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REVIEW

Cell death controlling complexes and their potential therapeutic role

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Abstract Programmed cell death plays a central role in the regulation of homeostasis and development of multicellular organisms. Deregulation of programmed cell death is connected to a number of disorders, including cancer and autoimmune diseases. Initiation of cell death occurs in the multiprotein complexes or high molecular weight platforms. Composition, structure, and molecular interactions within these platforms influence the cellular decision toward life or death and, therefore, define the induction of a particular cell death program. Here, we discuss in detail the key cell-death complexes—including DISC, complex II, and TNFRI complex I/II, and the necrosome, RIPoptosome, apoptosome, and PIDDosome—that control apoptosis or necroptosis pathways as well as their regulation. The possibility of their pharmacological targeting leading to the development of new strategies of interference with cell death programs via control of the high molecular weight platforms will be discussed.

Keywords Cell death · Protein complexes · DISC · Apoptosome · PIDDosome · RIPoptosome · Caspases

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Introduction

Programmed cell death (PCD) is an evolutionarily conserved process in multicellular organisms that regulates normal homeostasis in various tissues and cell types [1]. For many years, apoptosis was described as the only example of PCD, but nowadays several cell death modalities are known to represent the whole spectrum of PCD, including apoptosis, programmed necrosis, and autophagy [2]. Modes of PCD can be distinguished by their morphological and biochemical features. Apoptotic cell death is usually accompanied by membrane blebbing, cell shrinkage, chromatin condensation, DNA fragmentation, and engulfment of dying cells by macrophages or neighboring cells [3]. In contrast to apoptosis, programmed necrosis, or necroptosis, can be characterized by the rounding of cells, cytoplasmic swelling, dysfunction of cell organelles, rupture of the plasma membrane, and uncontrolled spilling of the intracellular content [4, 5]. The apoptotic program of cell death is mediated via specific regulatory and executory proteins, i.e., caspases. These enzymes belong to the family of cysteinyl-specific aspartate proteases [6, 7]. To date, 13 mammalian caspases have been identified as reviewed in [8, 9]. The caspase family of proteins can be classified into three groups based on their cell functions. The first group is the initiator caspases (caspase-2, -8, -9, -10), which are activated in multiprotein complexes resulting in the processing and activation of effector caspases, the second group of proteases. The latter group (caspase-3, -6, -7) are the key executioners in the cell-death machinery, which can cleave multiple cellular substrates leading to the destruction of the cell [10]. The third group comprises inflammatory caspases (caspase-1, -4, -5), which are involved in immune responses to microbial pathogens [9, 11]. Activation of inflammatory

caspases, such as caspase-1 and -5, also occurs in an intracellular complex, which is designated the inflammasome [9, 11].

Caspases are synthesized as inactive proforms (zymogen or procaspase) consisting of a prodomain, the large subunit p20, and the small subunit p10 [10, 12–15]. The large prodomain of initiator caspases contains specific structural motifs: DED (death effector domain) or CARD (caspase recruitment domain) [16]. These motifs are involved in the recruitment of caspases to the multiprotein complexes via homotypic interactions with other proteins [16, 17]. The assembly of these initiation platforms provides unique conditions for high local caspase concentration that allows the dimerization of inactive monomers, which is a prerequisite for their activation [17, 18]. During activation, the zymogen is cleaved at specific aspartate residues, large and small subunits are separated, and the N-terminal domain is removed to form an enzymatically active heterotetramer (p20–p10)*2 [19]. It has become increasingly evident that the multiprotein complexes play a crucial role in cell death signaling and that understanding the mechanism of the complex formation and activation may provide opportunities for the control of cell death processes. Here we discuss the current knowledge of mechanisms of formation of complexes, and their potential therapeutic benefit.

Caspase-activating platforms in death receptor signaling: DISC and complex I/complex II

Apoptosis can be induced via activation of extrinsic or intrinsic pathways. The extrinsic apoptotic pathway is triggered by binding of extracellular ligands to their cognate death receptors (DRs), such as CD95 (APO-1/Fas), TRAIL-R1/TRAIL-R2 (TRAIL receptor 1/2) and TNFR (tumor necrosis factor receptor) as discussed in [3]. DRs belong to the TNFR superfamily and are characterized by the presence of an intracellular death domain (DD) [16]. A number of adaptor molecules are recruited to the DD and participate in the transduction of the extrinsic death signal.

The activation of CD95 or TRAIL-R results in the formation of the DISC (death-inducing signaling complex). DISC comprises the oligomerized receptor, Fas-associated DD (FADD), initiator zymogens procaspase-8 and procaspase-10, and the regulatory protein cellular FLICE inhibitory protein (c-FLIP) [20, 21] (Fig. 1). The interactions between proteins at the DISC are based on the homotypic contacts [22]. The DD of the receptor interacts with the DD of FADD, whereas DED of FADD interacts with DED-containing procaspase-8/10 and c-FLIP. The ratio between the core DISC proteins DR, FADD, and procaspase-8 was considered to be 1:1:1 [3]. However, recently it was reported that the stoichiometry is different from that predicted and procaspase-8 exceeds FADD at the DISC by several fold [23, 24]. Furthermore, it was shown that at the DISC the DED-containing proteins procaspase-8/-10 and c-FLIP form so-called DED chains via interactions of their DEDs. In this way, procaspase-8 can form homodimers, leading to the efficient activation of caspase-8. Moreover, the length of procaspase-8 chains is controlled in a dynamic fashion and depends on several factors, such as CD95 stimulation strength, concentration of procaspase-8 in the cell, and the turnover rate of procaspase-8 at the DISC [23]. In particular, it was shown that longer chains could be formed upon low DR stimulation strength, reflecting the chain dynamics at the DISC. Thus, the control of the length of DED chains is one of the key factors in the regulation of procaspase-8 activation [23].

