Cell Death: A One-Way Journey to the Graveyard

Vincenzo Giansanti and Anna Ivana Scovassi*

Istituto di Genetica Molecolare CNR, Via Abbiategrasso 207, 27100 Pavia, Italy

Abstract: Tissue homeostasis is ensured by the correct balance between cell proliferation and death, the latter mainly occurring through a multi-step program, named apoptosis, which ultimately leads to the breakdown of cellular DNA and proteins. Apoptosis is activated under physiological developmental conditions, during metamorphosis and atrophy of tissues and organs, sexual differentiation and cell turnover, and can also be triggered by various external stimuli, including DNA damage, growth factor deprivation and metabolic stress. The main features of apoptosis will be described in detail. Although apoptosis is recognised as the main type of programmed cell death, cells may die by alternative mechanisms, e.g. autophagy and necrosis. Their properties will be discussed in this review.

APOPTOSIS: A HISTORICAL OVERVIEW

Individual cells may die through a finely orchestrated sequence of biochemical and morphological changes which define the so-called Programmed Cell Death (PCD). This notion was first developed when Lockshin and Williams described the controlled cell death occurring during insect development [1-4], and further extended to different organisms [5-8]. After the discovery of PCD in insects, the best-studied animal model was the nematode *Caenorhabditis elegans*, which is characterised by the selective elimination of 131 out of 1090 cells during embryo development [9]. Genetic studies in this organism allowed the identification of the four essential gene products governing PCD: CED-3 (Cell Death Abnormal), a cystein-protease involved in protein degradation; CED-4, the upstream activator of CED-3; CED-9, able to bind CED-4, thus abolishing its death function; EGL-1, a CED-9-interacting protein that prevents CED-9/CED-4 interaction, thereby promoting PCD [9-11].

Apoptosis is the most well known form of PCD, defined for the first time by Kerr, Currie and Wyllie as an event reminiscent of the falling of leaves from trees [12]. The greek word *apoptosis* was used by Hippocrates to describe the removal of bone cells after a fracture and by Galen to define the falling of scabs [13], and was found in a Spanish dictionary of medicine written in 1878 [14].

DISTINCTIVE FEATURES OF APOPTOTIC CELLS

Morphological Changes

Apoptotic cells can be easily recognised by morphological hallmarks, such as nuclear shrinkage, chromatin condensation and aggregation, changes in the surface of plasma membrane, appearance of blebs and consequent formation of the so-called apoptotic bodies. Apoptosis can trigger cell death without rising of any inflammatory response or autoimmune reaction, because apoptotic cells display "eat-me signals", including phosphatidylserine [15], annexin I [16], a large variety of sugars, such as mannose and galactose [17], and ICAM-3 [18] (reviewed in [19]), which favour their recognition by phagocytes [20-22]. However, as recently reviewed [23], the non-immunogenic nature of apoptosis is presently debated, given that the apoptotic response elicited by anti-cancer therapies could exert an immunostimulatory side effect [24].

Degradation of Proteins

Apoptotic cells undergo controlled and organised DNA and protein digestion, carried out by specific endonucleases and proteolytic enzymes, respectively [25]. Among the proteases that promote the orderly destruction of structural and regulatory proteins [25], the best known are *caspases*. Caspases are cytosolic proteases so-named because of their mechanism of action: they contain a cystein residue in their catalytic site and cleave their substrates after an aspartic acid [26]. To date, 14 distinct mammalian caspases have been identified. Their primary structure consists of an N-terminal pro-domain, a large subunit, and a small subunit linked by a region flanked by two aspartate residues [27]. As illustrated in Fig. (**1**), mammalian caspases with a long pro-domain are named *initiator* caspases; those with a short pro-domain are called *effector* caspases [28-30]. Caspases are present within the cell as inactive zymogens and are converted into active enzymes only in response to apoptotic stimuli. The conversion of initiator caspase-8 [31] and -9 [32] into active proteases occurs through their dimerisation, while the transformation of effector pro-caspases to mature enzymes requires one cleavage to remove the N-terminal pro-domain and one to separate the large from the small subunit. These cleavages are followed by heterodimerisation of the subunits (Fig. **1**). Once activated, caspases can in turn activate other caspases. Many targets of caspases are known, some of them involved in the maintenance of cell structure, such as actin, fodrin, tubulin and lamin; others, with an enzymatic activity, control DNA metabolism [33]. A recent survey revealed that at least 400 substrates of caspases exist [34]. The cleavage of structural proteins impairs their function in cytoskeleton dynamics. Analogously, most enzymes, once proteolysed, are generally inactive. The nuclear protein PARP-1 (poly(ADP-ribose) polymerase-1), which is involved in the recognition of DNA

*Address correspondence to this author at the IGM-CNR, *Via* Abbiategrasso 207, 27100 Pavia, Italy; Tel: +39-0382-546334; Fax: +39-0382-422286; E-mail: scovassi@igm.cnr.it

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single and double strand breaks, is the most utilised apoptotic marker. Its proteolysis by caspases converts the 113 kDa protein into two inactive fragments of 89 and 24 kDa easily detectable by immunological assays [35,36]. Although caspases held the predominant role in proteolysis, there is growing evidence that other proteolytic enzymes are involved in the execution of apoptosis. A family of serine proteases proved to degrade proteins during apoptosis [37]. Among them, the HtrA2/Omi (high temperature requirement protein A) protease plays a crucial role in mitochondrial homeostasis and is released from mitochondria into the cytosol during apoptosis, thus being able to antagonize anti-apoptotic factors [38]. Cathepsins are proteases normally localised intralysosomally; after a specifical signal that targets lysosomes, cathepsins are released into the cytoplasm, where they trigger apoptosis [39,40]. Another class of proteolytic enzymes involved in apoptosis are calpains (i.e. calcium-dependent proteases), which are present in the cytosol as zymogens and activated when Ca^{2+} is released from the endoplasmic reticulum into the cytosol [41,42].

