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Relevance of death receptors in nervous system: role in the pathogenesis of neurodegenerative diseases and targets for therapy

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Resum

L'apoptosi és un procés fisiològic que controla el nombre de cèl·lules en organismes superiors. L'apoptosi està estrictament regulada i s'ha vist que està implicada en la patogènesi d'algunes malalties del sistema nerviós. En aquest sentit, un excés de mort cel·lular contribueix a les malalties neurodegeneratives, mentre que, el seu dèficit és una de les raons del desenvolupament de tumors. El punt principal de regulació del procés apoptòtic és l'activació de les caspases, cisteïna-proteases que tenen especificitat pels residus aspàrtic. Les caspases es poden activar per dos mecanismes principals: (1) alliberament de citocrom C dels mitocondris alterats al citoplasma i (2) l'activació dels receptors de la membrana anomenats receptors de mort (DR, de l'anglès *death receptor*). Aquests receptors s'han caracteritzat extensament en el sistema immunitari, mentre que en el sistema nerviós les seves funcions són encara desconegudes. El present article se centra en el paper dels DR en la patogènesi de malalties neurodegeneratives i suggereix el seu potencial des del punt de vista terapèutic. També es descriuen diverses molècules intracel·lulars caracteritzades per la seva habilitat en la modulació dels DR. Entre elles, presentem dues noves proteïnes – *lifeguard* i FAIM – que s'expressen específicament al sistema nerviós.

Paraules clau: apoptosi · receptors de mort · neurones · FAIM · LFG

Abstract

Apoptosis is a strictly controlled, physiological process by which the number of cells in metazoan organisms is regulated. Recently, it has been shown that apoptosis is involved in the pathogenesis of certain nervous system diseases. Excess cell death is thought to contribute to neurodegenerative disorders while defects in apoptosis lead to the development of neoplasias. Regulation of apoptosis primarily occurs through the activation of caspases, cysteine proteases that specifically cleave aspartic acid residues. Caspases are activated by two mechanisms: (1) release of cytochrome C from mitochondria to the cytoplasm and (2) activation of plasma membrane death receptors (DRs). These latter proteins have been widely characterized in the immune system, whereas in the nervous system their function remains elusive.

In this article we focus on the role of DRs in the pathogenesis of neurodegenerative diseases and on the potential of these proteins as therapeutic targets. We also discuss several intracellular molecules that modulate DR activation. Among these, we introduce two novel proteins, Lifeguard and FAIM, which are specifically expressed in the nervous system.

Keywords: apoptosis · death receptors · neurons · FAIM · lifeguard

The integrity of a cell is regulated by internal sensors and extracellular signals. When a cell loses functional contact or adequate interaction with its surroundings or detects irreparable internal damage, apoptosis is activated. [1]. Mammals have developed another mechanism that allows the organism to actively drive individual cells to self-destruction. This type of "instructive" apoptosis is especially relevant in the immune system, where it has been characterized in detail. Nevertheless, it is also becoming highly relevant in other systems such as the nervous system.

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1 Death receptors

Death receptors (DRs) belong to the tumor necrosis factor (TNF) superfamily, which comprises receptors involved in proliferation, differentiation, and apoptosis. DRs are type I integral membrane proteins with a conserved extracellular domain containing two to four cysteine-rich pseudo-repeats (CRD), a single transmembrane region, and a conserved intracellular death domain (DD) of about 80 amino acids that binds to adaptor proteins and initiates

apoptosis [2]. To date, eight DRs containing DDs have been characterized: TNF receptor 1 (TNFR1; also known as DR1, CD120a, p55, and p60), Fas (DR2, APO-1, and CD95), DR3 (APO-3, LARD, TRAMP, and WSLT), TNF- related apoptosis-inducing ligand (TRAILR1; DR4 or APO-2), TRAILR2 (DR5, KILLER and TRICK2), DR6, ectodysplasin A receptor (EDAR), and the nerve growth factor receptor (p75NTR). The DDs of these proteins allow them to recruit adaptor molecules and to initiate activation of the apoptotic signaling cascade [3]. Ligands for these receptors are structurally related molecules that also belong to the TNF superfamily and include FasL, which binds to Fas; TNFα and lymphotoxin α, which bind TNFR1; TL1A, also called VEGI (vascular endothelial cell growth inhibitor), which interacts with DR3; and TRAIL, which activates DR4 and DR5 [4]. DR ligands cans also interact with "decoy" receptors (DcRs), which do not have DDs and therefore do not form death signaling complexes. Four DcRs have been described: TRAILR3 (also called DcR1), TRAILR4 (DcR2), DcR3, and osteoprotegrin (OPG).

