Cell Death and Differentiation (2008), 1–11

www.nature.com/cdd

Review

No death without life: vital functions of apoptotic effectors

L Galluzzi[1,2,3], N Joza[1,2,3], E Tasdemir[1,2,3], MC Maiuri[1,2,3,4], M Hengartner[5], JM Abrams[6], N Tavernarakis[7], J Penninger[8], F Madeo[9] and G Kroemer*[,1,2,3]

As a result of the genetic experiments performed in *Caenorhabditis elegans*, it has been tacitly assumed that the core proteins of the 'apoptotic machinery' (CED-3, -4, -9 and EGL-1) would be solely involved in cell death regulation/execution and would not exert any functions outside of the cell death realm. However, multiple studies indicate that the mammalian orthologs of these *C. elegans* proteins (i.e. caspases, Apaf-1 and multidomain proteins of the Bcl-2 family) participate in cell death-unrelated processes. Similarly, loss-of-function mutations of *ced-4* compromise the mitotic arrest of DNA-damaged germline cells from adult nematodes, even in a context in which the apoptotic machinery is inoperative (for instance due to mutations of *egl-1* or *ced-3*). Moreover, EGL-1 is required for the activation of autophagy in starved nematodes. Finally, the depletion of caspase-independent death effectors, such as apoptosis-inducing factor (AIF) and endonuclease G, provokes cell death-independent consequences, both in mammals and in yeast (*Saccharomyces cerevisiae*). These results corroborate the conjecture that any kind of protein that has previously been specifically implicated in apoptosis might have a phylogenetically conserved apoptosis-unrelated function, most likely as part of an adaptive response to cellular stress.
Cell Death and Differentiation advance online publication, 29 February 2008; doi:10.1038/sj.cdd.cdd200828

'Programmed cell death' (PCD), an expression that was admirably coined by Richard Lockshin in the 1960s,[1] is often resolved by apoptosis, a modality of cell death that is defined by characteristic biochemical and morphological features such as chromatin condensation (pyknosis) and nuclear fragmentation (karyorrhexis).[2–5] Perhaps, owing to our bilateral symmetry or our intrinsic intellectual limitations, we tend to categorize elements in dualistic terms. According to this one-dimensional and simplistic logic, cell biologists, biochemists and geneticists have assumed that the processes ensuring cell survival and those involved in cell death would rather be diametrically opposed than overlapping.

In apparent accord with this conjecture, genetic screens in *Caenorhabditis elegans* revealed the existence of genes that are required for developmental cell death of the nematode (and hence named *ced* genes, for '*C. elegans* death'), yet had no discernible function in its ordinary life.[6] The concept was born that the apoptotic machinery of *C. elegans*, as built up by CED-3 (a caspase), CED-4 (an adaptor molecule), CED-9 (a Bcl-2/Bcl-X_L ortholog) and EGL-1 (a Bcl-2 homology domain (BH3)-only protein), would solely exist for the regulation and execution of cell death, and would have no important roles in normal life. This concept was tacitly transferred to the mammalian system when effector caspases (i.e. a group of cysteine-aspartyl-proteases that account for the execution of cell death) were presumed to take part only in cell death and not to be involved in any death-unrelated process.[7] Similarly, for a long time, it has been assumed that proapoptotic proteins from the Bcl-2 family as well as apoptosis protease-activating factor 1 (Apaf-1) (the

[1]INSERM, U848, Villejuif, France; [2]Institut Gustave Roussy, Villejuif, France; [3]University Paris-Sud, Paris 11, Villejuif, France; [4]Department of Experimental Pharmacology, School of Biotechnological Sciences, University of Naples Federico II, Naples, Italy; [5]Institute of Molecular Biology, University of Zurich, Zurich, Switzerland; [6]Department of Cell Biology, UT Southwestern Medical Center, Dallas, USA; [7]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Heraklion Crete, Greece; [8]Institute of Molecular Biotechnology of the Austrian Academy of Science, Vienna, Austria and [9]Center of Molecular Biology, University of Graz, Graz, Austria

*Corresponding author: G Kroemer, U848, INSERM, Institut Gustave Roussy, Pavillon de Recherche 1, 39 rue Camille Desmoulins, Villejuif FR-94805, France. Tel: +33 1 4211 6046; Fax: +33 1 4211 6047; E-mail: kroemer@igr.fr

Keywords: Apaf-1; apoptosis; autophagy; Bcl-2; BH3-only proteins; caspases

Abbreviations: AIF, apoptosis-inducing factor; Aip1, actin-interacting protein 1; ANT, adenine nucleotide translocator; Apaf-1, apoptosis protease-activating factor 1; BH3, Bcl-2 homology domain 3; CARD, caspase-recruitment domain; *ced, Caenorhabditis elegans* death; CK, creatine kinase; CRADD, caspase-2 and RIPK1 domain containing adaptor with death domain; Csp12-L, full-length caspase-12; Csp12-S, truncated caspase-12; CypD, cyclophilin D; Cyt *c*, cytochrome *c*; DIABLO, direct IAP-binding protein with a low pI; DISC, death-induced signaling complex; Drp1, dynamin-like protein 1; EndoG, endonuclease G; ER, endoplasmic reticulum; FADD, Fas-associating death domain-containing protein; HK, hexokinase; HtrA2, high temperature requirement protei n A 2; IAP, inhibitor of apoptosis protein; IM, mitochondrial inner membrane; IMS, mitochondrial intermembrane space; IP_3R, inositol-1,4,5-trisphosphate receptor; MLS, mitochondrial localization signal; MMP, mitochondrial membrane permeabilization; *mnd2*, motor neuron degeneration 2; OM, mitochondrial outer membrane; Omi, Omi stress-regulated endoprotease; PARP-1, poly (ADP-ribose) polymerase 1; PBR, peripheral-type benzodiazepine receptor; PCD, programmed cell death; PIDD, p53-induced protein with a death domain; PTPC, permeability transition pore complex; RAIDD, RIP-associated ICH-1/CED-3 homologous protein with a death; RIP1, receptor-interacting protein 1; SERCA, sarco/endoplasmic reticulum Ca^{2+} ATPase; Smac, second mitochondria-derived activator of caspase; TNFR, tumor necrosis factor receptor; TRADD, TNFR-associated death domain protein; UV, ultraviolet; VDAC, voltage-dependent anion channel

Received 08.1.08; revised 01.2.08; accepted 04.2.08; Edited by G Melino

mammalian equivalent of CED-4) would play a part only in self-destructive phenomena.

During recent years, however, a paradigm shift has occurred as it becomes clear that proteins involved in the induction of apoptosis or in the apoptotic dismantling of cells also exhibit cell death-unrelated functions.[8] Indeed, it seems plausible that evolution has not 'invented' proapoptotic factors *ex nihilo* but rather has 'appropriated' molecules that already had a function in vital processes (such as adaptation to stress) into the service of PCD. This is the topic of the present review article.

Vital Functions of Mammalian Caspases

The first mammalian caspase to be cloned was caspase-1, which was initially named interleukin-1β-converting enzyme and hence constituted the first example of a 'proinflammatory caspase'.[9] Such enzymes (also known as group I caspases) participate in the maturation of cytokines and include caspase-1, -4 and -5 in humans, as well as caspase-1, -11 and -12 in mouse.[10] For a long while, all other caspases have been classified either as 'initiator' or 'executioner' caspases,[11] and it was initially thought that they would only contribute to cell death,[12] a concept that nowadays is amply invalidated (Table 1).

Caspase-2 is the only caspase found in the nucleus and can be directly activated by DNA damage.[13] Some data suggest that caspase-2 is involved in DNA repair. Thus, *caspase-2*$^{-/-}$ mice age prematurely, a phenotype that is often associated with deficient DNA repair.[14] Caspase-3 activation has also been implicated in the differentiation of multiple cell types. For instance, caspase-3 can participate in osteoblastic differentiation, and caspase-3-deficient mice exhibit delayed ossification and decreased bone mineral density.[15]

The involvement of caspases in differentiation was initially thought to be restricted to pathways involving partial self-destructive processes, in which entire organelles are eliminated. During terminal differentiation, some cell types, including erythroblasts, keratinocytes and lens epithelial cells, lose their nuclei and cytoplasmic organelles.[16,17] For example, erythropoiesis involves the sequential formation in the bone marrow of a series of red blood cell precursors (proerythroblasts, basophilic, polychromatophilic and orthochromatic erythroblasts), which extrude their nuclei and enter the circulation as mature, anucleate erythrocytes.[18] Caspase inhibitors arrest the maturation of erythroid progenitors at early stages of differentiation, well before nuclear shrinkage and chromatin condensation occur.[19] Effector caspases such as caspase-3 are transiently activated through the mitochondrial pathway during erythroblast differentiation.[19] In this context, caspase-3 cleaves proteins involved in nuclear envelope integrity (e.g. lamin B) and chromatin condensation (e.g. acinus), but spares other potential substrates (such as the major erythroid transcription factor GATA-1), presumably