Degradation of DNA

During the last steps of apoptosis, DNA is firstly degraded into molecules ranging from 50 to 300 kb; then, the cleavage at internucleosomal DNA regions generates oligonucleosomal-sized fragments (180 bp and multiples), giving rise to a typical ladder when analysed by agarose gel electrophoresis [43]. Many DNase activities involved in apoptotic DNA degradation have been described, including Ca^{2+}/Mg^{2+}-dependent endonucleases, Mg^{2+}-depen-dent DNases, acid DNases, DFF (DNA fragmenting factor), CAD (caspase-activated DNase) and L-DNase II [24]. It has also been reported that the Apoptosis Inducing Factor (AIF) is involved in the formation of high molecular weight DNA fragments, even if AIF does not have an intrinsic endonuclease property but could induce DNA fragmentation through the interaction with other proteins [44,45]. The activation of AIF requires its translocation from the mitochondria to the nucleus; this event is promoted by poly(ADP-ribosylation) [46]. Other nucleases (e.g. DFF40/CAD) are activated through the proteolysis of their specific inhibitors by caspases, thus demonstrating the existence of an interplay between protein and DNA degradation [47,48]. A particular acid DNase, that is L-DNase II, is involved in caspase-independent apoptosis [48].

APOPTOTIC CELL DEATH PATHWAYS

Apoptosis is a genetically-regulated and energy-dependent process that can be induced by a large number of pro-apoptotic stimuli through two distinct pathways: the extrinsic, activated

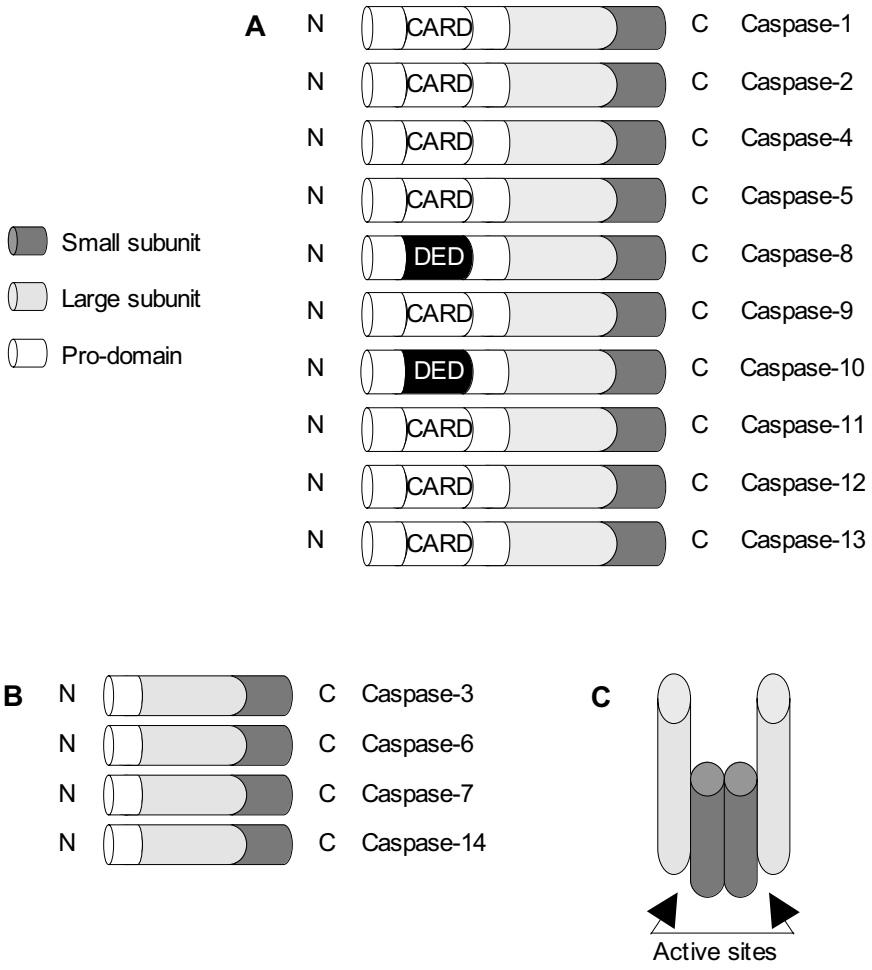

Fig. (1). Caspase family. (**A**) Initiator caspases show, from C- to N-terminal, a small subunit, a large subunit and a pro-domain containing either a CARD or a DED motif. (**B**) Effector caspases show a C-terminal small subunit, a large subunit and a short pro-domain. (**C**) Active caspase conformation.

