ application only to terminals of cultured neurons induce caspase-dependent nerve-terminal degeneration without necessarily killing the entire nerve cell, at least not immediately [51, 87].

Many pathways to explain the confusion

Despite the impressive amount of data implicating caspases and apoptosis as etiological factors in neurodegenerations, contradictory experiments and lack of morphologically convincing apoptotic neurons don't allow to fully understand the exact relationship between neurodegenerative processes and apoptosis.

This confusion can be explained by the fact that it is unlikely that a single linear execution system, even as diversified as apoptosis, is the sole responsible for

neuronal death. Most likely, many interrelated or independent pathways may have developed to regulate cell removal. In fact, it has been shown that deletion of a single caspase has only partial and localized effects on cell death. Moreover, some forms of cell death seem to be caspase-independent or dependent upon other proteases such as serine proteases, cathepsin and calpains. In addition, caspases effects can concern only the morphological features of the death (apoptosis, necrosis, paraptosis) but not the final outcome. But, to date, while the existence of alternative pathways is clear, the evidence for effective alternative execution system is limited; the only case known is the one involving AIF [18].

An example of cell removal due to interrelated execution systems is the death caused by the absence of ATP; in this case, cells triggered to undergo apoptosis cannot activate caspases (ATP is needed for apoptosome formation and, probably, in some steps upstream of cytochrome c release) but death is not blocked; it progresses in a caspase-3 independent way with features of necrosis or mixed features between apoptosis and necrosis [88, 89]. If apoptosis of injured neurons is blocked, functionally damaged neurons could persist and they would eventually lyse by necrosis and paradoxically promote the onset of inflammatory response with further progression of disease.

Cell death in onset or progression of neurodegenerations?

The confusion regards not only the form of death in neurodegenerations, but also its role in the onset or in the progression of the disease. In other words, is the abnormal cell death the cause of the disease or is cells removal a secondary consequence of upstream events that represent the real etiology of the disease? For example, a common feature of neurodegenerative disorders is the accumulation of intracellular inclusions mostly formed by protein aggregates that are usually difficult to unfold or degrade. The accumulation of misfolded proteins can cause alteration of axonal transport, cytoskeletal damage and, finally, loss of connectivity with target cells. Apoptosis could act to remove these dysfunctional neurons as final step of a process started by other causes. In such cases, by the time apoptosis occurs, neurons are already so severely damaged that they are not salvageable and indeed blocking apoptosis (a neat way of eliminating damaged cells) could make things worse, leading to necrotic death which triggers harmful inflammation.

Conclusive remarks

Since non functional apoptosis has often been associated to tumorigenesis and to therapy resistance and since overexpression of Apaf1 and other proapoptotic molecules increases tumor-sensitiveness to chemio- or radio- induced cell death, these apoptotic components could be considered a tool to achieve a pharmaceutical destruction of malignant cells. On the other hand, once the role of caspases-dependent apoptosis in neurodegenerations will be definitively demonstrated, caspases and molecules regulating caspases activity such as Apaf1 could offer a target for extending neuron survival and developing novel therapies.

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References

1. **Kerr J.F., Wyllie A.H., Currie A.R.,** Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics, *Br. J. Cancer*, **26**: 239-257, 1972
2. **Jacobson M.D., Weil M., Raff M.C.,** Programmed cell death in animal development, *Cell*, **88**: 347-354, 1997
3. **Earnshaw W.C., Martins L.M., Kaufmann S.H.,** Mammalian caspases: structure, activation, substrates, and functions during apoptosis, *Annu. Rev. Biochem.,* **68**: 383-424, 1999
4. **Martinou J.C., Green D.R.,** Breaking the mitochondrial barrier, *Nat. Rev. Mol. Cell Biol.,* **2**: 63-67, 2001
5. **Zamzami N., Kroemer G.,** The mitochondrion in apoptosis: how Pandora's box opens, *Nat. Rev. Mol. Cell. Biol.,* **2**: 67-71, 2001
6. **Li P., Nijhawan D., Budihardjo I., Srinivasula S.M., Ahmad M., Alnemri E.S., Wang X.,** Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade, *Cell*, **91**: 479-489, 1997