Table 1 Apoptosis-regulatory and vital functions of caspases

Caspase	Knockout phenotype	Role in apoptosis	Other roles
Caspase-1	Mice develop normally	Role in 'pyroptosis', i.e. cell death initiated by activation of the inflammasome	Proteolytic maturation of interleukins, in particular IL-1β and IL-18
Caspase-2	Mice have excess oocytes Premature aging	Initiator/executioner caspase Initiator of apoptosis after DNA damage and during mitotic catastrophe	DNA repair
Caspase-3	Perinatal lethality Brain hyperplasia	Executioner caspase	B cell proliferation Differentiation of erythroblasts, monocytes, keratinocytes, and epithelial, sperm, skeletal, muscle, osteoblast and trophoblast cells Platelet maturation
Caspase-6	Mice develop normally	Executioner caspase	Unknown
Caspase-7	Perinatal lethality	Executioner caspase	Unknown
Caspase-8	Embryonic lethality Impaired heart development and decreased pool of hematopoietic precursors In humans, familial mutation leads to immunodeficiency	Initiator caspase of the death receptor (extrinsic) apoptotic pathway Proteolytically activates downstream caspases and Bid	Cell cycle control Macrophagic differentiation NF-κB-mediated pro-survival pathways Placental trophoblast differentiation T-cell proliferation
Caspase-9	Perinatal lethality Excess brain tissue	Initiator caspase of the mitochondrial (intrinsic) apoptotic pathway	In most differentiation processes in which caspase-3 has been implicated
Caspase-10	In humans, a familial mutation is linked with type II autoimmune lymphoproliferative syndrome	Putative initiator caspase of the extrinsic pathway Proteolytically activates downstream caspases and Bid	Implicated in the activation of NF-κB-mediated pro-survival pathways
Caspase-11	Mice develop normally Lymphocytes have a defect in actin depolymerization	Involved in neuronal cell death induced by MPTP and other pathological stimuli	IL-1 production Interacts with Aip1 and promotes cofilin-mediated actin depolymerization
Caspase-12	Mice develop normally	Initiator caspase in ER stress-dependent apoptosis	Attenuates inflammation Innate immune response
Caspase-14	Mice skin exhibit reduced hydration levels and enhanced sensitivity to UVB-induced apoptosis	Unknown	Terminal differentiation of keratinocytes

Abbreviations: Aip1, actin interacting protein 1; ER, endoplasmic reticulum; IL, interleukin; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; UV, ultraviolet.

because these proteins are protected by the interaction with HSP70.[19,20] Interestingly, it has been suggested that caspase-3 involvement in erythropoiesis is restricted to the early phases of proerythroblast differentiation, with no major roles in the nuclear substructure reorganization and nuclear extrusion that characterize the late phases of the process.[21]

Caspase activation is also involved in thrombopoiesis. Platelets are formed from mature megakaryocytes and arise from the budding of thin, long cytoplasmic extensions called proplatelets. This process requires both localized caspase-3 and -9 processing and mitochondrial membrane permeabilization (MMP) and cytochrome *c* (Cyt *c*) release within the central body of the cell.[22] Caspase inhibitors, as well as Bcl-2 overexpression, block platelet formation *in vitro*.[23] Importantly, in the context of proplatelet generation, the activation of caspases is confined to granular, perinuclear structures. Conversely, widespread caspase activation is associated with megakaryocyte death and inefficient thrombopoiesis, as it occurs in myelodysplastic syndromes.[23,24]

Furthermore, caspases play a role in the differentiation of human blood monocytes into macrophages, a process exhibiting no morphological signs of apoptosis.[19] In this context, caspase activation involves Cyt *c* release from mitochondria and leads to the cleavage of a specific subset of caspase substrates, including the protein acinus but not poly (ADP-ribose) polymerase 1 (PARP-1).[25] Monocytic-macrophagic differentiation is repressed by pharmacological inhibitors of caspases as well as by overexpression of Bcl-2.[25] Apparently, the apical caspase involved in the macrophagic maturation of monocytes is caspase-8, which acts by cleaving the serine/threonine kinase receptor-interacting protein 1 (RIP1), thereby preventing sustained NF-κB activation and setting off downstream caspases.[26] Notably, caspase-8 has also been implicated in the differentiation of the human placental trophoblast, by mediating the syncytial fusion of the cytotrophoblast that accounts for the generation of the syncytiotrophoblast.[27]

Loss-of-function mutations in the human *caspase-8* gene cause defects in the activation of T, B and NK cells, culminating in immunodeficiency.[28] Similarly, knockout of *caspase-8* (or that of its cytosolic adaptor FADD, i.e. Fas-associating death domain-containing protein) in mice results in impaired heart muscle development and defects in the immune system, particularly the hematopoietic precursor and T-cell progenitor compartments.[29] Moreover, mice carrying a T-cell-specific inactivation of *caspase-8* develop impaired activation-induced expansion of peripheral T cells and an inability to clear lymphocyte choriomeningitis virus.[29] Caspase activation linked to the activation of T or B lymphocytes reportedly results in the intracellular proteolysis of a restricted panel of substrates, including PARP-1, lamin B and the kinase Wee1 (but not the DNA fragmentation factor subunit of 45 kDa, i.e. DFF45, nor replication factor C 140, i.e. RFC140, both of which are frequently cleaved in apoptosis).[30] In mice, liver-specific inactivation of *caspase-8* attenuates the first wave of hepatocyte proliferation after partial hepatectomy.[31] However, depending on the mouse genetic background, this may prompt an inflammatory response that eventually leads to enhanced proliferation and hepatomegaly.[31] While *caspase-8* deletion in bone marrow cells results in the functional impairment of hematopoietic progenitors, *caspase-8* loss in cells of the myelomonocytic lineage leads to an arrest of macrophagic differentiation and cell death.[32]

In humans, genetic alterations in *caspase-10* may be causative or protective in type II autoimmune lymphoproliferative syndrome, most likely due to its role in the initiation of the extrinsic apoptotic pathway.[33] Furthermore, caspase-10 has been implicated in the activation of NF-κB-dependent pro-survival signaling pathways, by a mechanism that may not require its catalytic domain.[34]

Caspase-11 was first described as an obligate activator of caspase-1.[35] More recently, caspase-11 has been reported to interact physically and functionally with actin-interacting protein 1 (Aip1), an activator of cofilin-mediated actin depolymerization.[36] This interaction is mediated by the caspase-recruitment domain (CARD) of caspase-11 and the C-terminal WD40 propeller domain of Aip1.[36] Thus, cells lacking Aip1 or caspase-11 exhibit similar defects in actin dynamics.[36]

Murine caspase-12 was initially reported to play a role in apoptosis induced by endoplasmic reticulum (ER) stress including disruption of Ca^{2+} homeostasis and accumulation of misfolded proteins.[37] Human caspase-12 may attenuate the inflammatory and innate immune response to endotoxins, and hence the loss of caspase-12 function may constitute a risk factor for developing sepsis.[38] In humans, a single nucleotide polymorphism in the *caspase-12* gene is responsible for the synthesis of either a truncated (Csp12-S) or a full-length proenzyme (Csp12-L). Interestingly, the read-through polymorphism resulting in the production of Csp12-L is confined to populations of African descent, and the frequency of the Csp12-L allele is particular high in African-American individuals characterized by severe septic responses.[38]

The activation of caspase-14 (whose expression is constitutively high during embryonic development but almost exclusively restricted to the suprabasal layers of the epidermis and the hair follicles in adult mice and humans) has been associated with the terminal differentiation of human keratinocytes and cornification.[39] *Caspase-14*$^{-/-}$ mice exhibit an altered composition of the stratum corneum, presumably due to an aberrant processing of filaggrin.[40] This results in reduced skin-hydration levels and enhanced sensitivity of the skin to UVB-induced photodamage and apoptosis.[40]

Altogether, these examples (Table 1) illustrate that most if not all caspases have cell death-unrelated functions.

Vital Functions of Mitochondrial Death Effectors

Mitochondria are crucial organelles in a cell's life and death. On the one hand, they act as major regulators of mitochondrial apoptosis, by integrating pro-survival and pro-death signals and ultimately sealing the cell's fate via MMP.[3,41,42] On the other hand, they generate the bulk of intracellular ATP via oxidative phosphorylation, and participate in multiple biosynthetic pathways.[43] Most mitochondrial death effectors exert also cell death-unrelated functions (Table 2).

Although Bcl-2 family proteins were initially characterized as cell death regulators, it has recently become clear that several members also control autophagy, either as part of a cell death or cell survival program.[5,44,45] Thus, antiapoptotic

proteins such as Bcl-2, Bcl-X_L, Bcl-w and Mcl-1 inhibit autophagy, presumably because they bind to the BH3 domain of Beclin 1,[46] an essential autophagy protein, thereby inhibiting the capacity of Beclin 1 to activate the phosphoinositide-3-kinase Vps34 (which participates in phagophore nucleation).[45,47–49] Conversely, proapoptotic BH3-only proteins from the Bcl-2 family such as BNIP3, Bad, Noxa, p53-upregulated modulator of apoptosis (Puma), Bim_{EL} and Bik can induce autophagy, likewise because they competitively disrupt the above-mentioned inhibitory interaction between their antiapoptotic relatives (e.g. Bcl-2, Bcl-X_L, and so on) and Beclin 1.[48,50,51] Importantly, both antiapoptotic and proapoptotic proteins from the Bcl-2 family contribute to the modulation of Ca^{2+} signaling at the ER.[52] In this context, Bcl-2 and Bcl-X_L have been reported to lower the luminal steady-state concentration of Ca^{2+}, by directly promoting Ca^{2+} leak into the cytosol,[53], by gating the response of the inositol-1,4,5-trisphosphate receptor (IP_3R) to inositol-1,4,5-trisphosphate,[54] or by destabilizing the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA).[55] Irrespective of their specific mechanism of action, Bcl-2 and Bcl-X_L act at the ER by dampening IP_3R-mediated Ca^{2+} release.[56] Conversely, Bax and Bak enhance the release of Ca^{2+} from ER stores, via a mechanism that implicates the SERCA.[57] Owing to the established role of Ca^{2+} overload in MMP,[58] the control of Ca^{2+} fluxes by members of the Bcl-2 family has been principally studied in the context of apoptosis.[57] However, since Ca^{2+} signaling impacts so many areas of cell biology, it seems appropriate to consider the relationship between Bcl-2-like proteins and Ca^{2+} from a broader point of view, which includes a plethora of apoptosis-unrelated processes (e.g. differentiation, regeneration, autophagy).[59,60] A similar consideration holds true for the role of proapoptotic Bcl-2 family members in the regulation of mitochondrial morphology and dynamics.[61] Thus, whereas the link between Bak, Bax and Bik and components of the mitochondrial fission/fusion machinery