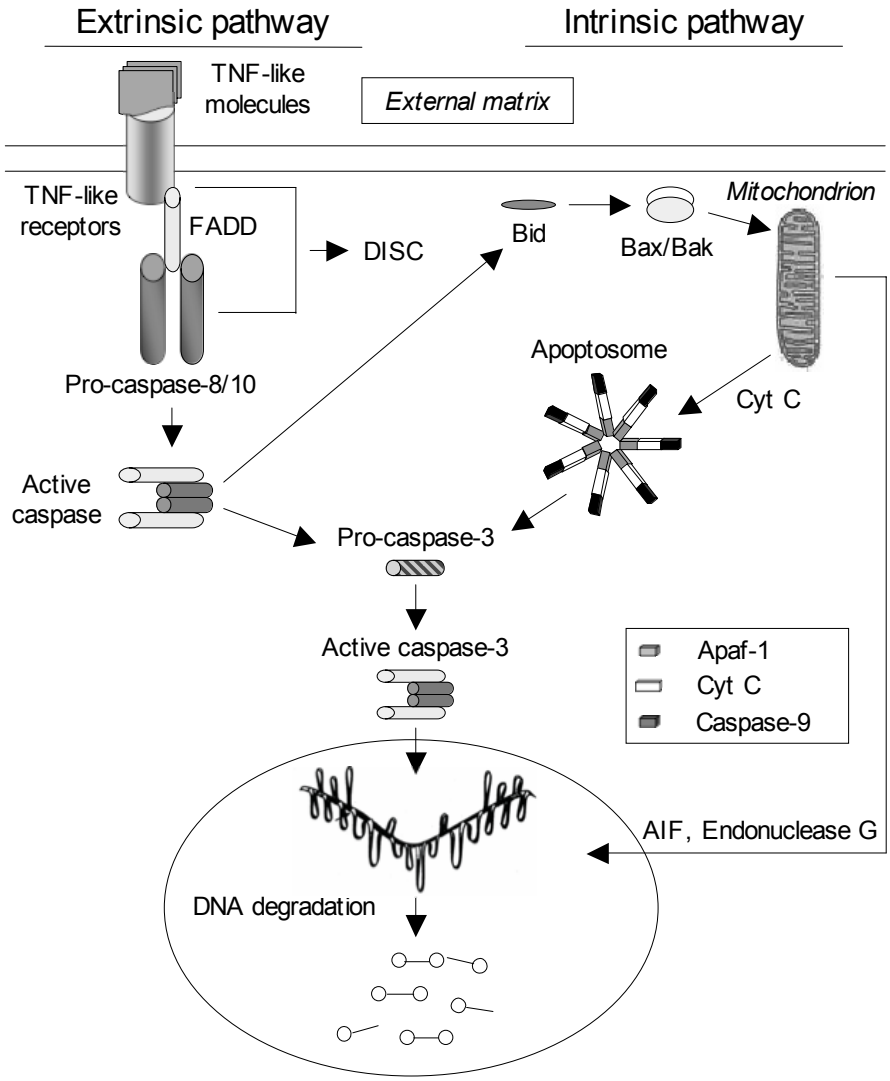

Fig. (2). Simplified view of the apoptotic pathways. The *extrinsic* pathway is triggered by TNF-like molecules that activate their receptors causing DISC formation and caspase-8/10 activation. The *intrinsic* pathway is induced by internal stimuli sensed by Bid, thus causing Bax/Bak activation, mitochondria permeabilisation, apoptosome formation and caspase-9 activation. Each pathway independently converges on caspase-3 that can promote DNA and cellular protein dismantling.

by soluble molecules that can bind to plasma membrane receptors, and the intrinsic, sensible to physical and chemical injuries. The main features of these pathways are schematised in Fig. (**2**).

The *extrinsic apoptotic pathway* is activated by soluble molecules belonging to the Tumor Necrosis Factor-α (TNF-α) superfamily, such as TNF-α, FasL (Fas Ligand, also known as CD95L), TRAIL (TNF-related apoptosis inducing ligand) and TNFSF10 (TNF ligand superfamily member 10). These molecules can bind to their respective receptors TNFR-1, Fas, TRAIL-R1,2 [49]. A common feature of death receptors is an 80 amino acids domain, named death domain (DD), in their cytoplasmic tail [50], which is crucial for the interaction with adaptor molecules that can transduce the death signal. Fas and TRAIL-R1,2 receptors can bind directly to the adaptor molecule FADD (Fas-associated molecule with death domain) [51,52], while the interaction between FADD and TNFR1 is mediated by TRADD (TNFR1 associated death domain protein) [53]. The interplay between death receptors and adaptor factors is regulated by homotypic interactions with the DD present in both classes of molecules [54].

FADD recruitment to death receptors is essential to transduce the death stimulus to the initiator caspase-8 or -10 to promote their activation through the formation of the supermolecular complex *DISC* (death-inducing signalling complex) [54], where FADD is essential, being able to interact with death receptors (thanks to its DD domain) and with initiator caspases through its *DED* domain (death effector domain). According to the first proposed "induced-proximity model" [55], once recruited to DISC, initiator caspases are activated through their FADD-induced dimerisation, thus being able to cleave each other promoting their complete activation. Based on this first model, two further models have been postulated, the "proximity-induced dimerisation" and the "induced conformation". According to the former model, caspase activation is achieved by dimerisation favoured by their close proximity promoted by FADD, whose role would

be to increase local concentration of inactive caspases [56], thus caspases would be activated only by dimerisation. The latter model implies that caspase binding to DISC allows conformational changes within the active site responsible for the activation [57]. After DISC formation, the initiator caspase-8 and -10 became active and can, in turn, activate initiator and effector caspases. Since apoptosis requires a tight regulation to be induced only when it is necessary, signal transduction mediated by DISC could be down-regulated or blocked by the so-called Decoy receptors, such as DcR1 and DcR2, also known as TRAIL-R3 and TRAIL-R4, respectively [58]. Both these inhibitors show high homology with the extracellular domain of death receptors, but they completely (DcR1) or partially (DcR2) lack the DD intracellular domain [58], thus being unable to transduce the apoptotic stimulus because they cannot trigger DISC formation.