7. **Acehan D., Jiang X., Morgan D.G., Heuser J.E., Wang X., Akey C.W.,** Three-dimensional structure of the apoptosome. Implications for assembly, procaspase-9 binding, and activation, *Mol. Cell*, **9**: 423-432, 2002
8. **Riedl S.J., Renatus M., Schwarzenbacher R., Zhou Q., Sun C., Fesik S.W., Liddington R.C., Salvesen G.S.,** Structural basis for the inhibition of caspase-3 by XIAP, *Cell*, **104**: 791-800, 2001
9. **Bratton S.B., Walker G., Srinivasula S.M., Sun X.M., Butterworth M., Alnemri E.S., Cohen G.M.,** Recruitment, activation and retention of caspases-9 and -3 by Apaf-1 apoptosome and associated XIAP complexes, *EMBO J.*, **20**: 998-1009, 2001
10. **Verhagen A.M., Ekert P.G., Pakusch M., Silke J., Connolly L.M., Reid G.E., Moritz R.L., Simpson R.J., Vaux D.L.,** Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins, *Cell*, **102**:43-53, 2000
11. **Verhagen A.M., Silke J., Ekert P.G., Pakusch M., Kaufmann H., Connolly L.M., Day C.L., Tikoo A., Burke R., Wrobel C., Moritz R.L., Simpson R.J., Vaux D.L.,** HtrA2 promotes cell death through its serine protease activity and its ability to antagonize inhibitor of apoptosis proteins, *J. Biol. Chem.*, **277**: 445-454, 2002
12. **Saleh A., Srinivasula S.M., Balkir L., Robbins P.D., Alnemri E.S.,** Negative regulation of the Apaf-1 apoptosome by Hsp70, *Nat. Cell. Biol.*, **2**: 476-483, 2000
13. **Pandey P., Saleh A., Nakazawa A., Kumar S., Srinivasula S.M., Kumar V., Weichselbaum R., Nalin C., Alnemri E.S., Kufe D., Kharbanda S.,** Negative regulation of cytochrome c-mediated oligomerization of Apaf-1 and activation of procaspase-9 by heat shock protein 90, *EMBO J.*, **19**: 4310-4322, 2000
14. **Bruey J.M., Ducasse C., Bonniaud P., Ravagnan L., Susin S.A., Diaz-Latoud C., Gurbuxani S., Arrigo A.P., Kroemer G., Solary E., Garrido C.,** Hsp27 negatively regulates cell death by interacting with cytochrome c, *Nat. Cell. Biol.,* **2**: 645-652, 2000
15. **Cecconi F.,** Apaf1 and the apoptotic machinery, *Cell Death Differ.*, **6**: 1087-1098, 1999
16. **Cecconi F., Alvarez-Bolado G., Meyer B.I., Roth K.A., Gruss P.,** Apaf1 (CED-4 homolog) regulates programmed cell death in mammalian development, Cell., 94(6): 727-37, 1998
17. **Sperandio S., de Belle I., Bredesen D.E.,** An alternative, nonapoptotic form of programmed cell death, *Proc. Natl. Acad. Sci. USA*, **97**: 14376-81, 2000
18. **Susin S.A., Lorenzo H.K., Zamzami N., Marzo I., Snow B.E., Brothers G.M., Mangion J., Jacotot E., Costantini P., Loeffler M., Larochette N., Goodlett D.R., Aebersold R., Siderovski D.P., Penninger J.M., Kroemer G.,** Molecular characterization of mitochondrial apoptosis-inducing factor., *Nature*, **397**: 441-6, 1999
19. **Honarpour N., Gilbert S.L., Lahn B.T., Wang X., Herz J.,** Apaf-1 deficiency and neural tube closure defects are found in fog mice, *Proc. Natl. Acad. Sci. USA*, **98**: 9683-9687, 2001
20. **Moreno S., Ferraro E., Eckert S., Cecconi F.,** Apaf1 reduced expression levels generate a mutant phenotype in adult brain and skeleton, *Cell Death Differ.*, 9: 340-342, 2002
21. **Vousden K.H.,** p53: death star, *Cell*, **103**: 691-694, 2000