2 Death receptor signaling pathways

Once death ligands (DLs) bind to their specific receptors, two different types of signaling complexes can be formed. The first, called DISC (death-inducing signaling complex), is typical of Fas, TRAILR1, and TRAILR2 receptors. DISC It leads directly to the recruitment and activation of caspase-8, which is the initiator caspase of the apoptotic process. The second type of complex is specific for TNFR1, DR3, and DR6 receptors and recruits a different set of adaptor proteins. Depending on the complex composition, Fas and TNFR can signal cell death or, surprisingly, cell survival (see below and Figure 1).

2.1 Fas signaling

FasL binds between the second and third CRD of the ligand-binding domain of Fas and induces clustering of the receptor at one pole of the cell [5], while, simultaneously, the DISC forms within the cell. During DISC formation, the adaptor FADD (Fas-associated death domain), procaspase-8 interacts with the Fas receptor through homotypic contacts. Local high concentrations of procaspase-8 induce autoproteolytic activation, which results in the formation of heterotetramers of two large subunits (p18) and two small subunits (p10) of active caspase-8. These are released into the cytoplasm, where they activate executor caspases such as caspase-3 and caspase-7. Assembly of the Fas-induced DISC differs between cell types. So-called type I cells are characterized by a high level of DISC formation and therefore strong caspase-8 activation, which allows for direct activation of executor caspases. At lower levels of caspase-8, apoptotic signaling requires a mitochondrial amplification loop [7]. In this case,