The *intrinsic apoptotic pathway* is activated by a variety of chemical and physical injures generating stimuli that converge on mitochondria [59]. The key event leading to the activation of this pathway is the mitochondrial outer membrane permeabilisation (*MOMP*) due to the activity of two pro-apoptotic proteins belonging to the Bcl-2 family, i.e. Bax and Bak [60]. On the basis of their function in apoptosis regulation, Bcl-2 family members can be divided into two classes: anti- and pro-apoptotic proteins. Bcl-2, Bcl-X_L, Bcl-W, MCL1 and A1, all characterised by four BH domains, contrast apoptosis [61]. On the contrary, pro-apoptotic members include BH3-only proteins, such as Bad, Bik, Bid, Bim, Noxa and Puma, which show only one BH domain, and proteins (Bax, Bak and Box) that contain three BH domains (reviewed in [62]). To date, two models have been proposed to explain Bax/Bak complex activation. According to one of them, given that anti-apoptotic members normally bind to and inhibit the Bax/Bak complex, the physical binding of pro-apoptotic members other than Bax and Bak to the anti-apoptotic ones could release Bax/Bak complex and promote its pro-apoptotic function [63]. An alternative model postulates a direct activation of Bax/Bak through their interaction with BH3-only proteins [64]. Once activated, Bax and Bak can insert themselves into the outer mitochondrial membrane [65,66] and form a homo- or hetero-dimer, the so-called *MAC* (mitochondrial apoptosis-induced channel), a pore large enough to release different pro-apoptotic factors into the cytosol [67-71]. Moreover, Bax and Bak can indirectly trigger *MPTP* (mitochondrial permeability transition pore) opening thanks to the ability to release Ca^{2+} from the endoplasmic reticulum [72]. After its release, Ca^{2+} can induce MPTP opening followed by transmembrane potential ($\Delta\psi m$) dissi-pation [72]. As a consequence of mitochondria permeabili-sation, some factors are released into the cytosol, including cytocrome c, AIF, endonuclease G, Smac/DIABLO (second mitochondria-derived activator of caspase) and HtrA2/Omi [73]; this event triggers cell death either in a intrinsic cell death or independent manner.

Intrinsic cell death goes through the activation of the initiator caspase-9, which requires the assembly of an activation platform called apoptosome, where the base is represented by the cytosolic protein *Apaf-1* (apoptotic protein activating factor-1). This protein exists in different spliced isoforms [30]; all of them contain an N-terminal *CARD* (caspase recruitment domain), a central *CED-4 like* domain and a 13-14 repetition of a *WD40* domain which can bind cytocrome c [74]. In its inactive form, Apaf-1 is present in the cytosol bound to an ATP molecule in a conformation that inhibits its activation; cytocrome c binding induces a conformational change in Apaf-1 that activates its intrinsic ATPase function [75]. Once activated, Apaf-1 recruits other Apaf-1 molecules to assemble the apoptosome that has, in its three-dimensional structure, a wheel-shaped form with 7-fold symmetry. In this conformation, CARD and CED-4 homology domains constitute the hub of the wheel, which binds to caspase-9 monomers, while the seven spokes are made of WD40 domains [74]. Many steps are required to drive correct apoptosome assembly, including Apaf-1 proteolysis by caspases, possibly serving as a negative feedback to counteract unwanted apoptosis activation [76]. Other factors that can prevent apoptosome formation are K^+ [77] and Ca^{2+} [78]; these ions can inhibit apoptosome function in a cytoplasmic concen-tration-dependent manner. Furthermore, apoptosome assembly could be avoided because of the action of chaperones Hsp70 and Hsp90, which prevent caspase-9 binding to apoptosome and/or cytocrome c-mediated oligomerisation of Apaf-1 molecules [79-81].

Many factors block either the activation or the function of zymogens and activated caspases; such proteins are called *IAPs* (inhibitors of apoptotic proteins) [82]. Human genome encodes for 8 different IAP proteins: X-linked IAP (the best characterised IAP, also known as XIAP), $cIAP_1$, $cIAP_2$, ML-IAP, NAIP, ILP2, Apollon/Bruce and Survivin [83-85]. These proteins contain 1-3 tandem repeated copies of the so-called *BIR* domain (baculovirus IAP repeat); some of them show a C-terminal domain with an E3 ubiquitin ligase activity possibly involved in promoting caspase destruction by the proteasome [83,84].

IAPs are not the only proteins involved in caspase regulation. Indeed, various pro-apoptotic proteins interact with them to establish if apoptosis can go forward or needs to be blocked. Such factors are Smac/DIABLO and HtrA2/Omi, that can bind to [86] or cleave some IAPs [87,88], respectively. These two classes of apoptotic proteins interact to regulate apoptosis execution and their interplay is controlled by their different localisation during cell life. Indeed, in viable cells Smac/DIABLO and HtrA2/Omi are confined to mitochondria while IAPs are in the cytosol, thus caspases are blocked in an inactive state. When a death stimulus triggers mitochondrial permeabili-sation, the release of pro-apoptotic factors such as Smac/DIABLO and HtrA2/Omi counteracts the inhibitory action of IAPs, thus making apoptosis possible.

IS APOPTOSIS THE ONLY PCD?

In recent years, it has been found that many forms of cell death other than apoptosis exist [20,89-91]. Among them, anoikis, autophagy, and necrosis are below described.

Anoikis

In normal cells, loss of adhesion is counteracted by a death mechanism termed *anoikis*, which plays an essential role to avoid the undesired metastastic spread of cancer cells through their detachment from the primary tumor followed by their migration to the lymphatic and circulatory systems [92]. The features of this process are reminiscent of the apoptotic ones,

being characterised by the activation of the extrinsic and/or intrinsic pathways, and the consequent involvement of caspase-8 and/or -9 [92]. Anoikis is accompanied by cytoskeleton perturba-tions and alterations of the integrin complex [93]. The mediators responsible for anoikis have not been well characterised. Among the putative crucial factors, reactive oxygen species (ROS) produced through the involvement of the small GTPase Rac-1 upon integrin engagement have been reported to be able to transduce a pro-survival signal that ensures that cells escape from anoikis [94]. Given that anoikis impairment copes with the ability of cancer cells to survive in a non-adherent status, the understanding of the molecular bases of this form of cell death will greatly help in designing strategies able to bypass this crucial point.