22. **Macleod K.,** Tumor suppressor genes, *Curr. Op. in Genet. Dev.,* **10**: 81-93, 2000
23. **Soengas M.S., Alarcon R.M., Yoshida H., Giaccia A.J., Hakem R., Mak T.W., Lowe S.W.,** Apaf-1 and Caspase-9 in p53-dependent apoptosis and tumor inhibition, *Science*, **284**: 156-159, 1999
24. **Guo Z., Yikang S., Yoshida H., Mak T.W., Zacksenhaus E.,** Inactivation of retinoblastoma tumor suppressor induces apoptosis protease-activating factor-1 dependent and independent apoptotic pathways during embryogenesis, *Cancer Res.*, **61**: 8395-8400, 2001
25. **Kimura M., Kurukawa T., Abe T., Yatsuoka T., Youssef E.M., Yokoyama T. et al.,** Identification of two common regions of allelic loss in chromosome arm 12q in human pancreatic cancer, *Cancer Res.*, **58**: 2456-2460, 1998
26. **Bala S., Oliver H., Renault B., Montgomery K., Dutta S., Rao P., Houldsworth J., Kucherlapati R., Wang X., Chaganti R.S.K., Murty V.V.V.S.,** Genetic analysis of the APAF1 Gene in male germ cells tumors, *Genes Chromosomes Cancer*, **28**: 258-268, 2000
27. **Yamamoto H., Gil J., Schwartz S. jr, Perucho M.,** Frameshift mutations in Fas, Apaf-1, and Bcl-10 in gastrointestinal cancer of the microsatellite mutator phenotype, *Cell Death Differ.*, **7**: 238-239, 2000
28. **Soengas M.S., Capodieci P., Polsky D., Mora J., Esteller M., Opitz-araya X., McCombie R., Herman J.G., Gerald W.L., Lazebnik Y.A., Cordòn-cardò C., Lowe S.W.,** Inactivation of the apoptosis effector Apaf-1 in malignant melanoma, *Nature*, **409**: 207-211, 2001
29. **Wolf B.B., Schuler M., Li W., Eggers-Sedlet B., Lee W., Tailor P., Fitzgerald P., Mills G.B., Green D.R.,** Defective cytochrome c-dependent caspase activation in ovarian cancer cell lines due to diminished or absent apoptotic protease activating factor-1 activity, *J Biol Chem.*, **276**: 34244-34251, 2001
30. **Liu J.R., Opipari A.W., Tan L., Jiang Y., Zhang Y., Tang H., Nunez G.,** Dysfunctional apoptosome activation in ovarian cancer: implications for chemoresistance, *Cancer Res.*, **62**: 924-931, 2002
31. **Gilberston R.,** Paediatric embryonic brain tumors: biological and clinical relevance of molecoular genetics abnormalities, *Eur J. Cancer*, **38**: 675-685, 2002
32. **Pomeroy S.L., Tamayo P., Gaasenbeek M., Sturla L.M., Angelo M., McLaughlin M.E., Kim J.Y.H., Goumnerova L.C., Black P.M. et al.,** Prediction of central nervous system embryonal tumour outcome based on gene expression, *Nature*, **415**: 436-442, 2002
33. **Teitz T., Wei T., Liu D., Valentine V., Valentine M., Grenet J., Lahti J.M., Kidd V. J.,** Caspase-9 and Apaf-1 are expressed and functionally active in human neuroblastoma tumor cell lines with 1p36 LOH and amplyfied MYCN, *Oncogene*, **21**: 1848-1858, 2002
34. **Fortin A., Cregan S.P., MacLaurin J.G., Kushwaha N., Hickman E.S., Thompson C.S., Hakim A., Albert P.R., Cecconi F., Helin K., Park D.S., Slack R.S.,** APAF1 is a key trascriptional target for p53 in the regulation of the neuronal cell death., *J. Cell Biol.*, **155**: 207-16, 2001
35. **Moroni M.C., Hickman E.S., Lazzarini Denchi E., Caprara G., Colli E., Cecconi F., Muller H., Helin K.,** Apaf-1 is a trascriptional target for e2f and p53, *Nature Cell Biol.*, **3**: 552-558, 2001