Table 2 Apoptosis-related and -unrelated functions of non-caspase cell death effectors in mammals

Protein	Role in apoptosis	Role in normal metabolism
AIF	IM-associated FAD-containing protein facing the IMS Translocates from mitochondria to nuclei and forms an active nuclease complex with CypA to promote DNA degradation	Redox-active enzyme (NADH oxidase) AIF depletion induces a defect in the respiratory chain (mainly complex I) Tissue-specific KO causes whole-body resistance against diabetes and obesity
Apaf-1	Cytosolic adaptor protein Forms the apoptosome in presence of dATP and Cyt *c*, thereby acting as an allosteric activator of caspase-9	Regulates the intra-S-phase checkpoint induced by DNA damage, downstream of ATM and upstream of Chk1
Bad	BH3-only protein Activated by dephosphorylation (upon growth factor withdrawal) Inhibits antiapoptotic Bcl-2 family proteins	Required for optimal induction of autophagy in response to starvation
Bak/Bax	Proapoptotic BH123 multidomain proteins of the Bcl-2 family Promote MMP directly, by creating pores in the OM, and/or by favoring the activation of the PTPC	Enhance agonist-induced Ca^{2+} release at the ER, with possible implications in several apoptosis-unrelated phenomena Interact with the mitochondrial fusion/fission machinery
Bcl-2, Bcl-XL Bcl-w, Mcl-1	Antiapoptotic BH1234 multidomain proteins of the Bcl-2 family Inhibit MMP by sequestering proapoptotic Bcl-2 family members and by modulating ER Ca^{2+} fluxes	Inhibit autophagy by sequestering the essential autophagy modulator Beclin 1 Modulate Ca^{2+} fluxes at the ER, with possible implications in several apoptosis-unrelated phenomena
Bid	BH3-only protein Activated by proteolytic processing (by caspase-8, cathepsins of calpains) Directly induces Bax/Bak oligomerization	Might be involved in ATM-dependent DNA damage response
Bik	BH3-only protein Induced by multiple stress signals Regulates Bax/Bak-dependent release Ca^{2+} release from ER, thereby favoring MMP	Involved in the optimal induction of autophagic responses Interacts with the mitochondrial fusion/fission machinery
BimEL	BH3-only protein Activated upon phosphorylation-dependent release from cytoskeletal components Direct inducer of Bax/Bak oligomerization	Involved in the induction of autophagy in the delayed DNA damage response
BNIP3	BH3-only protein Transactivated by HIF1 in response to oxygen deprivation Inhibits antiapoptotic Bcl-2 family proteins	Promotes a protective response against ischemia/reperfusion injury, by favoring the autophagy of damaged mitochondria
Cyt *c*	IM-associated heme-containing protein of the IMS Upon MMP, forms the apoptosome in presence of dATP and Apaf-1, thereby acting as an allosteric activator of caspase-9	Shuttles electrons between complex III and complex IV of the respiratory chain Involved in the differentiation associated with caspase-3 and -9 activation
EndoG	IMS protein Translocates from mitochondria to nuclei to mediate chromatinolysis	Implicated in DNA recombination and recombination-dependent DNA repair Required for the survival of tetraploid colon cancer cell clones
FADD	Cytosolic adaptor protein Component of the DISC, the complex promoting the activation of caspase-8	Development and homeostasis of the T-cell peripheral compartment Implicated in cell cycle progression Involved in innate immunity signaling
Noxa	BH3-only protein Transactivated by p53 upon DNA damage Inhibits antiapoptotic Bcl-2 family proteins	Involved in the induction of autophagy in the delayed DNA damage response

Table 2 *(Continued)*

Protein	Role in apoptosis	Role in normal metabolism
Omi/HtrA2	IMS serine protease Cleaves IAPs and caspase-unrelated substrates, thus promoting caspase-dependent and -independent apoptosis	Negative regulator of cell cycle Reduced activity is associated with several neurodegenerative disorders (role in the processing of mitochondrial proteins?)
PIDD	Nuclear and cytosolic adaptor protein Contribute to assemble the PIDDosome in response to DNA damage, thereby activating caspase-2	Activates NF-κB-dependent pro-survival pathways by stimulating the sumoylation and ubiquitination of NEMO
Puma	BH3-only protein Transactivated by p53 after DNA damage Inhibits antiapoptotic Bcl-2 family proteins	Involved in the induction of autophagy in the delayed DNA damage response
RAIDD/ CRADD	Cytosolic adaptor protein Involved in the PIDDosome, a complex for activating caspase-2 upon DNA damage	Possibly implicated in specific differentiation programs
Smac/ DIABLO	IMS protein Sequestrates IAPs and hence favors caspase activation	Putative role in the regulation of cell cycle
TRADD	Nuclear and cytosolic adaptor protein Contributes to the assembly of the DISC, and hence promotes the activation of caspase-8	Implicated in TNFR-derived NF-κB-dependent pro-survival pathways Nuclear TRADD interacts with Stat1, thus modulating its transcriptional activity

Abbreviations: AIF, apoptosis-inducing factor; Apaf-1, apoptotic protease activating factor 1; BH, Bcl-2 homology domain; CRADD, caspase-2 and RIPK1 domain containing adaptor with death domain; CypA, cyclophilin A; Cyt *c*, cytochrome *c*; DIABLO, direct IAP binding protein with a low pI; DISC, death-induced signaling complex; EndoG, endonuclease G; ER, endoplasmic reticulum; FAD, flavine adenine nucleotide; FADD, *i.e.* Fas-associating death domain-containing protein; HIF1, hypoxia-inducible factor 1; HtrA2, high temperature requirement protein A 2; IAP, inhibitor of apoptosis protein; IM, mitochondrial inner membrane; IMS, mitochondrial intermembrane space; KO, knockout; MMP, mitochondrial membrane permeabilization; NEMO, NF-κB essential modulator; OM, mitochondrial outer membrane; Omi, Omi stress-regulated endoprotease; PIDD, p53-induced protein with a death domain; PTPC, permeability transition pore complex; Puma, p53-upregulated modulator of apoptosis; RAIDD, receptor-interacting protein (RIP)-associated ICH-1/CED-3 homologous protein with a death; Smac, second mitochondria-derived activator of caspase; TNFR, tumor necrosis factor receptor; TRADD, TNFR-associated death domain protein.

(e.g. dynamin-like protein 1, i.e. Drp1, hFis1, mitofusins)[62,63] has been extensively characterized during cell death, it cannot be excluded that this interaction might as well account for apoptosis-unrelated changes in mitochondrial dynamics. As a final example, Bid, another BH3-only protein involved in the crosstalk between the extrinsic and intrinsic apoptotic pathways,[64] has also been suggested to take part in the DNA damage response activated by ATM.[65]

The prototype of non-caspase apoptotic effectors characterized by well-defined vital roles is Cyt *c*.[8] In healthy cells, Cyt *c* is associated with the outer surface of the mitochondrial inner membrane (IM), where it functions as an electron shuttle between complex III and complex IV of the respiratory chain.[66] This activity of Cyt *c* is necessary for life, as indicated by the fact that knockout mice embryos die *in utero* by midgestation.[67] After apoptosis-related mitochondrial outer membrane permeabilization (MOMP), Cyt *c* is released into the cytosol, where it interacts with Apaf-1 and dATP to form a large heptameric complex (the so-called apoptosome) that recruits and allosterically activates caspase-9 and hence sets off the caspase cascade.[68,69] However, cytosolic Cyt *c* has been implicated also in a number of cell death-unrelated processes including the fragmentation of mature megakaryocytes,[23] monocytic-macrophagic differentiation,[25] *Drosophila melanogaster* sperm cell differentiation,[70] and B-cell homeostasis.[71] In this context, whereas the sustained release of Cyt *c* following irreversible MOMP activates caspase-dependent apoptosis, lower amounts of cytosolic Cyt *c* may promote limited caspase activation, and hence the cleavage of a restricted subset of substrates involved in cell death-unrelated processes.[72]

Apoptosis-inducing factor (AIF) is a phylogenetically ancient protein essential for survival.[73] Murine AIF is synthesized from a nuclear gene as an immature precursor with a $\sim$100 amino-acid long N-terminal mitochondrial localization signal (MLS). Upon import into mitochondria, the MLS is removed and AIF inserts into the IM via an N-terminal transmembrane region. The rest of the protein, which refolds and incorporates flavine adenine nucleotide as a prosthetic group required for its NADH oxidase activity,[74,75] faces the mitochondrial intermembrane space (IMS).[76] After MOMP, AIF translocates from mitochondria to the cytosol and eventually is imported into the nucleus (together with its obligate cofactor cyclophilin A), where it participates in chromatin condensation and DNA degradation.[77,78] Mutational and biochemical analysis of AIF indicate that its apoptotic and redox functions reside in distinct domains of the protein.[79] Importantly, murine *aif* knockout causes a defect in oxidative phosphorylation, mainly due to the downregulation of components of complex I of the respiratory chain.[80,81] This explain why, in the Harlequin mutant mouse, reduced AIF expression (due to a retroviral insertion into the first intron of the *aif* gene) leads to cerebellar and retinal neurodegeneration.[82] Indeed, cells expressing little or no AIF are particularly vulnerable to oxidative stress, in line with the notion that AIF has an antioxidant function.[82] Tissue-specific deletion of *aif* in the muscle or liver provokes increased glycolytic rates, insulin hypersensitivity and significant resistance to diabetes and high lipid diet-induced obesity,[83] underscoring a major role for AIF in apoptosis-unrelated mitochondrial metabolism.