Autophagy

Autophagy is a genetically controlled mechanism present in different species, from yeast to humans. In all organisms, this special pathway takes care of cellular components recycling [95] that can be required under extreme conditions such as hypoxia, nutrient starvation and oxidative stress [96]. Although autophagy is a basic process mainly involved in degradation and recycling of macromolecules and organelles, its extensive activation can lead to the so-called *type II cell death* (to distinguish it from apoptosis, also defined as *type I cell death*). However, due to our limited knowledge of this process, it is very difficult to define the boundary between *survival* and *lethal* autophagy. From a morphological point of view, autophagy is characterised by the appearance within the cytosol of large vacuole-like structures with a double-layered membrane. These structures then fuse each other to form the so called autophagosome that in turn fuses with lysosomes to allow the digestion of their content [97]. The possible impact of mitochondria disruption (the so-called *mitophagy*) on the dynamics of autophagosome formation has been recently discussed with respect to the role of ROS production [98,99]. From a molecular point of view, autophagy involves the activation of various genes. Even if a well-established pathway has not been so far described, some important factors involved in autophagy regulation have been discovered. Various regulators of this process belong to the *ATG* family (autophagy-related genes), originally discovered in yeast and conserved among different species [100]. In mammals, one autophagic marker is Beclin-1 protein, an orthologue of the Atg-6 yeast protein. This protein seems to be a key regulator of the autophagic death machinery and, interestingly, proved to be involved in the cross-talk with apoptosis, given that it could interact with Bcl-2 [101-103]. In fact, Maiuri *et al.* [104] recently reported a physical association of Beclin-1 with Bcl-2 and Bcl-X_L, whose inhibition allows the increase of cell number with cytosolic aggregation of the autophagic marker LC3 (a protein involved in the autophagosome maturation). In addition, Bad interferes with Bcl-2/Beclin-1 interaction by binding to Bcl-2, thus stimulating autophagy [104]. Recent studies demonstrate that autophagy is regulated also by p53. Although it is not clear which is the precise mechanism by which p53 can regulate this pathway, probably it acts as an autophagy suppressor [105]; however, these emerging data need to be supported by other evidences. Taken together, these results show that a connection between autophagy and apoptosis exists, although the exact mechanisms leading to their interaction remain to be elucidated.

Necrosis

Necrosis, the first type of cell death discovered, is often correlated with pathological conditions, like inflammation and virus infection. From a morphological point of view, necrotic cells are characterised by the loss of control of intracellular ion concentration, leading to cellular volume increase and swelling, mitochondria swelling and perinuclear organelle clustering [106]. As for the biochemical features, proteases and DNases seem to be activated without a well defined program [106]. Given that necrosis occurs without energy requirement, it was often described as a *passive* form of cell death, but emerging data support the idea that necrosis would be programmed [91,107,108]. For this reason, necrosis is now established as *type III PCD*, although a well-defined molecular pathway leading to necrosis is still lacking. However, encouraging data support the existence of some markers able to discriminate necrosis from apoptosis [109]. One paradigm of programmed necrosis implies that increased cytosolic Ca^{2+} concentration could allow calpain activation and a further enhanced Ca^{2+} concentration by cleavage of some transmembrane channels that regulate intracellular concentration of this ion, leading to osmotic imbalance and, finally, cell lysis [110]. Moreover, necrosis could involve PARP-1 over-activation in response to DNA damage. Prolonged activation of PARP-1, which forms ADP-ribose from NAD, would cause intracellular NAD depletion that, in turn, impairs ATP synthesis, therefore leading to energetic imbalance and cell death by necrosis [107,111,112]. Additional evidence that necrosis would be a programmed cell death come from different reports showing that the inactivation of both apoptosis and autophagy triggers necrosis [106,113,114]; however, further experiments would be performed to increase our knowledge on necrosis.

CONCLUSION

Given that tissue homeostasis is maintained through a balance between proliferation and death, it is obvious that Programmed Cell Death is "*as intrinsic for cells as mitosis*" [115], and plays a crucial role in governing physiological and pathological conditions. In fact, a deregulated cell death could contribute to the ethiology of a number of disorders, including neurodegenerative diseases, where massive death of neurons occurs [116], and cancer, which is promoted by a defective removal of cells [89]. The growing body of evidence supporting the existence (and intercorrelation) of various forms of PCD renders this field extremely complex. Among the various forms of PCD, the interest towards apoptosis is still growing, due to the discovery that it plays an active role in cancer development. Indeed, tumor development is often characterised by an impaired apoptosis [74]; this behaviour stimulated the search for drugs able to antagonize anti-apoptotic factors and to restore the apoptotic potential of cancer cells [117,118].

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REFERENCES

[1] Lockshin RA, Williams CM. Programmed Cell Death-I. Cytology of degeneration in the intersegmental muscles of the Pernyi Silkmoth. J Insect Physiol 1965; 11: 123-33.

[2] Lockshin RA, William CM. Programmed Cell Death. 3. Neural control of the breakdown of the intersegmental muscles of Silkmoths. J Insect Physiol 1965; 11: 601-10.

[3] Lockshin RA, Williams CM. Programmed Cell Death. IV. The influence of drugs on the breakdown of the intersegmental muscles of silkmoths. J Insect Physiol 1965; 11: 803-9.

[4] Lockshin RA, Williams CM. Programmed Cell Death. V. Cytolytic enzymes in relation to the breakdown of the intersegmental muscles of silkmoths. J Insect Physiol 1965; 11: 831-44.

[5] Jacobson MD, Weil M, Raff MC. Programmed cell death in animal development. Cell 1997; 88: 347-54.

[6] Lockshin RA, Zakeri Z. Programmed cell death and apoptosis: origins of the theory. Nat Rev Mol Cell Biol 2001; 2: 545-50.

[7] Vaux DL, Korsmeyer SJ. Cell death in development. Cell 1999; 96: 245-54.

[8] Zakeri Z, Lockshin RA. Cell death during development. J Immunol Methods 2002; 265: 3-20.