36. **Kannan K., Kaminski N., Rechavi G., Jacob-Hirsch J., Amariglio N., Givol D.,** DNA microarray analysis of genes involved in p53 mediated apoptosis: activation of Apaf-1, *Oncogene*, **20**: 3449-3455, 2001
37. **Rozenfeld-granot G., Krishnamurthy J., Kannan K., Toren A., Amariglio N., Givol D., Rechavi G.,** A positive feedback mechanism in the trascriptional activation of Apaf-1 by p53 and the coactivator Zac-1, *Oncogene*, **21**: 1469-1476, 2002
38. **Coldwell M.J., Mitchell S.A., Stoneley M., MacFarlane M., Willis A.E.,** Initiation of Apaf-1 translation by internal ribosome entry, *Oncogene*, **19**: 899-905, 2000
39. **Mitchell S.A., Brown E.C., Coldwell M.J., Jackson R. J., Willis A.E.,** Protein factor requirements of the Apaf1 internal ribosome entry segment: roles of polypyrimidine tract binding protein and upstream of N-ras, *Mol. Cell Biol.*, **21**: 3364-3374, 2001
40. **Chauhan D., Hideshima T., Rosen S., Reed J.C., Kharbanda S., Anderson K.C.,** Apaf-1/cytochrome c-independent and Smac-dependent induction of apoptosis in multiple myeloma (MM) cells, *J. Biol. Chem.*, **276**: 24453-24456, 2001
41. **Shinoura N., Sakurai S., Asai A., Kirino T., Hamada H.,** Over-expression of APAF-1 and caspase-9 augments radiation-induced apoptosis in U-373MG glioma cells, Int. J. Cancer., 93: 252-261, 2001
42. **Mizutani Y., Sato N., Kawauchi A., Nonomura N., Fukushima M., Miki T.,** Cisplatin-induced in vivo differentiation of human embryonal carcinoma, *BJU international*, **89**: 454-458, 2002
43. **Jones P.A.,** Death and methilation, *Nature*, **409**: 141-144, 2001
44. **Troy C.M., Salvesen G.S.,** Caspases on the brain, *J. Neurosci. Res.*, **69**: 145-50, 2002
45. **Weidemann A., Paliga K., Durrwang U., Reinhard F.B., Schuckert O., Evin G., Masters C.L.,** Proteolytic processing of the Alzheimer's disease amyloid precursor protein within its cytoplasmic domain by caspase-like proteases, *J. Biol. Chem.*, **274**: 5823-5829, 1999
46. **Wellington C.L., Ellerby L.M., Hackam A.S., Margolis R.L., Trifiro M.A., Singaraja R., McCutcheon K., Salvesen G.S., Propp .SS., Bromm M., Rowland K.J., Zhang T., Rasper D., Roy S., Thornberry N., Pinsky L., Kakizuka A., Ross C.A., Nicholson D.W., Bredesen D.E., Hayden M.R.,** Caspase cleavage of gene products associated with triplet expansion disorders generates truncated fragments containing the polyglutamine tract, *J. Biol. Chem.,* **273**: 9158-9167, 1998
47. **Gervais F.G., Xu D., Robertson G.S., Vaillancourt J.P., Zhu Y., Huang J., LeBlanc A., Smith D., Rigby M., Shearman M.S., Clarke E.E., Zheng H., Van Der Ploeg L.H., Ruffolo S.C., Thornberry N.A., Xanthoudakis S., Zamboni R.J., Roy S., Nicholson D.W.,** Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid-beta precursor protein and amyloidogenic A beta peptide formation, *Cell*, **97**: 395-406, 1999
48. **Pellegrini L., Passer B.J., Tabaton M., Ganjei J.K., D'Adamio L.,** Alternative, non-secretase processing of Alzheimer's beta-amyloid precursor protein during apoptosis by caspase-6 and –8, *J. Biol. Chem.*, **274**: 21011-6, 1999
49. **Tanzi M., Bellelli E., Benaglia G., Cavatorta E., Merialdi A., Mordacci E., Ribero M.L., Tagger A., Verrotti C., Volpicelli A.,** Alternative cleavage of Alzheimer-associated presenilins during apoptosis by a caspase-3 family protease, *Science*, **277**: 373-376, 1997