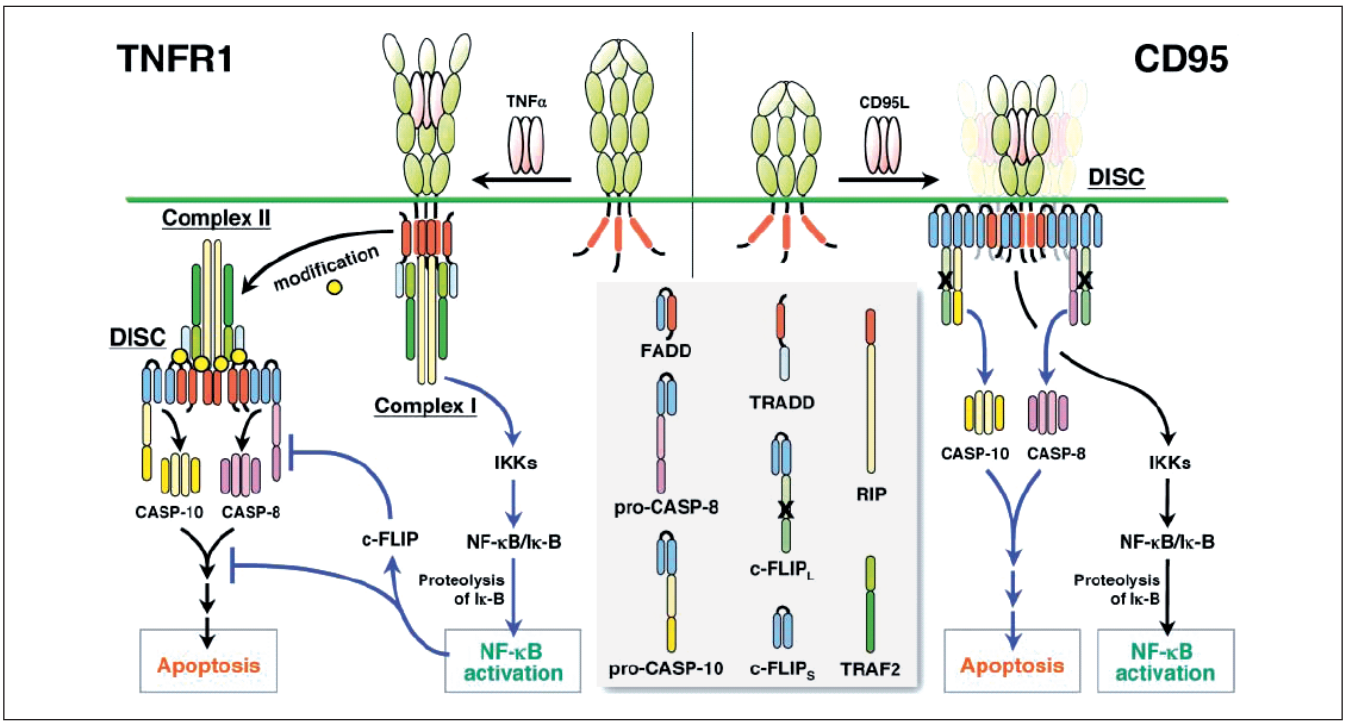

Figure 1. Comparison of TNFR1 and Fas signaling. Fas directly recruits FADD, procaspase-8, pro-caspase-10, and the caspase-8/10 regulator c-FLIP to the death-inducing signaling complex (DISC), resulting in the release of active caspase-8 and -10. This, in turn, induces apoptosis, the default pathway for Fas. Alternatively, in Fas apoptosis-resistant cells, Fas induces NF-kB activation. The TNFR1 default pathway activates NF-kB and other non-apoptotic pathways (not shown) through the formation of complex I, comprising TNFR1 and NF-kB activating signaling components such as RIP and TRAF2. Subsequently, there is transcriptional activation of a number of anti-apoptotic genes, such as c-FLIP and others that prevent the induction of apoptosis, in part by inhibiting caspase-8 activity. After receptor activation, some of the components of complex I are post-translationally modified. These modifications may serve as a cue for the signaling components but not the receptor (not shown) to leave the plasma membrane and form complex II (an intracellular DISC) by recruiting the apoptosis-inducing components FADD, procaspase-8, and either procaspase-10 (DISC on) or c-FLIP (DISC off). *Dark blue bold lines* indicate the default pathways, which are survival for TNFR1 and apoptosis for FAS. Only the membrane proximal events of apoptosis signaling are shown. *IKKs* IkB kinase complex; death domains are represented in *red* and death effector domains in *blue*. Barnhart, BC and Peter, ME (2003). The TNF receptor 1: a split personality complex. Cell.114 (2). 148-50

the few active molecules of caspase-8 process and activate Bid, a BH3-only member of the Bcl-2 family. Caspase-8 truncated Bid (tBid) is able to induce the release of cytochrome c and thus the activation of mitochondrial-based apoptosis [3].

2.2 Signaling by TNFRI

Signaling by TNFα occurs through two receptors, TNFR1 and TNFR2, although most of the biological effects are mediated by TNFR1 [6]. For more than a decade it has been known that ligand binding to TNFR1causes receptor trimerization [7] and release of the TNFR-inhibiting protein SODD (silencer of death domains) from the intracellular domain. This allows recruitment of the adaptor protein TRADD (TNFR-associated death domain protein), which serves as a common platform for the different molecules that execute the distinct functions of TNFα, either cell survival or apoptosis. In 2003, Micheau and Tschopp proposed a model to explain the detailed mechanisms of TNFR1 signaling [8], in which apoptotic signaling requires the formation of two successive complexes. The first one recruits TRADD, RIP1, TRAF2 (TNFR-associated factor), and IAP1. This complex activates NF-kB signaling but not apoptosis. NF-kB pathway activation contributes to blocking apoptotic signals by inducing the expression of anti-apoptotic proteins such as IAP1, IAP2, TRAF1, TRAF2, and FLIP [9]. Moreover, it seems that NF-kB can also block apoptosis by suppressing the constitutive activity of the Jun-kinase pathway [10]. If NF-kB activation is strong enough, the cell will not die. Otherwise, the first complex dissociates and the second complex forms, in which TRADD and RIP1 associate with FADD and procaspase-8. In this complex, TNFRI is absent. Subsequently, caspase-8 activates apoptosis by a mechanism similar to that of Fas-caspase-8 [8].

3 Death receptors in the nervous system

The role of the extrinsic pathway in the central nervous system (CNS) is still poorly characterized. However, there is increasing evidence demonstrating that DR signaling participates in normal development and in a large variety of pathological situations [11].

3.1 Role of DRs in the development of the nervous system

Programmed cell death (PCD) is a very relevant phenomenon during CNS development since roughly half the neurons that are generated during neurogenesis are eliminated by this process. PCD is regulated by neurotrophic factors released from cells innervated by a given neuronal population. [12]. Since the magnitude of neuronal death differs in different neuronal populations [13], the exact mechanisms regulating the death of these cells probably differ as well.