[9] Ellis RE, Yuan JY, Horvitz HR. Mechanisms and functions of cell death. Annu Rev Cell Biol 1991; 7: 663-98.

[10] Hengartner MO, Horvitz HR. Programmed cell death in Caenorhabditis elegans. Curr Opin Genet Dev 1994; 4: 581-6.

[11] Metzstein MM, Stanfield GM, Horvitz HR. Genetics of programmed cell death in C. elegans: past, present and future. Trends Genet 1998; 14: 410-6.

[12] Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972; 26: 239-57.

[13] Scovassi AI. In: Torriglia A, Crisanti-Lassiaz P, Eds. Apoptosis in the retina. Kerala, India, Transworld Research Network 2006; 1-11.

[14] Fernandez-Flores A, Aguilera B, Yau P, Oliva H. An old meaning of the word apoptosis. Lancet 2002; 359: 1072.

[15] Gardai SJ, Bratton DL, Ogden CA, Henson PM. Recognition ligands on apoptotic cells: a perspective. J Leukoc Biol 2006; 79: 896-903.

[16] Arur S, Uche UE, Rezaul K, *et al.* Annexin I is an endogenous ligand that mediates apoptotic cell engulfment. Dev Cell 2003; 4: 587-98.

[17] Dini L. In: Scovassi AI, Eds. Apoptosis. Kerala, India, Research Signpost 2005; 107-29.

[18] Moffatt OD, Devitt A, Bell ED, *et al.* Macrophage recognition of ICAM-3 on apoptotic leukocytes. J Immunol 1999; 162: 6800-10.

[19] Erwig LP, Henson PM. Clearance of apoptotic cells by phagocytes. Cell Death Differ 2008; 15: 243-50.

[20] Fink SL, Cookson BT. Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. Infect Immun 2005; 73: 1907-16.

[21] Savill J, Fadok V. Corpse clearance defines the meaning of cell death. Nature 2000; 407: 784-8.

[22] Manfredi AA, Iannacone M, D'Auria F, Rovere-Querini P. The disposal of dying cells in living tissues. Apoptosis 2002; 7: 153-61.

[23] Tesniere A, Apetoh L, Ghiringhelli F, *et al.* Immunogenic cancer cell death: a key-lock paradigm. Curr Opin Immunol 2008; DOI 10.1016/ j.coi.2008.05.007.

[24] Zitvogel L, Apetoh L, Ghiringhelli F, *et al.* The anticancer immune response: indispensable for therapeutic success? J Clin Invest 2008; 118: 1991-2001.

[25] Scovassi AI, Torriglia A. Activation of DNA-degrading enzymes during apoptosis. Eur J Histochem 2003; 47: 185-94.

[26] Kumar S. Caspase function in programmed cell death. Cell Death Differ 2007; 14: 32-43.

[27] Nicholson DW, Thornberry NA. Caspases: killer proteases. Trends Biochem Sci 1997; 22: 299-306.

[28] Degterev A, Boyce M, Yuan J. A decade of caspases. Oncogene 2003; 22: 8543-67.

[29] Fuentes-Prior P, Salvesen GS. The protein structures that shape caspase activity, specificity, activation and inhibition. Biochem J 2004; 384: 201-32.

[30] Bao Q, Shi Y. Apoptosome: a platform for the activation of initiator caspases. Cell Death Differ 2007; 14: 56-65.

[31] Pop C, Fitzgerald P, Green DR, Salvesen GS. Role of proteolysis in caspase-8 activation and stabilization. Biochemistry 2007; 46: 4398-407.

[32] Pop C, Timmer J, Sperandio S, Salvesen GS. The apoptosome activates caspase-9 by dimerisation. Mol Cell 2006; 22: 269-75.

[33] Scovassi AI. In: Scovassi AI, Eds. Apoptosis. Kerala, India, Research Signpost 2005; 41-55.

[34] Luthi AU, Martin SJ. The CASBAH: a searchable database of caspase substrates. Cell Death Differ 2007; 14: 641-50.

[35] Soldani C, Scovassi AI. Poly(ADP-ribose) polymerase-1 cleavage during apoptosis: an update. Apoptosis 2002; 7: 321-8.

[36] Scovassi AI, Diederich M. Modulation of poly(ADP-ribosylation) in apoptotic cells. Biochem Pharmacol 2004; 68: 1041-7.

[37] O'Connell AR, Stenson-Cox C. A more serine way to die: defining the characteristics of serine protease-mediated cell death cascades. Biochim Biophys Acta 2007; 1773: 1491-9.

[38] Vande Walle L, Lamkanfi M, Vandenabeele P. The mitochondrial serine protease HtrA2/Omi: an overview. Cell Death Differ 2008; 15: 453-60.

[39] Chwieralski CE, Welte T, Buhling F. Cathepsin-regulated apoptosis. Apoptosis 2006; 11: 143-9.

[40] Stoka V, Turk V, Turk B. Lysosomal cysteine cathepsins: signaling pathways in apoptosis. Biol Chem 2007; 388: 555-60.

[41] Breckenridge DG, Germain M, Mathai JP, *et al.* Regulation of apoptosis by endoplasmic reticulum pathways. Oncogene 2003; 22: 8608-18.

[42] Friedrich P. The intriguing Ca^{2+} requirement of calpain activation. Biochem Biophys Res Commun 2004; 323: 1131-3.

[43] Wyllie AH. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 1980; 284: 555-6.

[44] Boujrad H, Gubkina O, Robert N, *et al.* AIF-mediated programmed necrosis: a highly regulated way to die. Cell Cycle 2007; 6: 2612-9.

[45] Lorenzo HK, Susin SA, Penninger J, Kroemer G. Apoptosis inducing factor (AIF): a phylogenetically old, caspase-independent effector of cell death. Cell Death Differ 1999; 6: 516-24.