50. **Lunkes A., Lindenberg K.S., Ben-Haiem L., Weber C., Devys D., Landwehrmeyer G.B., Mandel J.L., Trottier Y.,** Proteases acting on mutant huntingtin generate cleaved products that differentially build up cytoplasmic and nuclear inclusions, *Mol. Cell.*, **10**: 259-269, 2002
51. **Marx J.,** Neuroscience. New leads on the 'how' of Alzheimer's, *Science*, **293**: 2192-2194, 2001
52. **Rohn T.T., Head E., Su J.H., Anderson A.J., Bahr B.A., Cotman C.W., Cribbs D.H.,** Correlation between caspase activation and neurofibrillary tangle formation in Alzheimer's disease, *Am. J. Pathol.*, **158**: 189-198, 2001
53. **LeBlanc A., Liu H., Goodyer C., Bergeron C., Hammond J.,** Caspase-6 role in apoptosis of human neurons, amyloidogenesis, and Alzheimer's disease, *J. Biol. Chem.*, **274**: 23426-36, 1999
54. **Stadelmann C., Deckwerth T.L., Srinivasan A., Bancher C., Bruck W., Jellinger K., Lassmann H.,** Activation of caspase-3 in single neurons and autophagic granules of granulovacuolar degeneration in Alzheimer's disease. Evidence for apoptotic cell death., *Am. J. Pathol.,* **155**: 1459-66, 1999
55. **Lu D.C., Rabizadeh S., Chandra S., Shayya R.F., Ellerby L.M., Ye X., Salvesen G.S., Koo E.H., Bredesen D.E.,** A second cytotoxic proteolytic peptide derived from amyloid beta-protein precursor, *Nat. Med.*, **6**:397-404, 2000
56. **Li M., Ona V.O., Guegan C., Chen M., Jackson-Lewis V., Andrews L.J., Olszewski A.J., Stieg P.E., Lee J.P., Przedborski S,. Friedlander R.M.,** Functional role of caspase-1 and caspase-3 in an ALS transgenic mouse model, *Science*, **288**: 335-9, 2000
57. **Pasinelli P., Houseweart M.K., Brown R.H. Jr, Cleveland D.W.,** Caspase-1 and -3 are sequentially activated in motor neuron death in Cu,Zn superoxide dismutase-mediated familial amyotrophic lateral sclerosis, *Proc. Natl. Acad. Sci. USA,* **97**: 13901-13906, 2000
58. **Sanchez I., Xu C.J., Juo P., Kakizaka A., Blenis J., Yuan J.,** Caspase-8 is required for cell death induced by expanded polyglutamine repeats, *Neuron*, **22**: 623-633, 1999
59. **Jeon B.S., Kholodilov N.G., Oo T.F., Kim S.Y., Tomaselli K.J., Srinivasan A., Stefanis L., Burke R.E.,** Activation of caspase-3 in developmental models of programmed cell death in neurons of the substantia nigra, *J. Neurochem.*, **73**: 322-333, 1999
60. **Viswanath V., Wu Y., Boonplueang R., Chen S., Stevenson F.F., Yantiri F., Yang L., Beal M.F., Andersen J.K.,** Caspase-9 activation results in downstream caspase-8 activation and bid cleavage in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease, *J. Neurosci.,* **21**: 9519-28, 2001
61. **Love S., Barber R., Wilcock G.K.,** Neuronal death in brain infarcts in man, *Neuropathol. Appl. Neurobiol.*, **26**: 55-66, 2000
62. **Nicotera P.,** Apoptosis and age-related disorders: role of caspase-dependent and caspase-independent pathways, *Toxicol. Lett.*, **127**: 189-195, 2002
63. **Forloni G., Bugiani O., Tagliavini F., Salmona M.,** Apoptosis-mediated neurotoxicity induced by beta-amyloid and PrP fragments, *Mol. Chem. Neuropathol.*, **28**: 163-171, 1996