One of the best systems to study PCD is spinal cord motoneurons. The FasL/Fas system has been clearly implicated in the apoptotic cell death of motoneurons induced in vitro by trophic factor deprivation, a system thought to recapitulate that of PCD in vivo [14]. However, it has not been possible to demonstrate the role of the FasL/Fas system in vivo. Transgenic mice expressing a dominant negative form of the Daxx protein (essential for Fas-induced cell death) do not show any alterations in spinal cord development, indicating that neither Daxx nor any of its associate molecules (Fas, ASK-1, etc.) are significantly involved in cell death during spinal-cord development [15]. Similarly, Kovac et al. reported that animals carrying either mutated Fas (*lpr* mice) or FasL (*gld* mice) do not differ from wild-type mice with respect to the number of neurons in different areas of the hippocampus [16]. It was therefore concluded that Fas is not important in the apoptotic cell death that occurs during the development of the nervous system, although it can not be ruled out that another DR is redundant regarding the function of Fas [17]. Particularly, the neurotrophin receptor p75 (p75NTR) has been implicated in PCD occurring in early stages of brain development.

Although, based on what is known thus far, DRs, with the exception of p75NTR, do not seem to have a clear role in the apoptosis of embryonic development, they may have other developmental functions. Recent results have shown that the Fas system is involved in the growth of neuronal processes [18]. Zuliani and colleagues demonstrated that exogenous FasL promotes the branching of neurites in embryonic cortical neurons. Accordingly, it has been reported that pyramidal neurons from 5-week-old *lpr* mice show a retraction of the dendritic tree and a loss of dendritic spines [19]. The regulation of neurite growth seems to be independent of caspase-8 and the functional DD of Fas and instead to depend on the MEK1/ERK/p35 pathway [20].

3.2 Implication of the DRs in pathologies

A large number of studies have examined the role of DRs under pathological situations. Increased levels of the components of the Fas/FasL system and other cytotoxic cytokines such as TNFα have been detected in inflammatory, degenerative, traumatic, ischemic, and neoplastic processes.

3.3 Ischemia

Cerebral ischemia is a restriction in the blood supply to the brain. The numerous causes include the spontaneous, *in situ*, total occlusion of an artery, after traumatic brain injury, by a reduction in arterial diameter of hereditary origin, or by embolism, generally of cardiac origin. The magnitude of the damage depends on the duration and severity of the insult. Although most of the affected cells will die by necrosis, a slower rate of neuronal death is associated within the so-called penumbral area [21]. During the cell-stress response to ischemia, c-Jun N-terminal kinases/stress-activated protein kinases (JNK/SAPKs) are activated. JNK/SAPKs are translocated to the nucleus, where they activate the transcription factor c-jun, with consequent induction of the expression of target genes such as FasL and TNFα [22]. It has also been demonstrated that glial cells can contribute to the progression of cell death in the later period of ischemia, through the production of nitric oxide or pro-inflammatory cytokines [23]. Hypoxia or the presence of reactive oxygen species (ROS) induces the expression of FasL in microglial cells [24]. Studies have shown that the levels of FasL and TNFα are elevated in post-ischemic brains, as is the capacity of these

proteins to induce cell death in primary cultures of neurons. The importance of these DLs is reinforced by the resistance of Fas-deficient mice to post-ischemic neuronal damage [25]. Nevertheless, the role of TNFR1 in vivo is controversial, because in some cases it has been observed that the blockade of signaling by the receptor reduces the size of the affected area, whereas mice deficient in TNFR1, TNFR2, or both have greater ischemic damage. With the purpose of clarifying the function of these receptors, Martin-Villalba and others evaluated the resistance of TNFα- and FasL-deficient mice to primary ischemic damage and the subsequent inflammatory reaction, using a model of occlusion of the middle cerebral artery. Their results demonstrated that hybrid TNFα- and FasL-deficient mice were more resistant to damage induced by arterial occlusion. These results were reinforced by observations that, in control mice injected with a combination of antibodies to neutralize FasL and TNFα, infarct volume was reduced by 70%. A smaller inflammatory response, as shown by a reduced invasion of granulocytes, was also observed. The combination of protection against primary ischemic damage along with a reduced inflammatory response resulted in longer survival of TNFα- FasL-deficient animals. Moreover, the mice recovered locomotive function to an extent practically indistinguishable from that of animals that had been operated on but without arterial occlusion [26].