[46] Hong SJ, Dawson TM, Dawson VL. Nuclear and mitochondrial conversations in cell death: PARP-1 and AIF signaling. Trends Pharmacol Sci 2004; 25: 259-64.

[47] Torriglia A, Padron L. In: Scovassi AI, Eds. Apoptosis. Kerala, India, Res Signpost 2005; 27-40.

[48] Counis MF, Torriglia A. Acid DNases and their interest among apoptotic endonucleases. Biochimie 2006; 88: 1851-8.

[49] Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. Science 1998; 281: 1305-8.

[50] Fulda S, Debatin KM. Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. Oncogene 2006; 25: 4798-811.

[51] Boldin MP, Varfolomeev EE, Pancer Z, *et al.* A novel protein that interacts with the death domain of Fas/APO1 contains a sequence motif related to the death domain. J Biol Chem 1995; 270: 7795-8.

[52] Chinnaiyan AM, O'Rourke K, Tewari M, Dixit VM. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. Cell 1995; 81: 505-12.

[53] Schwandner R, Wiegmann K, Bernardo K, *et al.* TNF receptor death domain-associated proteins TRADD and FADD signal activation of acid sphingomyelinase. J Biol Chem 1998; 273: 5916-22.

[54] Muzio M. Signalling by proteolysis: death receptors induce apoptosis. Int J Clin Lab Res 1998; 28: 141-7.

[55] Salvesen GS, Dixit VM. Caspase activation: the induced-proximity model. Proc Natl Acad Sci USA 1999; 96: 10964-7.

[56] Shi Y. Caspase activation: revisiting the induced proximity model. Cell 2004; 117: 855-8.

[57] Chao Y, Shiozaki EN, Srinivasula SM, *et al.* Engineering a dimeric caspase-9: a re-evaluation of the induced proximity model for caspase activation. PLoS Biol 2005; 3: e183.

[58] Falschlehner C, Emmerich CH, Gerlach B, Walczak H. TRAIL signalling: decisions between life and death. Int J Biochem Cell Biol 2007; 39: 1462-75.

[59] Khosravi-Far R, Esposti MD. Death receptor signals to mitochondria. Cancer Biol Ther 2004; 3: 1051-7.

[60] Green DR. Apoptotic pathways: ten minutes to dead. Cell 2005; 121: 671-4.

[61] Reed JC. Bcl-2-family proteins and hematologic malignancies: history and future prospects. Blood 2008; 111: 3322-30.

[62] Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. Nat Rev Mol Cell Biol 2008; 9: 47-59.

[63] Willis SN, Fletcher JI, Kaufmann T, *et al.* Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. Science 2007; 315: 856-9.

[64] Youle RJ. Cell biology. Cellular demolition and the rules of engagement. Science 2007; 315: 776-7.

[65] Packham G, Stevenson FK. Bodyguards and assassins: Bcl-2 family proteins and apoptosis control in chronic lymphocytic leukaemia. Immunology 2005; 114: 441-9.

[66] Newmeyer DD, Ferguson-Miller S. Mitochondria: releasing power for life and unleashing the machineries of death. Cell 2003; 112: 481-90.

[67] Kinnally KW, Antonsson B. A tale of two mitochondrial channels, MAC and PTP, in apoptosis. Apoptosis 2007; 12: 857-68.

[68] Bernardi P, Krauskopf A, Basso E, *et al.* The mitochondrial permeability transition from in vitro artifact to disease target. FEBS J 2006; 273: 2077-99.

[69] Forte M, Bernardi P. The permeability transition and BCL-2 family proteins in apoptosis: co-conspirators or independent agents? Cell Death Differ 2006; 13: 1287-90.

[70] Kroemer G, Reed JC. Mitochondrial control of cell death. Nat Med 2000; 6: 513-9.

[71] Skulachev VP. Why are mitochondria involved in apoptosis? Permeability transition pores and apoptosis as selective mechanisms to eliminate superoxide-producing mitochondria and cell. FEBS Lett 1996; 397: 7-10.

[72] Pizzo P, Pozzan T. Mitochondria-endoplasmic reticulum choreography: structure and signaling dynamics. Trends Cell Biol 2007; 17: 511-7.

[73] Armstrong JS. The role of the mitochondrial permeability transition in cell death. Mitochondrion 2006; 6: 225-34.

[74] Fadeel B, Ottosson A, Pervaiz S. Big wheel keeps on turning: apoptosome regulation and its role in chemoresistance. Cell Death Differ 2008; 15: 443-52.

[75] Riedl SJ, Li W, Chao Y, *et al.* Structure of the apoptotic protease-activating factor 1 bound to ADP. Nature 2005; 434: 926-33.

[76] Lauber K, Appel HA, Schlosser SF, *et al.* The adapter protein apoptotic protease-activating factor-1 (Apaf-1) is proteolytically processed during apoptosis. J Biol Chem 2001; 276: 29772-81.

[77] Cain K, Langlais C, Sun XM, *et al.* Physiological concentrations of K+ inhibit cytochrome c-dependent formation of the apoptosome. J Biol Chem 2001; 276: 41985-90.

[78] Bao Q, Lu W, Rabinowitz JD, Shi Y. Calcium blocks formation of apoptosome by preventing nucleotide exchange in Apaf-1. Mol Cell 2007; 25: 181-92.

[79] Beere HM, Wolf BB, Cain K, *et al.* Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. Nat Cell Biol 2000; 2: 469-75.

[80] Saleh A, Srinivasula SM, Balkir L, *et al.* Negative regulation of the Apaf-1 apoptosome by Hsp70. Nat Cell Biol 2000; 2: 476-83.

[81] Pandey P, Saleh A, Nakazawa A, *et al.* Negative regulation of cytochrome c-mediated oligomerization of Apaf-1 and activation of procaspase-9 by heat shock protein 90. EMBO J 2000; 19: 4310-22.