The activation of procaspase-8 is an autoproteolytic process that occurs upon its oligomerization at the receptor complex [18, 25, 26]. Caspase-8 processing is mostly described by a two-step model, which includes the cleavage between the protease domains, and between the prodomain and the large protease subunit. Initially, zymogen is cleaved to form the p43/41 and p10 subunits. Further, the p43/41 subunit is processed to produce the prodomain p26/p24 and p18. These cleavage processes lead to the formation of the caspase-8 active heterotetramer (p18/p10)*2 at the DISC. Subsequently, the mature caspase-8 is released into the cytosol to trigger the apoptotic pathways [21]. In addition to the classical two-step cleavage mechanism, an alternative cleavage mechanism of procaspase-8 processing at the DISC was described, which involves formation of the C-terminal cleavage product p30 [27, 28]. The importance of p30 in regulation of procaspase-8 activation at the DISC has to be further addressed.

The stimulation of TRAIL-R or CD95 results in the formation of not only membrane-associated DISC, but in the assembly of secondary cytoplasmic signaling complexes [29, 30]. According to the initial reports, the CD95 secondary complex includes FADD, procaspase-8, and c-FLIP and does not comprise CD95 (Fig. 1). This complex potentially enhances the activation of initiator caspase-8 in the cytosol and the apoptotic process [30]. Later, it was reported that the secondary complex also contains the protein receptor-interacting protein 1 (RIP1) and—depending upon its modification status, stimulation conditions, and cell line—it could promote both nuclear factor (NF)-κB activation and necroptotic cell death; the latter will be discussed below [31, 32]. The TRAIL-R secondary complex contains FADD, procaspase-8, and several factors, e.g., RIP1 (receptor-interacting serine/threonine-protein kinase 1), TNFR-associated factor 2 (TRAF2) and NF-κB essential modulator (NEMO), also known as IKKγ, which are involved in NF-κB activation [29]. Recently, it has been shown that TRAIL-R and CD95- induced complex II also mediate chemokine