Endonuclease G (EndoG) is a nuclear DNA-encoded nuclease that normally resides in IMS. After MOMP, EndoG translocates to the nucleus, where it mediates oligonucleosomal DNA fragmentation independently of caspases.[84] Importantly, EndoG is also involved in DNA recombination, and its deficiency reduces cell proliferation and favors the arrest of cells in the G_2 phase of the cell cycle.[85] In accord with

the observation that recombination-dependent DNA repair is essential for the survival of tetraploid cells,[86] EndoG knockdown causes the death of tetraploid tumor cells.[87]

The stress-activated endoprotease Omi stress-regulated endoprotease (Omi) (also known as high temperature requirement protein A 2, i.e. HtrA2) belongs to a family of serine proteases that is well conserved from bacteria to humans.[88] In bacteria, HtrA2 is localized within the periplasmic space and determines thermotolerance,[89] whereas in healthy eukaryotic cells Omi/HtrA2 is confined to the IMS. After MOMP, the protease is released into the cytosol and promotes apoptosis via caspase-dependent and -independent mechanisms.[90] Thus, Omi/HtrA2 indirectly favors the activation of caspases by sequestering and cleaving inhibitor of apoptosis proteins (IAPs),[91,92] but also contributes to the execution of apoptosis via the cleavage of caspase-unrelated substrates like cytoskeletal proteins.[93] However, Omi/HtrA2 has also been suggested to act as a negative regulator of cell cycle progression during interphase, presumably through the proteolytic processing of the mitotic kinase WTS/large tumor-suppressor 1 (WARTS).[94] Interestingly, a loss-of-function mutation in *omi* (Ser276Cys) has been shown to underlie the pathology of the *mnd2* (for motor neuron degeneration 2) mouse strain, which exhibit early onset neurodegeneration with parkinsonian features and juvenile lethality.[95] The same phenotype is observed in $omi^{-/-}$ mice, suggesting that the most important function of Omi/HtrA2 *in vivo* relates more to protection against stress than to apoptosis.[96] Mitochondria purified from *mnd2* mice are more susceptible to Ca^{2+}-mediated permeabilization *in vitro* than control organelles purified from wild-type animals. Thus, almost paradoxically, the loss of Omi/HtrA2 enhances the susceptibility to apoptosis, thereby provoking the degeneration of striatal neurons in *mnd2* mice.[95] Moreover, it has been recently found that Omi/HtrA2 is activated through phosphorylation by PTEN-induced putative kinase 1, a putative mitochondrial protein kinase that is mutated in some cases of familial early-onset Parkinson's disease.[97] Finally, it has also been shown that the protease activity of Omi/HtrA2 is necessary for the physiological processing of β-amyloid precursor protein at mitochondria, thus pointing to Omi/HtrA2 as a possible therapeutic target for Alzheimer's disease.[98] Thus, multiple links exist between reduced Omi/HtrA2 activity and neurodegeneration.

Murine second mitochondria-derived activator of caspase (Smac) and its human ortholog direct IAP-binding protein with a low pI (DIABLO) are encoded by the nuclear genome and synthesized as an immature precursor that harbors an N-terminal MLS.[99,100] After mitochondrial import, MLS is proteolytically removed to yield a mature polypeptide of 23 kDa localized in the IMS and exposing an IAP-binding domain.[100] After MOMP, Smac/DIABLO is released into the cytosol, homodimerizes and promotes apoptosis by sequestering different members of the IAP family, thereby favoring caspase activation.[101,102] Notably, cell death-unrelated functions for Smac/DIABLO have not been unambiguously identified yet. $Smac^{-/-}$ mice are viable, grow normally and exhibit no major phenotypic alterations.[103] Moreover, $smac^{-/-}$ cells responded normally to multiple apoptotic stimuli. These observations suggest the existence of a redundant molecule compensating for Smac/DIABLO loss *in vivo*, with regards to both its lethal and vital functions.[103] Ectopic overexpression of Smac/DIABLO in the cytosol has been reported to induce the arrest of cells at the G_0/G_1 cell cycle transition.[104] However, the actual significance of this observation in a physiological context remains to be established.

Vital Functions of Cell Death-Related Adaptor Molecules

In both the extrinsic (death receptor-mediated) and intrinsic (mitochondrial) apoptotic pathways, supramolecular complexes are assembled to facilitate the interaction (and hence the activation) of transducers of the lethal signal with molecules responsible for upstream (initiation) or downstream (execution) phases.[3] So far, a number of adaptor proteins have been characterized that assist in the assembly of these complexes, including (i) Apaf-1, which contributes to the formation of the 'apoptosome' to activate caspase-9;[68] (ii) FADD and tumor necrosis factor receptor (TNFR)-associated death domain protein (TRADD), which recruit pro-caspase-8 at the plasma membrane within the death-induced signaling complex (DISC), thus transducing proapoptotic signals from the extracellular to the intracellular milieu;[105] (iii) p53-induced protein with a death domain (PIDD) and caspase-2 and RIPK1 domain containing adaptor with death domain (CRADD, also known as RIP-associated ICH-1/CED-3 homologous protein with a death, i.e. RAIDD), both participating in the assembly of the 'PIDDosome', a protein complex implicated in caspase-2 activation following genotoxic stress.[106,107] These factors are implicated also in several processes distinct from cell death (Table 2).

Knockdown of Apaf-1 in human cells as well as knockout of *apaf-1* in mice implicated Apaf-1 in DNA damage-induced cell cycle arrest. Thus, Apaf-1 loss compromises the DNA damage checkpoints elicited by ionizing irradiation or chemotherapy.[108] Apaf-1 depletion also reduces the activating phosphorylation of the checkpoint kinase Chk1 provoked by DNA damage,[108,109] and knockdown of Chk1 abrogates Apaf-1-mediated cell cycle arrest. Morevoer, epistatic analyses revealed that Chk1 operates downstream of Apaf-1 to mediate the intra-S-phase DNA damage checkpoint.[108] Finally, the nuclear translocation of Apaf-1, which *in vitro* can be induced by exogenous DNA damaging agents in an ATM- or ATR-dependent fashion, correlates *in vivo* with the endogenous activation of Chk-1, as assessed in biopsies from non-small cell lung cancer patients.[108] The influence of Apaf-1 on the cell cycle is not modulated by pharmacological inhibitors of caspases like Z-VAD.fmk nor by antiapoptotic proteins such as the baculovirus-encoded IAP p35 and Bcl-2. Moreover, Apaf-1 mutants that lack the N-terminal CARD can replace endogenous Apaf-1 in the control of DNA damage-induced cell cycle-blockade. This indicates that the cell cycle-arresting function of Apaf-1 is independent of its caspase-activating (proapoptotic) role.[108]

Beyond its role within the DISC, FADD has been implicated in a number of cell death-unrelated processes.[110] $Fadd^{-/-}$ mice die early during embryogenesis, with signs of cardiac failure and abdominal hemorrhage.[111] To circumvent this problem, transgenic mice expressing a dominant negative mutant of FADD (lacking the caspase-dimerizing death effector domain) have been generated. These animals exhibit

retarded thymocyte development and peripheral lymphocyte pools devoid of T cells.[112] A similar phenotype is observed in transgenic mice engineered for the conditional, T-cell-specific knockout of *fadd*.[113] Taken together, these observations highlight an essential role for FADD in the development and homeostasis of peripheral T cells. Several other studies point to the implication of FADD in proliferation and cell cycle progression, mainly in hematopoietic progenitors and cells of the lymphocytic lineage.[114,115] As a possibility, such functions of FADD may be accounted for by a nuclear pool of the protein,[116] and may be regulated by cell cycle-dependent phosphorylation at multiple serine residues.[117] Interestingly, FADD is also involved in multiple innate immunity signaling pathways, either dependent or independent from the Toll-like receptor 4.[118] Upon TNFR activation, two sequential signaling complexes are formed, which may account for its dual role in promoting cell death and survival. Whereas complex I (which contains TRADD and RIP1 but not FADD) signals NF-κB to promote cell survival, complex II (involving FADD and caspase-8) triggers cell death.[119] siRNA-mediated depletion of TRADD (gene knockout is incompatible with life) suggested that this adaptor is dispensable for necrosis induction along the TNFR–RIP1 axis, but required for the activation of both NF-κB and caspase-8.[120] Interestingly, a nuclear pool of TRADD has been implicated in both cell death and survival pathways, possibly via the interaction with the transcriptional factor Stat1.[121]