64. **Ivins K.J., Thornton P.L., Rohn T.T., Cotman C.W.,** Neuronal apoptosis induced by beta-amyloid is mediated by caspase-8, *Neurobiol. Dis.*, **6**: 440-449, 1999
65. **Uetsuki T., Takemoto K., Nishimura I., Okamoto M., Niinobe M., Momoi T., Miura M., Yoshikawa K.,** Activation of neuronal caspase-3 by intracellular accumulation of wild-type Alzheimer amyloid precursor protein, *J. Neurosci.*, **19**: 6955-6964, 1999
66. **Kienlen-Campard P., Miolet S., Tasiaux B., Octave J.N.,** Intracellular amyloid-beta 1-42, but not extracellular soluble amyloid-beta peptides, induces neuronal apoptosis, *J. Biol. Chem.*, **277**: 15666-15670, 2002
67. **Zhang Y., McLaughlin R., Goodyer C., LeBlanc A.,** Selective cytotoxicity of intracellular amyloid beta peptide1-42 through p53 and Bax in cultured primary human neurons., *J. Cell Biol.*, **156**: 519-529, 2002
68. **Wei W., Norton D.D., Wang X., Kusiak J.W.,** Abeta 17-42 in Alzheimer's disease activates JNK and caspase-8 leading to neuronal apoptosis, *Brain*, **125**: 2036-2043, 2002
69. **Kim H.S., Lee J.H., Lee J.P., Kim E.M., Chang K.A., Park C.H., Jeong S.J., Wittendorp M.C., Seo J.H., Choi S.H., Suh Y.H.,** Amyloid beta peptide induces cytochrome c release from isolated mitochondria, *Neuroreport*, **13**: 1989-1993, 2002
70. **Allen J.W., Eldadah B.A., Huang X., Knoblach S.M., Faden A.I.,** Multiple caspases are involved in beta-amyloid-induced neuronal apoptosis, *J. Neurosci. Res.*, **65**: 45-53, 2001
71. **Nakagawa T., Zhu H., Morishima N., Li E., Xu J., Yankner B.A., Yuan J.,** Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta, *Nature*, **403**:98-103, 2000
72. **Troy C.M., Rabacchi S.A., Friedman W.J., Frappier T.F., Brown K., Shelanski M.L.,** Caspase-2 mediates neuronal cell death induced by beta-amyloid, *J. Neurosci.*, **20**: 1386-1392, 2000
73. **Morishima Y., Gotoh Y., Zieg J., Barrett T., Takano H., Flavell R., Davis R.J., Shirasaki Y., Greenberg M.E.,** Beta-amyloid induces neuronal apoptosis via a mechanism that involves the c-Jun N-terminal kinase pathway and the induction of Fas ligand, *J. Neurosci.,* **21**: 7551-7560, 2001
74. **Wolozin B., Iwasaki K., Vito P., Ganjei J.K., Lacana E., Sunderland T., Zhao B., Kusiak J.W., Wasco W., D'Adamio L.,** Participation of presenilin 2 in apoptosis: enhanced basal activity conferred by an Alzheimer mutation, *Science*, **274**: 1710-1713, 1996
75. **Kim M., Lee H.S., LaForet G., McIntyre C., Martin E.J., Chang P., Kim T.W., Williams M., Reddy P.H., Tagle D., Boyce F.M., Won L., Heller A., Aronin N., DiFiglia M.,** Mutant huntingtin expression in clonal striatal cells: dissociation of inclusion formation and neuronal survival by caspase inhibition., *J. Neurosci.*, **19**: 964-973, 1999
76. **Miyashita T., Matsui J., Ohtsuka Y., Mami U., Fujishima S., Okamura-Oho Y., Inoue T., Yamada M.,** Expression of extended polyglutamine sequentially activates initiator and effector caspases, *Biochem. Biophys. Res. Commun.*, **257**: 724-30, 1999
77. **Saudou F., Finkbeiner S., Devys D., Greenberg M.E.,** Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions, *Cell*, **95**: 55-66, 1998