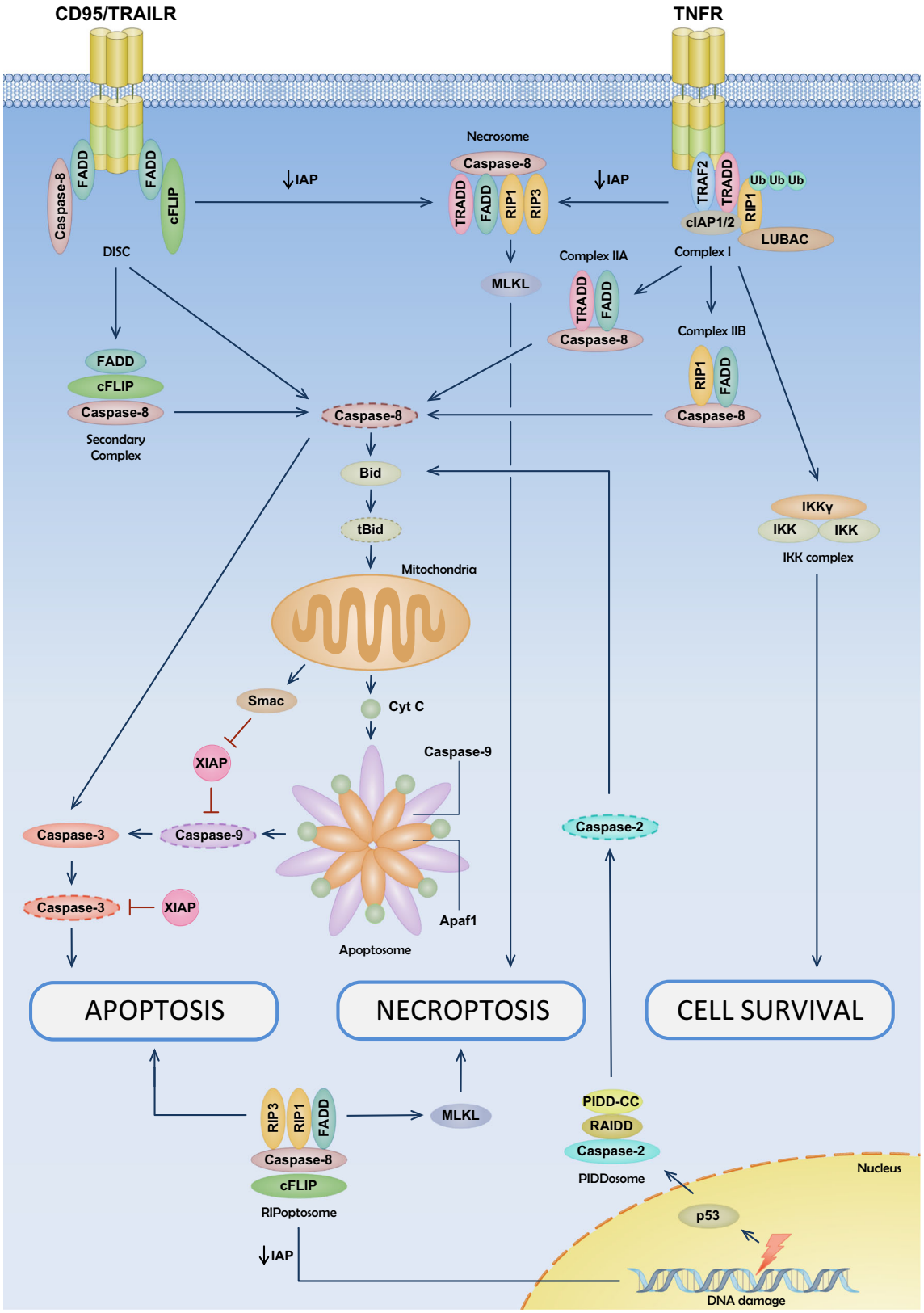

Fig. 1 Various high molecular weight-protein complexes, mechanisms of their activation and pathways regulation (for details, see text)

production and macrophage migration [29, 31]. Therefore, the stimulation of TRAIL-R/CD95 induces not only cell self-destruction, but also macrophage phagocytosis of apoptotic cells.

TNFR1 signaling has some similar and distinct features compared to CD95- or TRAIL-R1/2 pathways as reviewed in [33]. The stimulation of TNF-R1 triggers pro-inflammatory and immune-stimulatory activity, e.g., JNK, p38-MAPK, and NF-κB pathways [34]. The TNF-R1 stimulation results in the assembly of two signaling complexes: complex I and II (A/B) [17, 35]. Once stimulated, TNF-R1 recruits the adaptor molecule TNFR-associated death domain protein (TRADD), RIP1, TRAF-1/2, cellular inhibitor of apoptosis (cIAP-1/2), and the linear ubiquitin chain assembly complex (LUBAC) complex containing heme-oxidized IRP2 ligase (HOIL), HOIP, and Sharpin, leading to formation of TNF-R1 complex I [36–38] (Fig. 1). Complex I can promote efficient NF-κB activation via attachment of polyubiquitin chains to RIP1 and recruitment of NEMO [37]. Recently, the key role in this process has been attributed to the linear ubiquitination mediated by the LUBAC complex, and the functional details of this process are currently at the center of intense investigation [37, 38].

According to a number of reports, procaspase-8 could not be found in TNFRI complex I [36, 39]. These observations led to the search for the alternative platforms of caspase-8 activation and resulted in the elucidation of the TNF-R complex II, which is formed upon TNF-R1 stimulation, but lacks receptor [35]. The mechanistic details of complex II formation remain unclear. It was suggested that dissociation of RIP1 and TRAF2 from complex I facilitates the release of TRADD into the cytoplasm and its association with FADD via DD interactions. Further, procaspase-8 is recruited to the FADD/TRADD complex to form complex IIA (or TRADDosome) [17]. Activation of procaspase-8 occurs in the TRADDosome and triggers downstream death signaling (Fig. 1). Notably, NF-κB system can regulate c-FLIP expression. Consequently, apoptotic signals from complex IIA are suggested to be prevented by NF-κB activation, resulting from complex I, while a low activity of complex I permits complex IIA to induce apoptosis, unhindered by c-FLIP [35]. Apoptotic death can also be induced by a TRADD-independent platform, named complex IIB, which consists of RIP1, FADD, and procaspase-8 as reviewed in [17].

The regulation of caspase activity at the DISC and complex II TNFR-1 is provided by the DED protein c-FLIP. Alternative splicing leads to formation of three c-FLIP isoforms: L (long), S (short), and R (Raji) as discussed in [40]. All three isoforms contain tandem DEDs, while c-FLIP$_L$ additionally has the C-terminal inactive caspase-like domain. The isoforms c-FLIP$_S$ and c-FLIP$_R$ can be recruited to the DISC via DED interactions and negatively regulate the activation of procaspase-8 [41]. The isoform c-FLIP$_L$ has two functions: pro-apoptotic and anti-apoptotic [42]. The long isoform can increase activation of caspase-8 via formation of catalytically active procaspase-8–c-FLIP$_L$ heterodimers [43]. The optimal amount of these heterodimers for pro-apoptotic function of c- FLIP$_L$ is achieved under conditions of strong stimulation of the DR or a high concentration of c-FLIP$_{S/R}$ [42]. It has been shown that c-FLIP controls the threshold behavior of DR-induced apoptosis, and thereby it is likely that c-FLIP can block apoptosis upon spontaneous activation of DR as described in [40].

Thus, the main function of the multiprotein platforms DISC or TNFR (IIA/B) complexes is to generate optimal conditions for activation of initiator caspase-8, which triggers downstream death pathways.

Necrosome/RIPoptosome as necroptosis-initiating platforms

Programmed necrosis or necroptosis is a highly regulated multistep program of cell death that intersects with the regulation of apoptosis. Necroptosis is crucially different from apoptosis by the cellular morphology and drastically different signaling pathways, e.g., necroptosis is independent of effector caspase activation as reviewed in [4, 5, 44]. In many cells, the mode of PCD can be transformed from apoptosis to necroptosis by blocking of the former by synthetic pancaspase inhibitor zVAD-fmk or viral proteins, e.g., serpins.