According to recent reports, PIDD represents a master switch in the response to DNA damage. On the one hand, PIDD (transactivated by p53) can cooperate with CRADD/RAIDD to assemble the PIDDosome, thereby activating caspase-2 and promoting apoptosis.[106,107] As an alternative, PIDD is able to enhance genotoxic stress-induced NF-κB activation by augmenting the sumoylation and ubiquitination of NF-κB essential modulator.[122] This pro-survival role of PIDD depends on a 51-kDa C-terminal fragment including the death domain (PIDD-C), generated by an auto-proteolysis mechanism. Further processing of PIDD-C would then result in the formation of a 37-kDa fragment (PIDD-CC) responsible for caspase-2 activation. A non-cleavable PIDD mutant fails to translocate from the cytoplasm to the nucleus, thereby losing both activities.[123] So far, three isoforms of PIDD have been described, of which all are capable of activating NF-κB upon DNA damage, but only isoform 1 interacts with RAIDD/CRADD and activate caspase-2.[124] RAIDD/CRADD cell death-unrelated functions are still poorly characterized, but may include the regulation of (at least some) differentiation programs.[125] Notably, *raidd*$^{-/-}$ mice are not viable, yet this presumably depends on RAIDD/CRADD involvement in apoptosis-mediated embryo remodeling rather than in other processes (as assessed by its expression pattern during organogenesis, which correlates with profound morphological changes occurring in the developing embryo).[126]

Vital Functions of Components of the Permeability Transition Pore Complex

In some models of cell death characterized by excessive Ca^{2+} fluxes and reactive oxygen species overproduction, the cascade of events leading to MMP and apoptosis is set off at the IM, due to the activation of the permeability transition pore complex (PTPC), a large multiprotein complex formed at the contact sites between the mitochondrial outer membrane (OM) and IM.[3,127] Despite considerable effort to determine its exact molecular structure, the precise composition of the PTPC still remains elusive.[128] In this context, numerous studies suggest that the PTPC might be formed by the dynamic interaction of several partners, including the adenine nucleotide translocase (ANT, in the IM), the voltage-dependent anion channel (VDAC, in the OM), cyclophilin D (CypD, in the mitochondrial matrix), creatine kinase (CK, in the IMS), the peripheral-type benzodiazepine receptor (PBR, in OM) as well as hexokinase isoforms (HKI and HKII, in the cytosol).[128,129]

However, since 'housekeeping' genes (such as those coding for the majority of putative PTPC components) cannot be easily manipulated by genetic means (because their knockout is incompatible with life), PTPC constituents have been rather poorly investigated for their role in lethal processes, with the notable exception of CypD.[127,130,131] Thus, mice knockout for all isoforms of VDAC,[132,133] ANT[132,134] and other putative PTPC components have not yet been generated, despite the fact that there is firm evidence (obtained in cell lines) that numerous PTPC components do indeed play a role in cell death.[3] Nevertheless, all these proteins are known to participate into a number of mitochondrial and extramitochondrial metabolic pathways, reinforcing the concept that proapoptotic effectors also exert vital functions (Table 3). Thus, (i) ANT mediates ATP/ADP exchange between the mitochondrial matrix and the cytosol, thereby ensuring an adequate cytosolic energy supply while maintaining high rates of oxidative phosphorylation (by releasing substrate inhibition);[135] (ii)VDAC is the most abundant OM protein and, in healthy cells, exists as a large, voltage-gated channel accounting for OM permeability properties;[136] (iii) CypD exhibits a peptidyl-prolyl *cis–trans* isomerase activity, which contributes to the correct folding of mitochondrial matrix proteins;[137] (iv) CK catalyzes the ATP-dependent conversion of creatine into phosphocreatine, to constitute a highly diffusible intracellular energy buffer;[138] (v) PBR regulates cholesterol transport from OM to IM, the rate-limiting step in steroidogenesis;[139] and (vi) HK isoforms catalyze the production of glucose-6-phosphate, the first intermediate in glucose metabolism.[140]

Vital Functions of Evolutionarily Distant Death Effectors

The cell death-unrelated functions of apoptotic proteins are so conserved among evolutionarily distant species that it has been possible to predict them in model organisms other than mammals, based on the data that had been obtained in the human and murine systems (Table 4).

Based on the observation that BH3-only proteins induce autophagy in mouse and human cells, we made the prediction that the sole BH3-only protein present in *C. elegans*, that is EGL-1, would also regulate autophagy. Indeed, gain-of-function mutations of *egl-1* induce a maximum degree of autophagy that cannot be further enhanced by starvation, the physiological inducer of autophagy. Conversely, the *egl-1* knockout strongly limits starvation-induced autophagic responses.[51] In view of the fact that autophagy is mostly

Table 3 Cell death-regulatory and normal functions of PTPC constituents

PTPC component	Mitochondrial localization	Interacting partners	Role in cell death	Role in normal metabolism
ANT	IM	Bak, Bax Bcl-2 VDAC	ANT1 and ANT3 are proapoptotic upon overexpression ANT2 depletion is proapoptotic Forms non-specific channels in IM, modulated by Bcl-2-like proteins	ADP/ATP antiporter
CK	IMS	ANT VDAC	Antiapoptotic	Energy transfer from ATP to phosphocreatine stores
CypD	IM matrix face	ANT	Antiapoptotic, presumably by chaperoning misfolded IM proteins	Peptidyl-prolyl cis/trans isomerase Assists correct protein folding in mitochondrial matrix
HK	OM cytosolic face	VDAC	Antiapoptotic, presumably by inhibiting Bax binding to VDAC	Rate-limiting enzyme of the glycolytic pathway
PBR	OM	VDAC	Antiapoptotic when overexpressed Proapoptotic upon interaction with some ligands	Receptor for endozepine (CoA-binding protein) Ensures cholesterol transport from OM to IM during steroidogenesis
VDAC	OM	ANT Bak, Bax Bcl-2, Bcl-X_L	Regulates OM permeability VDAC1 cooperates with Bax VDAC2 inhibits Bak	Transport metabolites across OM

Abbreviations: AIF, apoptosis-inducing factor; ANT, adenine nucleotide translocase; CK, creatine kinase; CoA, coenzyme A; CypD, cyclophilin D; HK, hexokinase; IM, mitochondrial inner membrane; IMS, mitochondrial intermembrane space; OM, mitochondrial outer membrane; PBR, peripheral-type benzodiazepine receptor; PTPC, permeability transition pore complex; VDAC, voltage-dependent anion channel.

Table 4 Lethal and vital functions of evolutionarily distant cell death effectors

Protein	Species	Role in apoptosis	Role in normal metabolism
AIF	*Saccharomyces cerevisiae*	IM-associated FAD-containing protein facing the IMS Translocates from mitochondria to nuclei to form an active nuclease with CypA Participates in chronological aging	AIF KO induces a defect in the respiratory chain (mainly complex III)
CED-4	*Caenorhabditis elegans*	Ortholog of mammalian Apaf-1 Cytosolic allosteric activator of the caspase CED-3	Participates in DNA-damage induced cell cycle arrest of germ cells downstream of ATM and ATL
CED-9	*Caenorhabditis elegans*	Ortholog of mammalian Bcl-2/Bcl-X_L Sequester CED-4, thereby inhibiting the activation of CED-3 caspase	Involved with EGL-1 in the regulation of mitochondrial fusion/fission dynamics
EGL-1	*Caenorhabditis elegans*	BH3-only protein Interacts with CED-9 (a Bcl-2 ortholog) to competitively displace CED-4	Required for optimal induction of autophagy in response to starvation Involved with CED-9 in the regulation of mitochondrial fusion/fission dynamics
EndoG	*Saccharomyces cerevisiae*	IMS protein Translocates from mitochondria to nuclei to mediate chromatinolysis Participates in chronological aging	Required for the survival of tetraploid cells

Abbreviations: AIF, apoptosis-inducing factor; Apaf-1, apoptotic protease activating factor 1; BH, Bcl-2 homology domain; CypA, cyclophilin A; EndoG, endonuclease G; FAD, flavine adenine nucleotide; IM, mitochondrial inner membrane; IMS, mitochondrial intermembrane space; KO, knockout.

a cytoprotective mechanism,[44] these results suggest that BH3-only proteins may be required for an optimal adaptation to nutrient depletion. Recently, it has been proposed that EGL-1 also plays a role in the regulation of mitochondrial dynamics, by preventing CED-9 from binding to (and hence inhibiting) components of the mitochondrial fission/fusion machinery.[141]

Starting from the observation that Apaf-1 translocates to the nucleus of human DNA-damaged cells and participates in the intra-S-phase DNA damage checkpoint upstream of Chk1, we wanted to determine whether the *C. elegans* Apaf-1 ortholog CED-4 would have a similar function. Indeed, we found that CED-4 translocates to the nuclei of germline cells upon exposure of nematodes to ionizing irradiation, in an ATM- and ATL (the worm ortholog of ATR)-dependent fashion. Moreover, CED-4 was required for the proliferation arrest of *C. elegans* germline cells induced by γ-irradiation or UVC light.[108] Two independent lines of evidence indicate that the cell cycle-modulatory effect of CED-4 does not require the activation of apoptotic effectors: (i) the absence of CED-4 continues to reduce the DNA damage-induced cell-cycle arrest when the absence of proapoptotic proteins such as EGL-1 or CEP-1 (the *C. elegans* ortholog of p53) does not influence the cell cycle; and (ii) loss-of-function mutations in *ced-4* affects the cell cycle also in a genetic background where CED-3 cannot be activated and hence caspase-dependent apoptosis does not occur.[108]

Since mammalian AIF possesses a bona fide yeast ortholog[142] and since AIF depletion causes a respiratory defect in mouse and human cells,[81] we made the prediction that the knockout of yeast *aif1* gene would also lead to deficient oxidative phosphorylation. Indeed, *aif1*$^-$ yeast cells exhibited normal growth in rich media, yet proliferated less efficiently than isogenic controls in non-fermentable energy sources such as lactate or glycerol.[81] This is remarkable because, in mammalian cells, AIF depletion causes mostly a defect in respiratory complex I, indicating a major phylogenetic conservation of the contribution of AIF to optimal mitochondrial function. As an aside, it should be noted that the knockout of the *D. melanogaster* homolog of *aif1* also causes a major respiratory defect,[73] underscoring the importance of AIF in normal mitochondrial function.