3.4 Inflammatory diseases

Fas also appears to play a role in inflammatory diseases such as multiple sclerosis (MS). MS is an autoimmune pathology that affects the brain and spinal cord and is mediated by T-lymphocytes. Small areas of inflammation are formed and correlate with demyelinization caused by the specific death of oligodendrocytes. This progressive demyelinization interrupts the transmission of electric signals affecting different areas of the CNS, leading to alterations in sensation, visual problems, muscle weakness, depression, difficulties with coordination and speech, severe fatigue, cognitive impairment, problems with balance, overheating, and pain [27]. An experimental model widely used for the study of the MS is experimental allergic encephalomyelitis (EAE). EAE is induced by the injection of myelin-specific peptides into mice. After 10–14 days, the animals develop a loss of muscular tone in their tails and coordinated muscular movements deteriorate [28]. In this experimental model, the Fas/FasL system produces two different responses: (1) It acts as an inducer of cell death, since T-cells expressing FasL on their surfaces target those cells with surface expression of Fas, such as neurons and oligodendrocytes. The injection of specific anti-FasL antibodies just after the acute phase of EAE destabilizes the Fas/FasL system and results in a clinical improvement of symptoms [29]. (2) The immune system exerts protective functions in later phases of EAE, regulating autoimmune complications that arise after the appearance of self-antigens [11].

3.5 Traumatic brain injury

Traumatic brain injury (TBI) represents one of the main causes of death in the population below 45 years. Cellular damage is, in part, due to glutamate toxicity and a deregulation of calcium homeostasis [30]. Moreover, experimental evidence has shown that DRs participate in neuronal death. Data from animal models as well as from patients with severe trauma showed that TBI induces formation of the DISC complex and promotes activation of the caspase cascade. Studies of Fas and FasL expression showed co-localization in neurons, where the assembly of DISC may cause cell death by an autocrine mechanism. [31].

High levels of TNFα have been detected in extracts of traumatic cerebral cortex and in the cephalo-rachidian fluid of patients with TBI [32]. In experimental models, an increase in TNFR1 mRNA and protein beginning 1 h post-trauma was observed [33]. Therefore, TNFR1 and TNFα may constitute suitable targets for therapeutic strategies [34].

Another pathology associated with trauma is spinal cord injury (SCI). One of the main therapeutic goals is the recovery of motility after injury, by reestablishing neuronal connections. This can reportedly be attained by blocking FasL with specific antibodies. The mechanism behind this effect could involve a greater regenerative capacity of axons or the blockade of apoptosis by neurons and oligodendrocytes. [35].

3.6 Neurodegenerative diseases

Neurodegenerative diseases results from the death of specific neuronal populations. Alzheimer's disease (AD) is characterized by the deposition of β-amyloid fibers and an extensive loss of neurons. Many efforts have focused on the mechanism of fiber deposition, but it is still not clear how the formation of these so-called senile plaques results in neuronal death. Peculiarly, it seems that the loss of neurons is selective for certain areas of the brain (frontal cortex and hippocampus), whereas subcortical areas and the cerebellum remain unaffected [36]. Apoptotic features have been observed in affected neurons [37]. Among the different stimuli that induce the neuronal death seen in AD, the activation of DRs in microglial cells seems to be responsible for the neurotoxic effects. The β-amyloid fibers induce the production of cytokines, for example TNFα, through a tyrosine-kinase signaling-dependent mechanism [38].

Along with the production of cytokines, the formation of ROS contributes to neurotoxicity [39]. In mice overexpressing the human amyloid precursor protein, increased expression of Fas led to increased neuronal death. By contrast, Fas expression is reduced in hybrid mice expressing the protein superoxide dismutase (SOD), an enzyme able to process ROS to less toxic products [40]. These results suggest that antioxidant therapy may provide a strategy for reducing Fas expression.

Unlike the neurotoxic effects mediated by DRs in neurodegenerative diseases, these proteins play a neuroprotective role in Parkinson's disease (PD). PD involves a loss of dopaminergic neurons of the substantia nigra pars compacta, giving rise to the characteristic symptoms of tremor, bradykinesia, rigidity, and postural instability. A well-established model of PD in rodents and primates consists of the administration of 1-methyl-4-fenil-1,2,3,6-tetrahidropiridium dopaminergic toxin (MPTP) [41]. MPTP toxin metabolizes into 1-methyl-4-phenylpyridinium (MPP+), which by selectively concentrating in dopaminergic neurons causes the death of these cells. In patients with PD, both Fas expression and the anchored form to FasL are dimin-