As described above, the stimulation of TNFR1 leads to the formation of complex I, which promotes NF-κB activation or transition to complex II. Under conditions of apoptosis induction, active caspase-8 cleaves RIP1 and inactivates it, but when caspase activity is blocked, necroptotic cell death is triggered. RIP1 plays the central role in the transduction of the necroptotic signal as discussed in [44, 45]. Upon necroptosis induction, RIP1 interacts with kinase RIP3 via RHIM domains, forming amyloid fibers according to the recent X-ray structure of this complex [46–48]. Since caspase-8 is inhibited, RIP1 and RIP3 participate in the formation of the necroptosis-inducing complex, the so-called “necrosome” [46]. This complex includes FADD, caspase-8, RIP1, and RIP3 (Fig. 1). The adaptor molecule TRADD also may be a part of this complex, but this requires further clarification [46]. The post-translational modification of RIP1 plays a key role in necroptosis induction: its deubiquitination and phosphorylation seem to be essential for necroptosis initiation [45]. The first inhibitor of RIP1 developed by chemical screening [49], Nec-1 (necrostatin-1), blocks phosphorylation of RIP1 and thereby necroptosis [50, 51]. Recently, a number

of more effective inhibitors of necroptosis including Nec-3 were developed and successfully used in in vivo experiments [50, 52]. Importantly, necroptosis induction promotes RIP3 phosphorylation and activation by RIP1, which in turn phosphorylates the mixed-lineage kinase domain-like (MLKL) protein, the crucial protein for necroptosis death signal transduction [48, 53, 54]. The N-terminal region of MLKL is reported to be required for oligomerization and triggering cell death [55–57]. The positively charged amino acids of this region bind to phosphatidylinositol phosphate, which allows the recruitment of MLKL to the plasma membrane and necroptosis induction by directly permeabilizing the cell membrane [55]. However, recent studies have shown that RIP3 might also induce necroptosis by a RIP1-independent mechanism [58]. Thus, the molecular execution mechanism of necroptosis remains unclear and requires further detailed investigation.

Another important necroptosis-inducing multiprotein platform is the "RIPoptosome" [59, 60]. Its formation depends on the activity of cellular inhibitors of apoptosis proteins (cIAPs) 1/2. cIAPs ubiquitinate core components of the complex, in particular, RIP1, which leads to the proteosomal degradation of the complex and suppresses RIPoptosome formation [59, 60]. The downregulation of cIAPs by Smac (second mitochondria-derived activator of caspases) mimetics or genotoxic stress triggers formation of the RIP1/FADD/caspase-8 complex independently of the involvement of TNF, TRAIL, CD95L, or mitochondria, and induces caspase-8-mediated apoptosis or caspase-independent necroptosis. Interestingly, it has been shown that the RIPoptosome can be recruited to other signaling platforms, such as the Toll-like receptor-3, and therefore, could initiate necroptosis upon immune or pro-inflammatory signals [59].

The cross-talk between necroptosis and apoptosis is controlled by the balance of caspase-8 and c-FLIP isoforms [59, 61]. It has been proposed that formation of caspase-8 homodimers within the RIPoptosome results in its catalytic activity and induces apoptosis. In contrast, formation of caspase-8–c-FLIP$_L$ heterodimers results in limited catalytic activity, which is not sufficient to trigger apoptosis but could cleave RIP1. This in turn leads to RIPoptosome disassembly and cell survival. Upon interaction of heterodimerized caspase-8 with the short c-FLIP isoform, caspase-8 activation and RIP1 cleavage are inhibited, thereby promoting RIPoptosome formation. In this situation, apoptosis is blocked and necroptotic cell death is activated [59, 61] (Fig. 1). Recently, it has been shown that depletion of RIP1 can switch TNF-induced necroptosis to apoptosis [62]. This fact demonstrates that RIP1 is a negative regulator of FADD–caspase-8-mediated apoptosis and shows that the absence of RIP1 can promote the interaction of pro-apoptotic proteins, and can facilitate the formation of complex IIA. Furthermore, the depletion of RIP3 or MLKL can also shift TNF-induced necroptosis to apoptosis, which indicates that RIP3 and MLKL also negatively control FADD–caspase-8-mediated apoptosis [63]. Interestingly, apoptosis induction upon depletion of RIP3 or MLKL can be inhibited by Nec-1. These data demonstrate that RIP1 kinase activity is involved in the regulation of the switch between apoptosis and necroptosis [63].

Although the core components of the RIPoptosome and the necrosome have been identified, the stoichiometry of these complexes is still unknown. It is clear that further investigation of complex components will help to fully understand the mechanism of PCD regulation.