EndoG has also been investigated for its putative cell death-unrelated roles in non-mammals organisms. Interestingly, it has been demonstrated that EndoG is required for the survival of tetraploid *S. cerevisiae* cells, an observation that was confirmed in tetraploid human colon cancer cell clones.[87]

Finally, metacaspases (which may represent a phylogenetic ancestor of mammalian caspases) are not only implicated in various cell death scenarios,[143] but have been recently shown to modulate cell cycle progression and stress responses in fungal and protozoan models.[144,145]

Concluding Remarks

The results outlined above suggest that cell death regulators have vital (as opposed to the lethal) functions that are phylogenetically conserved. Although formal proof for this concept is lacking, it appears plausible that the cell death-unrelated function of such proteins is actually the most ancestral one and that the lethal function has been acquired later during evolution. In particular, it seems that apoptosis effectors exhibit vital functions that are prominently involved in the adaptation to stress such as redox stress (AIF), metabolic stress (BH3-only proteins/EGL-1), DNA damage (Apaf-1/CED-4, EndoG) or thermotolerance (Omi/HtrA2). This applies also to caspases, which play a role in inflammation and immunity, the host responses to pathogen invasion-induced stress.[146] Based on this concept, it is tempting to speculate that proteins involved in stress adaptation of individual cells might become potential death effectors later during evolution.

As a caveat to this speculation, however, it should be noted that the contribution to lethal processes of 'housekeeping' genes (which cannot be genetically manipulated because their depletion leads to cell death) is difficult to be investigated. Thus, it remains possible that – for methodological reasons – we are grossly underestimating the proteins and processes involved in the regulation and execution of cell death. The adventure that started in the 1960s with the examination of insect intersegmental muscle cells undergoing PCD[1] is only in its infancy. There is no death without life.

Acknowledgements. G Kroemer expresses his gratitude to Zahra Zakeri and Richard Lockshin for their constant enlightenment and long-standing friendship. This work has been supported by grants from the Ligue Nationale contre le Cancer (équipe labelliseé), Institut National du Cancer (INCa), Cancéropôle Ile-de-France, Agence Nationale de Recherche (ANR), the European Union (Active p53, Apo-Sys, ChemoRes, Death-Train, RIGHT, Trans-Death), ARC (Association pour la Recherche contre le Cancer) and INSERM (Institut National de Santé et de la Recherche Médicale).

Conflict of interest

The authors declare no competing financial interests.

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**: 123–133.
2. Castedo M, Ferri K, Roumier T, Metivier D, Zamzami N, Kroemer G. Quantitation of mitochondrial alterations associated with apoptosis. *J Immunol Methods* 2002; **265**: 39–47.
3. Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. *Physiol Rev* 2007; **87**: 99–163.
4. Kroemer G, El-Deiry WS, Golstein P, Peter ME, Vaux D, Vandenabeele P *et al.* Classification of cell death: recommendations of the Nomenclature Committee on Cell Death. *Cell Death Differ* 2005; **12** (Suppl 2): 1463–1467.
5. Galluzzi L, Maiuri MC, Vitale I, Zischka H, Castedo M, Zitvogel L *et al.* Cell death modalities: classification and pathophysiological implications. *Cell Death Differ* 2007; **14**: 1237–1243.
6. Horvitz HR. Nobel lecture. Worms, life and death. *Biosci Rep* 2003; **23**: 239–303.
7. Cohen GM. Caspases: the executioners of apoptosis. *Biochem J* 1997; **326** (Part 1): 1–16.
8. Garrido C, Kroemer G. Life's smile, death's grin: vital functions of apoptosis-executing proteins. *Curr Opin Cell Biol* 2004; **16**: 639–646.
9. Martinon F, Tschopp J. Inflammatory caspases and inflammasomes: master switches of inflammation. *Cell Death Differ* 2007; **14**: 10–22.
10. Martinon F, Tschopp J. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell* 2004; **117**: 561–574.
11. Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. *Nat Rev Mol Cell Biol* 2008; in press.
12. Marzo I, Susin SA, Petit PX, Ravagnan L, Brenner C, Larochette N *et al.* Caspases disrupt mitochondrial membrane barrier function. *FEBS Lett* 1998; **427**: 198–202.
13. Zhivotovsky B, Orrenius S. Caspase-2 function in response to DNA damage. *Biochem Biophys Res Commun* 2005; **331**: 859–867.
14. Zhang Y, Padalecki SS, Chaudhuri AR, De Waal E, Goins BA, Grubbs B *et al.* Caspase-2 deficiency enhances aging-related traits in mice. *Mech Ageing Dev* 2007; **128**: 213–221.
15. Miura M, Chen XD, Allen MR, Bi Y, Gronthos S, Seo BM *et al.* A crucial role of caspase-3 in osteogenic differentiation of bone marrow stromal stem cells. *J Clin Invest* 2004; **114**: 1704–1713.
16. Weil M, Raff MC, Braga VM. Caspase activation in the terminal differentiation of human epidermal keratinocytes. *Curr Biol* 1999; **9**: 361–364.
17. Wride MA, Parker E, Sanders EJ. Members of the bcl-2 and caspase families regulate nuclear degeneration during chick lens fibre differentiation. *Dev Biol* 1999; **213**: 142–156.
18. Daugas E, Cande C, Kroemer G. Erythrocytes: death of a mummy. *Cell Death Differ* 2001; **8**: 1131–1133.
19. Zermati Y, Garrido C, Amsellem S, Fishelson S, Bouscary D, Valensi F *et al.* Caspase activation is required for terminal erythroid differentiation. *J Exp Med* 2001; **193**: 247–254.
20. Ribeil JA, Zermati Y, Vandekerckhove J, Cathelin S, Kersual J, Dussiot M *et al.* Hsp70 regulates erythropoiesis by preventing caspase-3-mediated cleavage of GATA-1. *Nature* 2007; **445**: 102–105.
21. Krauss SW, Lo AJ, Short SA, Koury MJ, Mohandas N, Chasis JA. Nuclear substructure reorganization during late-stage erythropoiesis is selective and does not involve caspase cleavage of major nuclear substructural proteins. *Blood* 2005; **106**: 2200–2205.
22. Clarke MC, Savill J, Jones DB, Noble BS, Brown SB. Compartmentalized megakaryocyte death generates functional platelets committed to caspase-independent death. *J Cell Biol* 2003; **160**: 577–587.
23. De Botton S, Sabri S, Daugas E, Zermati Y, Guidotti JE, Hermine O *et al.* Platelet formation is the consequence of caspase activation within megakaryocytes. *Blood* 2002; **100**: 1310–1317.
24. Braun T, Carvalho G, Grosjean J, Ades L, Fabre C, Boehrer S *et al.* Differentiating megakaryocytes in myelodysplastic syndromes succumb to mitochondrial derangement without caspase activation. *Apoptosis* 2007; **12**: 1101–1108.
25. Sordet O, Rebe C, Plenchette S, Zermati Y, Hermine O, Vainchenker W *et al.* Specific involvement of caspases in the differentiation of monocytes into macrophages. *Blood* 2002; **100**: 4446–4453.
26. Rebe C, Cathelin S, Launay S, Filomenko R, Prevotat L, L'Ollivier C *et al.* Caspase-8 prevents sustained activation of NF-kappaB in monocytes undergoing macrophagic differentiation. *Blood* 2007; **109**: 1442–1450.
27. Black S, Kadyrov M, Kaufmann P, Ugele B, Emans N, Huppertz B. Syncytial fusion of human trophoblast depends on caspase 8. *Cell Death Differ* 2004; **11**: 90–98.
28. Chun HJ, Zheng L, Ahmad M, Wang J, Speirs CK, Siegel RM *et al.* Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency. *Nature* 2002; **419**: 395–399.