78. **Wellington C.L., Singaraja R., Ellerby L., Savill J., Roy S., Leavitt B., Cattaneo E., Hackam A., Sharp A., Thornberry N., Nicholson D.W., Bredesen D.E., Hayden M.R.,** Inhibiting caspase cleavage of huntingtin reduces toxicity and aggregate formation in neuronal and nonneuronal cells, *J. Biol. Chem.*, **275**: 19831-8, 2000
79. **Gervais F.G., Singaraja R., Xanthoudakis S., Gutekunst C.A., Leavitt B.R., Metzler M., Hackam A.S., Tam J., Vaillancourt J.P., Houtzager V., Rasper D.M., Roy S., Hayden M.R., Nicholson D.W.,** Recruitment and activation of caspase-8 by the Huntingtin-interacting protein Hip-1 and a novel partner Hippi, *Nat. Cell Biol.*, **4**: 95-105, 2002
80. **Zhu S., Stavrovskaya I.G., Drozda M., Kim B.Y., Ona V., Li M., Sarang S., Liu A.S., Hartley D.M., Wu du C., Gullans S., Ferrante R.J., Przedborski S., Kristal B.S., Friedlander R.M.,** Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice, *Nature*, **417**: 74-78, 2002
81. **Guegan C., Vila M., Teissman P., Chen C., Onteniente B., Li M., Friedlander R.M., Przedborski S.,** Instrumental activation of bid by caspase-1 in a transgenic mouse model of ALS, *Mol. Cell Neurosci.*, **20**: 553-562, 2002
82. **Mochizuki H., Hayakawa H., Migita M., Shibata M., Tanaka R., Suzuki A., Shimo-Nakanishi Y., Urabe T., Yamada M., Tamayose K., Shimada T., Miura M., Mizuno Y.,** An AAV-derived Apaf-1 dominant negative inhibitor prevents MPTP toxicity as antiapoptotic gene therapy for Parkinson's disease, *Proc. Natl. Acad. Sci. USA*, **98**: 10918-10923, 2001
83. **Yakovlev A.G., Ota K., Wang G., Movsesyan V., Bao W.L., Yoshihara K., Faden A.I.,** Differential expression of apoptotic protease-activating factor-1 and caspase-3 genes and susceptibility to apoptosis during brain development and after traumatic brain injury, *J. Neurosci.*, **21**: 7439-7446, 2001
84. **Chapman P.F., White G.L., Jones M.W., Cooper-Blacketer D., Marshall V.J., Irizarry M., Younkin L., Good M.A., Bliss T.V., Hyman B.T., Younkin S.G., Hsiao K.K.,** Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice, *Nat. Neurosci.*, **2**: 271-276, 1999
85. **Carter R.J., Lione L.A., Humby T., Mangiarini L., Mahal A., Bates G.P., Dunnett S.B., Morton A.J.,** Characterization of progressive motor deficits in mice transgenic for the human Huntington's disease mutation, *J. Neurosci.*, **19**: 3248-3257, 1999
86. **Hansson O., Petersen A., Leist M., Nicotera P., Castilho R.F., Brundin P.,** Transgenic mice expressing a Huntington's disease mutation are resistant to quinolinic acid-induced striatal excitotoxicity, *Proc. Natl. Acad. Sci. USA*, **96**: 8727-8732, 1999
87. **Ivins K.J., Bui E.T., Cotman C.W.,** Beta-amyloid induces local neurite degeneration in cultured hippocampal neurons: evidence for neuritic apoptosis, *Neurobiol. Dis.*, **5**: 365-378, 1998
88. **Ankarcrona M., Dypbukt J.M., Bonfoco E., Zhivotovsky B., Orrenius S., Lipton S.A., Nicotera P.,** Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function, *Neuron*, **15**: 961-973, 1995
89. **Volbracht C., Leist M., Kolb S.A., Nicotera P.,** Apoptosis in caspase-inhibited neurons, *Mol. Med.*, **7**: 36-48, 2001