Apoptosome: the caspase activation platform in intrinsic apoptosis

The intrinsic (or mitochondrial) apoptotic pathway can be triggered by different cellular stresses, including DNA damage, heat shock, accumulation of unfolded proteins in the endoplasmic reticulum, or nutrition deprivation [64]. These apoptotic stimuli lead to the mitochondrial outer-membrane permeabilization (MOMP) and the release of cytochrome *c* from the intermembrane space into the cytosol. Cytochrome *c* is associated with binding sites on the inner membrane that contain cardiolipin molecules. The oxidation of cardiolipin leads to dissociation of cytochrome *c* from this phospholipid [65]. Upon release from the mitochondria, cytochrome *c* binds to Apaf-1 in a dATP/ATP-dependent manner and triggers nucleotide exchange and the stepwise assembly of the ring structure [66]. Multiple copies of procaspase-9 bind to the Apaf-1 ring to form the apoptosome, the central signaling initiator platform for caspase activation in the mitochondria-mediated pathway (Fig. 1). The core apoptosome components have been characterized using proteomics approaches [67, 68]. These studies have demonstrated that Apaf-1, caspase-9, caspase-3 and XIAP are the major constituents of native apoptosomes and that cytochrome *c* is not stably associated with the active complex. A direct comparison of the structure of Apaf-1 in the apoptosome to the Apaf-1 monomer reveals conformational changes that occur during the first two steps of assembly. This includes an induced-fit mechanism for cytochrome *c* binding to regulatory β-propellers, which is dependent on shape and charge complementarity, and a large rotation of the nucleotide-binding module during nucleotide exchange. These conformational changes create an extended Apaf-1 monomer and drive apoptosome assembly. The Apaf-1 CARD has to be free to interact with a procaspase-9 CARD either before

or during apoptosome assembly. Irrespective of the timing, the end product of assembly is a holo-apoptosome with an acentric CARD–CARD disk and tethered procaspase-9 catalytic domains [69]. The interaction of procaspase-9 with Apaf-1 within the apoptosome increases procaspase-9 local concentration, which allows formation of catalytically active caspase-9. Malladi et al. [70] have shown that procaspase-9 is processed only through the CARD-displacement mechanism. It is a continuous cycle of procaspase-9 recruitment/activation in which cleaved caspase-9 is substituted by procaspase-9 and released from the apoptosome. Five to seven procaspase-9 molecules seem to be recruited to the apoptosome at the same time [71–73]. Even though multiple caspase-9 molecules can bind to the apoptosome, at any time period only 1–2 molecules of caspase-9 are active [74]. Importantly, the Apaf-1 apoptosome functions as a proteolytic-based "molecular timer" [70]. The intracellular concentration of procaspase-9 sets the overall duration of the timer, procaspase-9 autoprocessing activates the timer, and the rate at which the processed caspase-9 dissociates from the complex dictates how fast the timer "ticks" over.

Until recently, a number of observations and experiments supported the proximity-induced dimerization model of procaspase-9 activation in which catalytic domains are brought into close contact with each other within the apoptosome. It is thought that the active caspase is a dimer in which one monomer is catalytically active, whereas the second monomer is inactive. Proximity-induced dimerization would induce conformational changes in appropriate regions in procaspase-9 to form the active sites [75, 76]. However, recent detailed studies of the apoptosome suggest an alternative allosteric model of procaspase-9 activation. In this case, the main driving force for caspase activation is not dimerization, but a direct activation of caspase-9 upon binding to the apoptosome [66]. Catalytic domains from a single procaspase-9 may be bound directly to the apoptosome platform to become active; this action induces a conformational change of the caspase, which in turn increases its activity. Furthermore, it has been shown that autoproteolytic processing of caspase-9 is essential for its activation [77]. As soon as the apoptosome is formed and procaspase-9 is processed, downstream executioner caspase-3 and -7 are recruited to the apoptosome via Apaf-1 interactions. Further, caspase-9 activates effector caspases and initiates their release from the apoptosome.

Proteolytic function of the apoptosome might be regulated via binding of caspase-3 to the apoptosome and subsequent inhibition of caspase-9 activity. Indeed, it has been shown in vitro that high rates of caspase-3 activation or high amounts of caspase-3 decrease the duration of apoptosome activity. Caspase-3 and -9 have overlapping binding sites on the central hub of Apaf-1, which might allow the formation of hybrid apoptosomes with proteolytic activities of both enzymes [75]. Importantly, caspase-3 can also cleave Apaf-1, and cleaved Apaf-1 eliminates binding with caspase-9. On the other hand, the regulation of caspase activity and "apoptosome timer" duration is controlled by XIAP (X-linked inhibitor of apoptosis protein), a natural inhibitor of caspases-9 and -3, and its antagonist Smac, as well as the other players involved in IAP-dependent ubiquitination and post-translational modifications [74] (Fig. 1).

PIDDosome as a platform for caspase-2 activation

The PIDDosome was first described as a multiprotein complex that can act as a procaspase-2 activation platform upon genotoxic stress, by enabling autocatalytic procaspase-2 activation through induced proximity [78, 79]. The complex assembly is provided by interactions between the C-terminal fragment of PIDD (p53-induced protein with a DD), RAIDD (receptor-interacting protein-associated ICH-1/CED-3 homologous protein with a DD), and procaspase-2 through their CARD and DD interactions. RAIDD plays the role of a bridge that binds PIDD via DD:DD interactions and procaspase-2 via CARD:CARD interactions. In vitro experiments revealed that caspase-2 CARD is an insoluble protein that can be solubilized by its binding partner, RAIDD CARD, but not by the full-length RAIDD [80], indicating that the full-length RAIDD in the closed confirmation cannot interact with caspase-2 CARD. The latter can be solubilized and can interact with the full-length RAIDD in the presence of PIDD DD. Thus, PIDD DD initially binds to RAIDD, after which caspase-2 can be recruited to RAIDD via a CARD:CARD interaction. It is important to note that PIDD can undergo sequential autoproteolytic cleavage to produce two different fragments: PIDD-C and PIDD-CC. PIDD-C is the fragment that is involved in maintenance of pro-survival and repair functions, while PIDD-CC binds RAIDD and initiates procaspase-2 activation [81] (Fig. 1). The X-ray crystal structure of the PIDDosome reveals a ring-like structure of the complex that includes five molecules of PIDD-CC, seven molecules of RAIDD, and seven molecules of procaspase-2 [82].