29. Salmena L, Lemmers B, Hakem A, Matysiak-Zablocki E, Murakami K, Au PY *et al.* Essential role for caspase 8 in T-cell homeostasis and T-cell-mediated immunity. *Genes Dev* 2003; **17**: 883–895.
30. Beisner DR, Chu IH, Arechiga AF, Hedrick SM, Walsh CM. The requirements for Fas-associated death domain signaling in mature T cell activation and survival. *J Immunol* 2003; **171**: 247–256.
31. Ben Moshe T, Barash H, Kang TB, Kim JC, Kovalenko A, Gross E *et al.* Role of caspase-8 in hepatocyte response to infection and injury in mice. *Hepatology* 2007; **45**: 1014–1024.
32. Kang TB, Ben-Moshe T, Varfolomeev EE, Pewzner-Jung Y, Yogev N, Jurewicz A *et al.* Caspase-8 serves both apoptotic and nonapoptotic roles. *J Immunol* 2004; **173**: 2976–2984.
33. Zhu S, Hsu AP, Vacek MM, Zheng L, Schaffer AA, Dale JK *et al.* Genetic alterations in caspase-10 may be causative or protective in autoimmune lymphoproliferative syndrome. *Hum Genet* 2006; **119**: 284–294.
34. Shikama Y, Yamada M, Miyashita T. Caspase-8 and caspase-10 activate NF-kappaB through RIP, NIK and IKKalpha kinases. *Eur J Immunol* 2003; **33**: 1998–2006.
35. Wang S, Miura M, Jung YK, Zhu H, Li E, Yuan J. Murine caspase-11, an ICE-interacting protease, is essential for the activation of ICE. *Cell* 1998; **92**: 501–509.
36. Li J, Brieher WM, Scimone ML, Kang SJ, Zhu H, Yin H *et al.* Caspase-11 regulates cell migration by promoting Aip1-Cofilin-mediated actin depolymerization. *Nat Cell Biol* 2007; **9**: 276–286.
37. Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA *et al.* Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. *Nature* 2000; **403**: 98–103.
38. Saleh M, Vaillancourt JP, Graham RK, Huyck M, Srinivasula SM, Alnemri ES *et al.* Differential modulation of endotoxin responsiveness by human caspase-12 polymorphisms. *Nature* 2004; **429**: 75–79.
39. Eckhart L, Declercq W, Ban J, Rendl M, Lengauer B, Mayer C *et al.* Terminal differentiation of human keratinocytes and stratum corneum formation is associated with caspase-14 activation. *J Invest Dermatol* 2000; **115**: 1148–1151.
40. Denecker G, Hoste E, Gilbert B, Hochepied T, Ovaere P, Lippens S *et al.* Caspase-14 protects against epidermal UVB photodamage and water loss. *Nat Cell Biol* 2007; **9**: 666–674.
41. Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. *Science* 2004; **305**: 626–629.
42. Ferri KF, Kroemer G. Mitochondria – the suicide organelles. *Bioessays* 2001; **23**: 111–115.
43. Saraste M. Oxidative phosphorylation at the fin de siecle. *Science* 1999; **283**: 1488–1493.
44. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell* 2008; **132**: 27–42.
45. Galluzzi L, Vicencio JM, Kepp O, Tasdemir E, Maiuri MC, Kroemer G. To die or not to die: that is the autophagic question. *Curr Mol Med* 2008; in press.
46. Oberstein A, Jeffrey PD, Shi Y. Crystal structure of the Bcl-XL-Beclin 1 peptide complex: Beclin 1 is a novel BH3-only protein. *J Biol Chem* 2007; **282**: 13123–13132.
47. Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N *et al.* Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* 2005; **122**: 927–939.
48. Maiuri MC, Criollo A, Tasdemir E, Vicencio JM, Tajeddine N, Hickman JA *et al.* BH3-only proteins and BH3 mimetics induce autophagy by competitively disrupting the interaction between Beclin 1 and Bcl-2/Bcl-X(L). *Autophagy* 2007; **3**: 374–376.
49. Maiuri MC, Le Toumelin G, Criollo A, Rain JC, Gautier F, Juin P *et al.* Functional and physical interaction between Bcl-X(L) and a BH3-like domain in Beclin-1. *EMBO J* 2007; **26**: 2527–2539.
50. Abedin MJ, Wang D, McDonnell MA, Lehmann U, Kelekar A. Autophagy delays apoptotic death in breast cancer cells following DNA damage. *Cell Death Differ* 2007; **14**: 500–510.
51. Maiuri MC, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nat Rev Mol Cell Biol* 2007; **8**: 741–752.
52. Pinton P, Rizzuto R. Bcl-2 and Ca2+ homeostasis in the endoplasmic reticulum. *Cell Death Differ* 2006; **13**: 1409–1418.
53. Oakes SA, Scorrano L, Opferman JT, Bassik MC, Nishino M, Pozzan T *et al.* Proapoptotic BAX and BAK regulate the type 1 inositol trisphosphate receptor and calcium leak from the endoplasmic reticulum. *Proc Natl Acad Sci USA* 2005; **102**: 105–110.
54. White C, Li C, Yang J, Petrenko NB, Madesh M, Thompson CB *et al.* The endoplasmic reticulum gateway to apoptosis by Bcl-X(L) modulation of the InsP3R. *Nat Cell Biol* 2005; **7**: 1021–1028.
55. Dremina ES, Sharov VS, Kumar K, Zaidi A, Michaelis EK, Schoneich C. Anti-apoptotic protein Bcl-2 interacts with and destabilizes the sarcoplasmic/endoplasmic reticulum Ca2+-ATPase (SERCA). *Biochem J* 2004; **383**: 361–370.
56. Oakes SA, Opferman JT, Pozzan T, Korsmeyer SJ, Scorrano L. Regulation of endoplasmic reticulum Ca2+ dynamics by proapoptotic BCL-2 family members. *Biochem Pharmacol* 2003; **66**: 1335–1340.
57. Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, Pozzan T *et al.* BAX and BAK regulation of endoplasmic reticulum Ca2+: a control point for apoptosis. *Science* 2003; **300**: 135–139.
58. Demaurex N, Distelhorst C. Cell biology. Apoptosis – the calcium connection. *Science* 2003; **300**: 65–67.
59. Jiao J, Huang X, Feit-Leithman RA, Neve RL, Snider W, Dartt DA *et al.* Bcl-2 enhances Ca(2+) signaling to support the intrinsic regenerative capacity of CNS axons. *EMBO J* 2005; **24**: 1068–1078.
60. Hoyer-Hansen M, Bastholm L, Szyniarowski P, Campanella M, Szabadkai G, Farkas T *et al.* Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-beta, and Bcl-2. *Mol Cell* 2007; **25**: 193–205.
61. Perfettini J, Roumier T, Kroemer G. Mitochondrial fusion and fission in the control of apoptosis. *Trends Cell Biol* 2005; **15**: 179–183.
62. Germain M, Mathai JP, McBride HM, Shore GC. Endoplasmic reticulum BIK initiates DRP1-regulated remodelling of mitochondrial cristae during apoptosis. *EMBO J* 2005; **24**: 1546–1556.
63. Wasiak S, Zunino R, McBride HM. Bax/Bak promote sumoylation of DRP1 and its stable association with mitochondria during apoptotic cell death. *J Cell Biol* 2007; **177**: 439–450.
64. Zamzami N, El Hamel C, Maisse C, Brenner C, Munoz-Pinedo C, Belzacq AS *et al.* Bid acts on the permeability transition pore complex to induce apoptosis. *Oncogene* 2000; **19**: 6342–6350.
65. Kamer I, Sarig R, Zaltsman Y, Niv H, Oberkovitz G, Regev L *et al.* Proapoptotic BID is an ATM effector in the DNA-damage response. *Cell* 2005; **122**: 593–603.
66. Hosler JP, Ferguson-Miller S, Mills DA. Energy transduction: proton transfer through the respiratory complexes. *Annu Rev Biochem* 2006; **75**: 165–187.
67. Li K, Li Y, Shelton JM, Richardson JA, Spencer E, Chen ZJ *et al.* Cytochrome *c* deficiency causes embryonic lethality and attenuates stress-induced apoptosis. *Cell* 2000; **101**: 389–399.
68. Cain K, Bratton SB, Cohen GM. The Apaf-1 apoptosome: a large caspase-activating complex. *Biochimie* 2002; **84**: 203–214.
69. Garrido C, Galluzzi L, Brunet M, Puig PE, Didelot C, Kroemer G. Mechanisms of cytochrome *c* release from mitochondria. *Cell Death Differ* 2006; **13**: 1423–1433.
70. Arama E, Agapite J, Steller H. Caspase activity and a specific cytochrome *c* are required for sperm differentiation in *Drosophila*. *Dev Cell* 2003; **4**: 687–697.
71. Woo M, Hakem R, Furlonger C, Hakem A, Duncan GS, Sasaki T *et al.* Caspase-3 regulates cell cycle in B cells: a consequence of substrate specificity. *Nat Immunol* 2003; **4**: 1016–1022.
72. Yang JY, Widmann C. A subset of caspase substrates functions as the Jekyll and Hyde of apoptosis. *Eur Cytokine Netw* 2002; **13**: 404–406.
73. Joza N, Pospisilik JA, Benit P, Rangachari M, Nakashima Y, Rustin P *et al.* The molecular archeology of a caspase-independent death effector: AIF in *Drosophila*. *Cell Death Differ* 2008; in press.
74. Miramar MD, Costantini P, Ravagnan L, Saraiva LM, Haouzi D, Brothers G *et al.* NADH oxidase activity of mitochondrial apoptosis-inducing factor. *J Biol Chem* 2001; **276**: 16391–16398.
75. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM *et al.* Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* 1999; **397**: 441–446.
76. Modjtahedi N, Giordanetto F, Madeo F, Kroemer G. Apoptosis-inducing factor: vital and lethal. *Trends Cell Biol* 2006; **16**: 264–272.
77. Cande C, Vahsen N, Kouranti I, Schmitt E, Daugas E, Spahr C *et al.* AIF and cyclophilin A cooperate in apoptosis-associated chromatinolysis. *Oncogene* 2004; **23**: 1514–1521.
78. Zhu C, Wang X, Deinum J, Huang Z, Gao J, Modjtahedi N *et al.* Cyclophilin A participates in the nuclear translocation of apoptosis-inducing factor in neurons after cerebral hypoxia–ischemia. *J Exp Med* 2007; **204**: 1741–1748.
79. Cande C, Vahsen N, Metivier D, Tourriere H, Chebli K, Garrido C *et al.* Regulation of cytoplasmic stress granules by apoptosis-inducing factor. *J Cell Sci* 2004; **117**: 4461–4468.
80. Joza N, Oudit GY, Brown D, Benit P, Kassiri Z, Vahsen N *et al.* Muscle-specific loss of apoptosis-inducing factor leads to mitochondrial dysfunction, skeletal muscle atrophy, and dilated cardiomyopathy. *Mol Cell Biol* 2005; **25**: 10261–10272.
81. Vahsen N, Cande C, Briere JJ, Benit P, Joza N, Larochette N *et al.* AIF deficiency compromises oxidative phosphorylation. *EMBO J* 2004; **23**: 4679–4689.
82. Klein JA, Longo-Guess CM, Rossmann MP, Seburn KL, Hurd RE, Frankel WN *et al.* The harlequin mouse mutation downregulates apoptosis-inducing factor. *Nature* 2002; **419**: 367–374.
83. Pospisilik JA, Knauf C, Joza N, Benit P, Orthofer M, Cani PD *et al.* Targeted deletion of AIF decreases mitochondrial oxidative phosphorylation and protects from obesity and diabetes. *Cell* 2007; **131**: 476–491.
84. Li LY, Luo X, Wang X. Endonuclease G is an apoptotic DNase when released from mitochondria. *Nature* 2001; **412**: 95–99.
85. Huang KJ, Ku CC, Lehman IR. Endonuclease G: a role for the enzyme in recombination and cellular proliferation. *Proc Natl Acad Sci USA* 2006; **103**: 8995–9000.
86. Storchova Z, Breneman A, Cande J, Dunn J, Burbank K, O'Toole E *et al.* Genome-wide genetic analysis of polyploidy in yeast. *Nature* 2006; **443**: 541–547.
87. Buttner S, Carmona-Gutierrez D, Vitale I, Castedo M, Ruli D, Eisenberg T *et al.* Depletion of endonuclease G selectively kills polyploid cells. *Cell Cycle* 2007; **6**: 1072–1076.
88. Gray CW, Ward RV, Karran E, Turconi S, Rowles A, Viglienghi D *et al.* Characterization of human HtrA2, a novel serine protease involved in the mammalian cellular stress response. *Eur J Biochem* 2000; **267**: 5699–5710.
89. Lipinska B, Zylicz M, Georgopoulos C. The HtrA (DegP) protein, essential for *Escherichia coli* survival at high temperatures, is an endopeptidase. *J Bacteriol* 1990; **172**: 1791–1797.
90. Verhagen AM, Silke J, Ekert PG, Pakusch M, Kaufmann H, Connolly LM *et al.* HtrA2 promotes cell death through its serine protease activity and its ability to antagonize inhibitor of apoptosis proteins. *J Biol Chem* 2002; **277**: 445–454.