Table 1. Main death receptors antagonists

Name	*Description*	*Expresse d in NS*	*References*	*Comments*
A20	Zinc Finger Protein A20	YES	[66],[67],[68],[69]	
BAR	Bifunctional Apoptosis Regulator	YES	[701,[71],[72]	
FAIM	Fas Apoptosis Inhibitory Molecule	YES	[551,[561,[57]	FAIML exclusivve of NS
FAP-1	Fas Associated Phosphatase-1	YES	[73],[74],[75],[76],[77],[78]	
FLIP	FLICE Inhibitory Protein	YES	[59],[60],[61],[62],[63],[64],[65]	
LFG	Lifeguard	YES	[51],[52],[53],[54]	Exclusive of NS
PEA-15	Phosphoprotein Enriched in Astrocytes	YES	[79],[80],[81],[82],[83]	
SODD	Silencer Of Death Domains	YES	[84],[85],[86],[87]	
SUMO-1	Small Ubiquitin-related Modifier 1	YES	[88],[89],[90],[91]	
BTK	Burton's tyrosine kinase	NO	[92],[93]	
Toso	Cell surface protein	NO	[941,[95]	
E6	Viral protein	NO	[96],[97]	
E3 RID	Adenoviral protein	NO	[98]	
HPV E7	Adenoviral protein	NO	[99]	

ished [42], whereas the soluble form of FasL, which block Fas signaling, is increased [43]. Landau et al. demonstrated that in Fas-deficient mice (*lpr*) treated with suboptimal doses of MPTP there is a dramatic loss of dopaminergic neurons whereas the same doses do not cause alterations in wild-type mice. Neuronal loss is accompanied with hypokinesia, tremors, and discoordination. In vitro, it has also been demonstrated that Fas activation is able to protect against MPTP-induced cell death by a caspase-8 independent mechanism. However, other experiments carried out in animal models of PD showed an increase in TNFα shortly after MPTP administration. Mice deficient in TNFR1 and TNFR2 were completely resistant to death induced by MPTP, implying that TNFα is an effector of the neurodegenerative process [44].

4 Death receptors antagonists

The function of the DRs can be modulated by posttranslational modifications of the receptor, such as glycosylation [45], or by interfering in the apoptotic cascade. Table 1 lists the proteins that have been described to antagonize DR function. The table does not include those molecules that antagonize DR indirectly, that is, by interfering in the mitochondrial or intrinsic pathway, such as the anti-apoptotic Bcl-2 family members and IAPs.

4.1 FLICE inhibitory protein

Searches for proteins able to interact with Fas DD have led to the isolation of a new protein family characterized by the presence of dead effector domains (DEDs). These proteins, collectively called viral inhibitors of FLICE (vFLIPs), have been detected in several viruses, including herpesvirus Saimiri, herpesvirus human 8, and the herpesvirus associated with Karposi's sarcoma. Up to 13 different splicing variants have been described but only three are translated into proteins. Structurally, all isoforms consist of two DEDs, which are similar to the N-terminal end of caspase-8. The C-terminal of FLIPL also consists of two caspase-like domains (p20 and p12), although they do not have the amino acids critical for caspase activity. FLIPS and FLIPR [46] contain DEDs at their C-terminal ends; the DED in FLIPR is shorter than the one in FLIPS and different from FLIPL, caspase-8, and caspase-10 [47]. Generally, all FLIP isoforms play an anti-apoptotic role in DR-induced apoptosis. Results obtained from loss-of-function experiments for FLIPS and for FLIPL, together [48] or separately [49], showed that FLIP has inhibitory consequences in the processing of caspase-8. In addition, mouse embryonic fibroblasts isolated from FLIP-deficient mice showed high sensitivity to DR-induced apoptosis [48]. Once FLIPL is recruited to the DISC, it is processed into two subunits: p43, which remains in the DISC, and p12, which is released into the cystosol. In the presence of FLIPL, caspase-8 is also processed into two fragments of p43 and p41 kDa, whereas a p10 subunit is released to the cytoplasm. According to the model of autocatalytic cleavage induced by proximity, proposed for caspase-8 [50], autocatalytic activation takes place first; this is followed, in the presence of an adjacent caspase domain, by a transcatalytic cut that leads to complete activation of the caspase. The resulting heterotetramer, composed of two p18 and to p10 subunits, is then ready to cleave downstream substrates. If the adjacent molecule in this model is FLIPL, without a functional caspase domain, the autocatalytic cut of caspase-8 will occur but not the transcatalytic cut, therefore preventing total activation of the enzyme [47].