The PIDDosome formation depends on the process of PIDD cleavage. As mentioned above, the PIDD-CC fragment can interact with RAIDD leading to the formation of the "death-related" multiprotein complex. The PIDD-C fragment is known to be a positive regulator of NF-κB signaling [83, 84]. Therefore, PIDD can play the role of a molecular switch between cell survival and death. Recent studies have shown a phosphorylation-dependent regulatory mechanism for this switch [85]. The phosphorylation

of PIDD is controlled by Ataxia telangiectasia mutated (ATM) and is necessary for the conformational change of PIDD that allows interaction with RAIDD. The ATM/ATR-caspase-2 pathway can be blocked by Chk1 (checkpoint kinase-1), which suppresses PIDD phosphorylation and inhibits PIDDosome formation. Interestingly, structural studies of PIDDosome have revealed the importance of RAIDD conformation and microenvironmental variants inside the cell upon genotoxic stress for the formation of the PIDDosome [80].

Intriguingly, the activation of caspase-2 may occur regardless of PIDD and RAIDD. There is a lot of evidence that MEFs from PIDD-deficient mice undergo PIDD-independent apoptosis that occurs normally in response to various stress signals [86, 87]. The absence of PIDD does not affect the normal mouse development, caspase-2 activation, or cell death induced by apoptotic stimuli. The downregulation of PIDD and RAIDD by siRNA also failed to influence processing and activation of caspase-2 after treatment with 5-fluorouracil [88]. Furthermore, Manzl et al. [86] reported the formation of high molecular weight (HMW) complex, which contains caspase-2, in wild-type, pidd$^{-/-}$, or raidd$^{-/-}$ SV40-immortalized MEFs after a temperature shift. The HMW complex containing caspase-2, but not PIDD or RAIDD, could also be formed upon cisplatin treatment (our unpublished data). Imre et al. [89] demonstrated the initiator role of caspase-2 in pore-forming toxin-mediated apoptosis and purified HMW complex containing caspase-2 under these conditions. This platform seems to activate caspase-2 in a PIDD/RAIDD-independent manner. The obtained data indicate the existence of alternative cellular platforms for caspase-2 activation. However, the protein components of these complexes and the molecular mechanisms are still unknown and demand further investigation.

When and why is each platform activated?

The formation of multiprotein platforms is accompanied by the transduction of death signals, and it can be initiated by different external or internal events, such as crosslinking of death receptors to their ligands, nutrition deprivation, treatment with damaging agents, cell stresses, accumulation of unfolded proteins, or heating [90]. However, the induction of PCD and complexes assembly requires appropriate conditions. It was suggested that the formation of DISC depends on the conformation and plasma membrane localization of death receptors. In particular, the pre-association of CD95 and TNFR via the PLAD domain (pre-ligand binding assembly domain) was proposed to play an important role in DISC activation [91, 92]. Nevertheless, the processes of membrane localization, association with lipid rafts, and diffusion of death receptors in the cellular membrane require further investigation. In addition, the degree of the crosslinking of the death ligands seems to play a key role in apoptosis induction. Interestingly, there are a number of studies demonstrating that only the trimerized or oligomerized ligand is capable of inducing cell death, while the monomeric ligand induces the NF-κB signaling pathway [93]. The activation of caspase-8 at the DISC is controlled by several mechanisms, including DED chain formation, post-translational modifications of the core DISC proteins, and the amount of c-FLIP proteins in the cells [21]. The ratio between procaspase-8 and c-FLIP at the DISC as a key factor controlling apoptosis onset was extensively addressed by systems biology approaches [94]. Activation of caspase-8 downstream from the DISC is controlled differently in the so-called type I and type II cells. The DISC formation in type I cells is characterized by a high level of activated caspase-8, which is able to directly cleave effector caspase-3 and -7. In contrast, in type II cells, the signals of PCD are reinforced through apoptosome activation [95, 96].

The supramolecular necroptotic platforms can be formed in response to ligation of death receptors (CD95, TRAIL-R, or TNFR) with cognate death ligands or genotoxic stress as well as TLR stimulation [61, 97–99]. When apoptotic cell death is blocked by a pancaspase inhibitor or viral proteins, leads to assembly of the necroptosis complex core RIP1/RIP3, followed by necroptotic cell death; however, the exact mechanism of RIPoptosome/necrosome formation and induction is still elusive.

The assembly of the apoptosome starts with the permeabilization of the mitochondrial outer membrane (MOMP), which is controlled by Bcl-2 family members [100, 101]. The Bcl-2 family includes three groups of proteins: pro-apoptotic BH3-only proteins, e.g. Bad, Bid and others, anti-apoptotic multidomain Bcl-2 proteins, such as Bcl-2, Bcl-w and Bcl-xL, and multidomain pro-apoptotic proteins, e.g. Bax, Bak. The last group of proteins undergoes oligomerization upon interaction with BH3-only proteins, leading to induction of intrinsic apoptosis pathway. The proteins of the first group can promote the conformational changes of pro-apoptotic Bcl-2 proteins and, following oligomerization of Bax and Bak, lead to MOMP [102, 103]. However, anti-apoptotic Bcl-2 proteins can prevent Bax-Bak oligomerization, and therefore, the interactions between Bcl-2 family members regulate mitochondrial membrane status and subsequently the apoptosome assembly.