[82] Vaux DL, Silke J. IAPs, RINGs and ubiquitylation. Nat Rev Mol Cell Biol 2005; 6: 287-97.

[83] Hunter AM, LaCasse EC, Korneluk RG. The inhibitors of apoptosis (IAPs) as cancer targets. Apoptosis 2007; 12: 1543-68.

[84] Pennati M, Folini M, Zaffaroni N. Targeting survivin in cancer therapy: fulfilled promises and open questions. Carcinogenesis 2007; 28: 1133-9.

[85] Srinivasula SM, Ashwell JD. IAPs: what's in a name? Mol Cell 2008; 30: 123-35.

[86] Chai J, Du C, Wu JW, *et al.* Structural and biochemical basis of apoptotic activation by Smac/DIABLO. Nature 2000; 406: 855-62.

[87] Jin S, Kalkum M, Overholtzer M, *et al.* CIAP1 and the serine protease HTRA2 are involved in a novel p53-dependent apoptosis pathway in mammals. Genes Dev 2003; 17: 359-67.

[88] Suzuki Y, Imai Y, Nakayama H, *et al.* A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death. Mol Cell 2001; 8: 613-21.

[89] Jäättela M. Multiple cell death pathways as regulators of tumour initiation and progression. Oncogene 2004; 23: 2746-56.

[90] Lockshin RA, Zakeri Z. Apoptosis, autophagy, and more. Int J Biochem Cell Biol 2004; 36: 2405-19.

[91] Degterev A, Yuan J. Expansion and evolution of cell death programmes. Nat Rev Mol Cell Biol 2008; 9: 378-90.

[92] Simpson CD, Anyiwe K, Schimmer AD. Anoikis resistance and tumor metastasis. Cancer Lett 2008; doi:10.1016/j.canlet.2008.05.029.

[93] Frisch SM, Screaton RA. Anoikis mechanisms. Curr Opin Cell Biol 2001; 13: 555-62.

[94] Giannoni E, Buricchi F, Grimaldi G, *et al.* Redox regulation of anoikis: reactive oxygen species as essential mediators of cell survival. Cell Death Differ 2008; 15: 867-78.

[95] Levine B, Klionsky DJ. Development by self-digestion: molecular mechanisms and biological functions of autophagy. Dev Cell 2004; 6: 463-77.

[96] Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell 2008; 132: 27-42.

[97] Kelekar A. Autophagy. Ann NY Acad Sci 2005; 1066: 259-71.

[98] Kim I, Rodriguez-Enriquez S, Lemasters JJ. Selective degradation of mitochondria by mitophagy. Arch Biochem Biophys 2007; 462: 245-53.

[99] Scherz-Shouval R, Elazar Z. ROS, mitochondria and the regulation of autophagy. Trends Cell Biol 2007; 17: 422-7.

[100] Klionsky DJ. Autophagy: from phenomenology to molecular understanding in less than a decade. Nat Rev Mol Cell Biol 2007; 8: 931-7.

[101] Liang XH, Jackson S, Seaman M, *et al.* Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 1999; 402: 672-6.

[102] Pattingre S, Tassa A, Qu X, *et al.* Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell 2005; 122: 927-39.

[103] Takacs-Vellai K, Vellai T, Puoti A, *et al.* Inactivation of the autophagy gene bec-1 triggers apoptotic cell death in *C. elegans*. Curr Biol 2005; 15: 1513-7.

[104] Maiuri MC, Le Toumelin G, Criollo A, *et al.* Functional and physical interaction between Bcl-X(L) and a BH3-like domain in Beclin-1. EMBO J 2007; 26: 2527-39.

[105] Tasdemir E, Maiuri MC, Galluzzi L, *et al.* Regulation of autophagy by cytoplasmic p53. Nat Cell Biol 2008; 10: 676-87.

[106] Golstein P, Kroemer G. Cell death by necrosis: towards a molecular definition. Trends Biochem Sci 2007; 32: 37-43.

[107] Chiarugi A. In: Scovassi A, Eds. Apoptosis. Kerala, India, Res Signpost 2005; 13-26.

[108] Blank M, Shiloh Y. Programs for cell death: apoptosis is only one way to go. Cell Cycle 2007; 6: 686-95.

[109] Krysko DV, Vanden Berghe T, D'Herde K, Vandenabeele P. Apoptosis and necrosis: detection, discrimination and phagocytosis. Methods 2008; 44: 205-21.

[110] Criddle DN, Gerasimenko JV, Baumgartner HK, *et al.* Calcium signalling and pancreatic cell death: apoptosis or necrosis? Cell Death Differ 2007; 14: 1285-94.

[111] Nicotera P, Leist M, Manzo L. Neuronal cell death: a demise with different shapes. Trends Pharmacol Sci 1999; 20: 46-51.

[112] Berger NA. Poly(ADP-ribose) in the cellular response to DNA damage. Radiat Res 1985; 101: 4-15.

[113] Shimizu S, Kanaseki T, Mizushima N, *et al.* Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. Nat Cell Biol 2004; 6: 1221-8.

[114] Degenhardt K, Mathew R, Beaudoin B, *et al.* Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell 2006; 10: 51-64.

[115] Bongaerts GP. What of apoptosis is important: the decay process or the causative origin? Med Hypotheses 2008; 70: 482-7.

[116] Krantic S, Mechawar N, Reix S, Quirion R. Molecular basis of programmed cell death involved in neurodegeneration. Trends Neurosci 2005; 28: 670-6.

[117] Letai AG. Diagnosing and exploiting cancer's addiction to blocks in apoptosis. Nat Rev Cancer 2008; 8: 121-32.

[118] Giansanti V, Scovassi A. In: Mondello C, Eds. Multiple pathways in cancer development. Kerala, India, Transworld Research Network 2008; in press.

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