Besides studies on the anti-apoptotic function of FLIP, the phenotype of FLIP null mice has opened up new perspectives. These mice die at day 10.5 of embryogenesis due to a failure in cardiac development not related to a lack of apoptosis, suggesting apoptosis-independent roles for FLIP. Moreover, FLIP is expressed in different cell types, ones unrelated to DR sensitivity [51]. Little or almost nothing is known about the physiological function of FLIP in the nervous system. Its expression in cortical neurons during development has been detected [52]

although expression has not been correlated with the resistance or sensitivity to Fas of the different neuronal populations. In granular neurons of the cerebellum, which are resistant to Fas, it has been observed that FLIP expression diminishes during *in vitro* differentiation while LFG, another recently described Fas antagonist, is up-regulated [53]. Therefore, FLIP expression could regulate the activities of DRs during the development of distinct cellular types and during specific periods.

4.2 Zinc finger protein A20

A20, one of the primary response genes to TNFα, encodes a protein of 80 kDa, with a C-terminal domain that contains seven zinc finger motifs (Cys2/Cys2) [54]. This protein is able to inhibit TNFα- and interleukin-1-induced apoptosis. The ability to block the signaling induced by these inflammatory cytokines constitutes a mechanism of negative regulation in the inflammatory response [55]—a function that is essential during development since A20-deficient mice die prematurely from severe inflammation and cachexia [56]. Although the mechanisms of molecular action are still unclear, it seems that A20 interferes in TRADD and RIP binding to TNFRI [57].

4.3 Bifunctional apoptosis regulator

The bifunctional apoptosis regulator (BAR) protects cells against extrinsic or intrinsic apoptotic stimuli. It is a protein of 450 kDa, located in the endoplasmic reticulum of neurons [58]. BAR was firstly described in a two-hybrid assay aimed at identifying Bax antagonists. The protein contains a sterile alpha motif (SAM) in the middle part of its structure that allows its interaction with anti-apoptotic Bcl-2 family members such as Bcl-2 or BclXL, but not with Bax or Bak [59]. In addition to the SAM domain, BAR contains a domain similar to the DED. That domain interacts with caspase-8, thus inhibiting the extrinsic pathway. One possibility is that BAR captures fragments of active caspase-8 into intracellular membranes, neutralizing the enzyme's effect and preventing further cleavage of vital substrates [60].

4.4 Fas-associated phosphatase-1

Fas-associated phosphatase-1 (FAP-1) was identified in a two-hybrid assay to detect proteins associated with Fas. FAP-1 encodes a tyrosine phosphatase that interacts with the last 15 amino acids of the Fas C-terminus, through a PDZ3 domain. FAP-1 overexpression reduces FasL-induced cell death in T-lymphocytes [61] and in other human cell lines. One of the antagonistic mechanisms that have been proposed is based on the capacity of FAP-1 to dephosphorylate tyrosines that are important for the recruitment of adaptor proteins (e.g., FADD) to Fas [62, 63]. Later studies proposed that strong expression of FAP-1 diminishes the presence of Fas in the plasma membrane but increases levels of the protein in intracellular reservoirs. Experiments with dominant negatives of FAP-1 or in which endogenous levels of the phosphatase are reduced showed increased transport of Fas to the surface of the plasma membrane [64]. FAP-1 has been identified as one of the genes responsible for tumor resistance to Fas-induced cell death. [65]. This effect could also be the basis of the high aggressiveness and resistance to treatment of astrocytomas [62] or colon carcinomas [66].

4.5 Phosphoprotein enriched in astrocytes-1 (PEA-15)

PEA-15 is a 15-kDa phosphoprotein ubiquitously expressed in cells but enriched in astrocytes [67]. It contains a DD that allows its interaction with Fas [68] and TNFR1. PEA-15 is able to inhibit Fas-induced apoptosis but enhances TNFα-induced cell death. However, results in PEA-15 deficient mice shows that PEA-15 increases astrocyte survival after exposure of the cells to TNFα [69]. Phosphorylation at Ser-104 blocks the interaction between PEA-15 and ERK, whereas phosphorylation at Ser-116 is necessary for the interaction of PEA-15 with FADD and the capacity of this complex to inhibit apoptosis [70].