Another way for the initiation of apoptosome formation is the mitochondrial permeability transition. The cell stresses (such as ROS or high concentration of Ca^{2+}) cause the opening of non-specific pores that are formed by at least three proteins: the voltage-dependent anion channel

(VDAC), the adenine nucleotide translocator (ANT), and cyclophilin D [104]. This process induces the loss of membrane potential, mitochondria swelling, and rupture of the membrane that leads to the release of apoptosome components and cell death.

Interestingly, apoptosome formation plays an important role in differentiation. A link between apoptosis and differentiation was suggested even in the seminal paper on apoptosis [105]. However, until recently, the mechanism that separated these two biological processes was unclear. Analysis of mouse embryonic stem cells undergoing differentiation into cardiomyocytes and their apoptosis revealed a significant time shift in apoptosome formation during these events, discriminating apoptosis from differentiation [106].

Recently a new role for apoptosome has been suggested [107]. The authors found that Tango7, an effector of cell death in Drosophila, collaborates with the active apoptosome compartment via its C terminus and directly stimulates the activity of this complex. Such kind of stimulation led to caspase-dependent cell remodeling and discriminated process of cell death from remodeling. In Drosophila this sequence of events was essential to resolve individual sperm from a syncytium.

The initial steps of PIDDosome formation have not been clarified as well as the association of DISC or the apoptosome. As has been noted above, the PIDDosome activation depends on the processing of PIDD, which might be regulated by ATM kinase upon genotoxic stress [85]. However, molecular mechanisms regulating PIDD processing and association of PIDDosome components are poorly understood and require further investigations.

Is it possible to specifically activate any of the platforms for therapeutic benefit?

During 40 years of research into various aspects of programmed cell death, including its signaling pathways, it has been shown that alterations or abnormal activation of PCD could lead to the development of a number of human diseases. For example, inefficient activation of PCD is associated with cancer, autoimmune disorders, and infection, while enhanced activation of many cell-death modalities could result in the development of neurological disorders [108, 109]. Therefore, regulation of PCD by affecting the platforms of caspase activation and their assembly could be one of the potential roads for treatment of a wide spectrum of human disorders.

The targeted activation of the multiprotein platforms requires special triggers that can promote formation of complexes. DR ligands (e.g., TRAIL, FasL) seem to be the most preferable and simple therapeutic strategy because they directly induce the assembly of the DISC and promote PCD through activation of the extrinsic pathway. Thus, TRAIL agonist molecules are being developed and tested as therapeutics in the treatment of human cancer [110, 111]. Importantly, although TRAIL was suggested to be an efficient and specific trigger of cancer cell death, accumulating evidence revealed that its killing capacity alone is limited. However, TRAIL inefficiently sensitizes even resistant tumor cells to treatment with various anticancer drugs. [112]. The application of mutant variants of TRAIL with superior selectivity (WO2009077857) or in combination with chemical therapeutic agents (WO2000048619) might improve the efficiency of the treatment and solve resistance problems [113, 114].

Approaches that trigger the apoptotic intrinsic pathway through apoptosome formation might also be used for successful therapeutic strategies. Agents promoting the process of MOMP and assembly of the apoptosome can target Bcl-2 family proteins and/or mitochondria [102]. These agents can downregulate the expression of Bcl-2 (antisense oligonucleotides) or activate pro-apoptotic factors (Bak-delivery vector and BH3-only mimetics) [101]. The BH3 mimetics have demonstrated a high potential for cancer therapy. In particular, a number of studies were devoted to the development of ABT-737 and ABT-263 that are two well-established inhibitors of Bcl-2, Bcl-XL and Bcl-W interactions, which in turn trigger pro-apoptotic effects. Clinical trials of ABT-263 in blood cell cancers or small cell lung cancers have demonstrated considerable action. Furthermore, recent preclinical trials of ABT-263 in combination with other drugs have shown increased efficacy. Thus, the further development of BH3 mimetics, in particular, in combinatory therapy is undoubtedly a promising strategy [101]. The agents activating pro-apoptotic factors are able to interact with mitochondrial membrane components (lipid bilayer, VDAC, ANT) and promote MOMP and the release of cytochrome *c*. Another promising opportunity for the development of the antitumor drugs includes targeting glycolytic pathways in tumor cells, that are characterized by high level of glycolysis known as “Warburg effect” [115]. At present, many agents that block glycolysis, such as inhibitors of hexokinase, are being tested in clinical trials.

The activity of the death platforms is also strictly controlled at the level of their post-translational modifications. Recently, an elegant way of the apoptosome control by ubiquitination has been described in breast cancer cells. It has been shown that the ubiquitin E3 ligases, MDM2 and HUWE1, inhibit the function of the apoptosome complex by either directly ubiquitinating the apoptosome activator CAS or indirectly via ubiquitinating anti-apoptotic Mcl-1 and PP5 (protein phosphatase 5) [116]. These findings specifically suggest the potential for therapy directed

against MDM2 to activate the apoptosome in breast cancer cells.