4.6 Silencer of death domains (SODD)

It has been demonstrated that the increase in the density of receptors containing DDs in certain areas of the plasma membrane cause DD aggregation and activation of the caspase cascade in the absence of ligand binding. The fact that DRs are commonly expressed on the cell surface, where they form oligomers [71], suggests the existence of molecules that maintain them in their inactive state. SODD (silencer of death domains) was isolated as a DR3-binding protein. It is 457 amino acids long and also binds DR3, TNFR1, and TNFR2 but not Fas, DR4, or DR5. SODD blocks all TNFR1 functions, including apoptosis and activation of the NF-kB or JNK pathways in vitro [72]. SODD null mice appear normal, thus suggesting the existence of redundant or compensatory mechanisms, such as the A20 or TRAF2 proteins [73].

4.7 Small ubiquitin-related to modifier 1

Small ubiquitin-related to modifier 1 (SUMO) is another molecule that interacts with the intracellular domain of Fas. It consists of 101 amino acids and has high homology with ubiquitin [74]. SUMO overexpression protects cells from both Fas- and TNFRI-induced apoptosis; however, the exact mechanism remains unknown. SUMO1 is able to induce post-translational modifications that affect the stability or intracellular location of many proteins, among them those participating in apoptotic pathways, such as caspase-2, caspase-7 [75], caspase-8 [76], protein ASK-1 [77], and the NEMO/IKKγ complex.

4.8 Lifeguard

Lifeguard (LFG) was cloned in 1999. The protein protects against Fas-induced cell death but not against death induced by other DRs such as TNFR1 [78]. A comparison with sequences in the databases showed that LFG is the human homologous of rat NMP35 (neural membrane protein 35) [79]. The latter protein was identified by differential display experiments targeting genes that are regulated during development of the rat sciatic nerve. NMP35 is predominantly expressed in the nervous system. Expression of the protein increases during development, reaching maximum levels in the adult.

The first attempts to understand LFG function were carried out in HeLa and Jurkat cell lines, both of which are sensitive to FasL-induced apoptosis. LFG was reported to be responsible for maintaining the resistance of neurons to Fas in the cerebel-

lum [53]. We subsequently demonstrated that LFG regulates Fas activity in other neuronal populations, such as cortical neurons [80]. In those studies we also showed that an endogenous decrease of LFG in granular neurons of cerebellum and cortical neurons sensitizes these neurons to Fas-induced apoptosis. In summary, these results locate LFG as one of the main proteins responsible for the maintenance of Fas-resistance in neurons, particularly when other antagonists, such as FLIP, are not or poorly expressed.

4.9 Fas apoptosis inhibitory molecule (FAIM)

Fas apoptosis inhibitory molecule (FAIM) was first identified as a Fas antagonist in B-cells. FAIM has been highly conserved during the evolution, from *Caenorhabditis elegans* to humans [81]. A longer, alternative splicing isoform of the protein but with unknown function was identified and named FAIML. FAIMS is widely expressed, including in the nervous system. We demonstrated that overexpression of FAIMS, but not FAIML, enhances NGF-induced neurite outgrowth in different neuronal populations through activation of the NF-kB pathway. No anti-apoptotic function has been described in the nervous system. However, FAIML is specifically expressed in neurons and its expression is regulated during development. FAIML does not affect neurite outgrowth, nor does it modulate NF-kB activation. However, cells overexpressing FAIML are resistant to apoptotic cell death induced by DRs such as Fas or TNFR1. Reduction of endogenous expression shows that endogenous FAIML protects primary neurons against DR-induced cell death. FAIML normally binds the Fas receptor and prevents its activation. FasL binding to Fas induces the release of FAIML from Fas, allows the binding of FADD and caspase-8, and leads to apoptosis activation [82].

5 Conclusions and prospectives

Signaling pathways controlling neuronal death and survival are crucial for the normal development and function of the nervous system. In contrast to most cell types, neurons survive for the lifetime of the organism and therefore require powerful intracellular mechanisms to antagonize cell death stimuli.

Death receptors are widely expressed in the nervous system, in both neurons and glial cells. However, DR-induced apoptosis does not seem to play a role in regulating cell death in the developing nervous system, although, under certain pathological conditions, DR-induced apoptosis might be involved in cell death of mature neurons. The deleterious functions of DRs in the nervous system require a fine tuning mechanism, which might be achieved by different regulatory proteins. Restriction of DR-mediated signaling to specific neurons could control neuronal development and the selectivity of neuronal loss in degenerative diseases. In addition, under certain pathological conditions, DRs are clearly involved either in inducing or preventing apoptosis.

Further research efforts are needed to expand our understanding of the molecular basis of DR activity regulation in the nervous system. Such efforts will provide a starting point for the development of new therapeutic strategies for DR-associated neuropathologies.

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