It was also found that rapid leukemia engraftment (short time to leukemia, TTL(short)) in the NOD/SCID/huALL (non-obese diabetic/severe combined immuno-deficiency/human acute lymphoblastic leukemia) xenograft model is indicative of early patient relapse [117]. Intact apoptosome function, as reflected by cytochrome *c*-related activation of caspase-3 (CRAC-positivity), was strongly associated with prolonged NOD/SCID engraftment (long time to leukemia, TTL(long)) of primary leukemia cells, good treatment response, and superior patient survival. Conversely, deficient apoptosome function (CRAC-negativity) was associated with rapid engraftment (TTL(short)) and early relapse. These data strongly emphasize the impact of correct apoptosome activation for ALL cells on the engraftment phenotype in the NOD/SCID/huALL model, and most importantly also on patient outcome.

Similarly, a systematic immunohistochemical analysis of the expression of the key proteins involved in apoptosome-dependent caspase activation (Apaf-1, procaspase-9 and -3, SMAC, and XIAP) in a cohort of stage II and III colorectal cancer patients from a phase III trial of adjuvant 5-fluorouracil-based chemotherapy vs. postoperative observation revealed the importance of the apoptosome activation pathway for predicting both prognosis and response to therapy [118].

The inhibition of necroptotic cell death can help to control the inflammatory response found in many chronic conditions. Preventing RIP3-mediated programmed necrosis by inhibition of RIP1 activity and complex II could be crucial in the treatment of inflammatory conditions such as Crohn's disease, irritable bowel syndrome, and chronic skin inflammatory diseases; thus, necrostatin-like drugs could be potential therapeutic agents for a range of inflammatory and degenerative disorders [45]. Interestingly, RIP1 was shown to maintain DNA integrity and cell proliferation by regulation of mitochondrial oxidative phosphorylation and glycolysis [119]. In lung cancer, loss of RIP1 led to suppression of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) resulting in accelerated glycolysis, spontaneous DNA damage, and p53-mediated inhibition of cell proliferation. It is very likely that maintenance of glycolysis by RIP1 is pivotal to cancer cell energy homeostasis and may be exploited for use in anticancer treatment.

However, there are still puzzles regarding the application of targeting other platforms, such as the PIDDosome. Many studies have demonstrated that a reduced level of caspase-2 is associated with childhood acute lymphoblastic leukemia and drug resistance of this disease, and gastric carcinoma [120–122]. Thus, the data characterizing the expression level of PIDDosome components may be applicable for cancer prediction, but the question about mechanisms of this platform activation and PCD induction is still open.

It is obvious that a complete understanding of how different platforms are regulated and how they can be activated in various modalities of PCD can give us not only fundamental information on PCD, but also may be a potential clue toward the development of therapeutic drugs.

Concluding remarks

Being involved in maintaining cellular homeostasis, cell death is a well-organized and regulated process. The mechanism of its activation/processing is controlled at several levels. Caspases are one of the main families of proteins involved in regulation of apoptotic cell death. Accordingly, many studies have been performed to examine the caspase activation mechanism. As a result of these studies, it has become clear that formation of high molecular weight complexes is an essential step for caspase activation. Although the structural/functional organization of two complexes, DISC and apoptosome, was well described, recent publications revealed the significance of the presence of different amounts of proteins and the chains of their interactions for proper function of DISC. Moreover, these original data permitted us to understand how at least the DISC complex is dynamically regulated. The recruitment of caspases to the DISC, apoptosome, and PIDDosome complexes results in a local increase in the concentration of caspases, which leads to their activation by proximity-induced oligomerization.

It is now clear that several distinct, yet intimately connected, signal transduction cascades are in place to trigger apoptotic cell death under some circumstances. Moreover, accumulated evidence indicates the existence of cross-talk between various cell-death modalities. Consequently, several regulators and executors interact not only with platforms initiating apoptotic cell death, but are also involved in formation of non-apoptotic high molecular weight complexes. This switch-like behavior might be regulated by, for example, proteolysis as exemplified by cleavage of PIDD. A list of PIDD-interacting proteins is growing, leading to identification of new PIDD-containing complexes, which may allow us to reveal yet unknown functions of this interesting protein. Although in this review we discussed complexes essential for activation of apoptotic caspases, it is necessary to mention the existence of caspases involved in regulation of inflammation, including caspase-1 and -5 [11]. Similar to the activation process of apoptotic caspases, activation of inflammatory caspases occurs through the formation of high molecular

weight complexes, called inflammasomes. Although the principle of organization and activation of inflammasomes is similar to the DISC, apoptosome, and PIDDosome, it is unclear whether the structures of these complexes are also similar.

Clearly, one of the biggest challenges is to understand what drives the same proteins to be a part of various complexes and how to influence these processes. The recently introduced protein–protein database for the death domain superfamily will be helpful to study the death domain superfamily-mediated formation of cell death-activating complexes. By analyzing the structural basis for the assembly mechanism of cell death-activating complexes, we hope to provide a comprehensive understanding of their function. The switch of death modes has differential effects on physiological outcomes according to the stimuli and tissue niche. Beyond being solely of academic interest, obtained data might allow us to therapeutically manipulate cell death in various pathological processes, including cancer and autoimmune diseases.

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