91. Suzuki Y, Imai Y, Nakayama H, Takahashi K, Takio K, Takahashi R. A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death. *Mol Cell* 2001; **8**: 613–621.
92. Yang QH, Church-Hajduk R, Ren J, Newton ML, Du C. Omi/HtrA2 catalytic cleavage of inhibitor of apoptosis (IAP) irreversibly inactivates IAPs and facilitates caspase activity in apoptosis. *Genes Dev* 2003; **17**: 1487–1496.
93. Vande Walle L, Van Damme P, Lamkanfi M, Saelens X, Vandekerckhove J, Gevaert K *et al.* Proteome-wide Identification of HtrA2/Omi Substrates. *J Proteome Res* 2007; **6**: 1006–1015.
94. Kuninaka S, Iida SI, Hara T, Nomura M, Naoe H, Morisaki T *et al.* Serine protease Omi/HtrA2 targets WARTS kinase to control cell proliferation. *Oncogene* 2007; **26**: 2395–2406.
95. Jones JM, Datta P, Srinivasula SM, Ji W, Gupta S, Zhang Z *et al.* Loss of Omi mitochondrial protease activity causes the neuromuscular disorder of mnd2 mutant mice. *Nature* 2003; **425**: 721–727.
96. Martins LM, Morrison A, Klupsch K, Fedele V, Moisoi N, Teismann P *et al.* Neuroprotective role of the Reaper-related serine protease HtrA2/Omi revealed by targeted deletion in mice. *Mol Cell Biol* 2004; **24**: 9848–9862.
97. Plun-Favreau H, Klupsch K, Moisoi N, Gandhi S, Kjaer S, Frith D *et al.* The mitochondrial protease HtrA2 is regulated by Parkinson's disease-associated kinase PINK1. *Nat Cell Biol* 2007; **9**: 1243–1252.
98. Vaux DL, Silke J. HtrA2/Omi, a sheep in wolf's clothing. *Cell* 2003; **115**: 251–253.
99. Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, Reid GE *et al.* Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. *Cell* 2000; **102**: 43–53.
100. Du C, Fang M, Li Y, Li L, Wang X. Smac, a mitochondrial protein that promotes cytochrome *c*-dependent caspase activation by eliminating IAP inhibition. *Cell* 2000; **102**: 33–42.
101. Wu G, Chai J, Suber TL, Wu JW, Du C, Wang X *et al.* Structural basis of IAP recognition by Smac/DIABLO. *Nature* 2000; **408**: 1008–1012.
102. Vaux DL, Silke J. Mammalian mitochondrial IAP binding proteins. *Biochem Biophys Res Commun* 2003; **304**: 499–504.
103. Okada H, Suh WK, Jin J, Woo M, Du C, Elia A *et al.* Generation and characterization of Smac/DIABLO-deficient mice. *Mol Cell Biol* 2002; **22**: 3509–3517.
104. Jia L, Patwari Y, Kelsey SM, Srinivasula SM, Agrawal SG, Alnemri ES *et al.* Role of Smac in human leukaemic cell apoptosis and proliferation. *Oncogene* 2003; **22**: 1589–1599.
105. Algeciras-Schimnich A, Shen L, Barnhart BC, Murmann AE, Burkhardt JK, Peter ME. Molecular ordering of the initial signaling events of CD95. *Mol Cell Biol* 2002; **22**: 207–220.
106. Tinel A, Tschopp J. The PIDDosome, a protein complex implicated in activation of caspase-2 in response to genotoxic stress. *Science* 2004; **304**: 843–846.
107. Berube C, Boucher LM, Ma W, Wakeham A, Salmena L, Hakem R *et al.* Apoptosis caused by p53-induced protein with death domain (PIDD) depends on the death adapter protein RAIDD. *Proc Natl Acad Sci USA* 2005; **102**: 14314–14320.
108. Zermati Y, Mouhamad S, Stergiou L, Besse B, Galluzzi L, Boehrer S *et al.* Nonapoptotic role for apaf-1 in the DNA damage checkpoint. *Mol Cell* 2007; **28**: 624–637.
109. Mouhamad S, Galluzzi L, Zermati Y, Castedo M, Kroemer G. Apaf-1 deficiency causes chromosomal instability. *Cell Cycle* 2007; **6**: 3103–3107.
110. Park SM, Schickel R, Peter ME. Nonapoptotic functions of FADD-binding death receptors and their signaling molecules. *Curr Opin Cell Biol* 2005; **17**: 610–616.
111. Yeh WC, Pompa JL, McCurrach ME, Shu HB, Elia AJ, Shahinian A *et al.* FADD: essential for embryo development and signaling from some, but not all, inducers of apoptosis. *Science* 1998; **279**: 1954–1958.
112. Walsh CM, Wen BG, Chinnaiyan AM, O'Rourke K, Dixit VM, Hedrick SM. A role for FADD in T cell activation and development. *Immunity* 1998; **8**: 439–449.
113. Zhang Y, Rosenberg S, Wang H, Imtiyaz HZ, Hou YJ, Zhang J. Conditional Fas-associated death domain protein (FADD): GFP knockout mice reveal FADD is dispensable in thymic development but essential in peripheral T cell homeostasis. *J Immunol* 2005; **175**: 3033–3044.
114. Pellegrini M, Bath S, Marsden VS, Huang DC, Metcalf D, Harris AW *et al.* FADD and caspase-8 are required for cytokine-induced proliferation of hemopoietic progenitor cells. *Blood* 2005; **106**: 1581–1589.
115. Arechiga AF, Bell BD, Leverrier S, Weist BM, Porter M, Wu Z *et al.* A Fas-associated death domain protein/caspase-8-signaling axis promotes S-phase entry and maintains S6 kinase activity in T cells responding to IL-2. *J Immunol* 2007; **179**: 5291–5300.
116. Sheikh MS, Huang Y. The FADD is going nuclear. *Cell Cycle* 2003; **2**: 346–347.
117. Alappat EC, Feig C, Boyerinas B, Volkland J, Samuels M, Murmann AE *et al.* Phosphorylation of FADD at serine 194 by CKIalpha regulates its nonapoptotic activities. *Mol Cell* 2005; **19**: 321–332.
118. Balachandran S, Venkataraman T, Fisher PB, Barber GN. Fas-associated death domain-containing protein-mediated antiviral innate immune signaling involves the regulation of Irf7. *J Immunol* 2007; **178**: 2429–2439.
119. Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell* 2003; **114**: 181–190.
120. Zheng L, Bidere N, Staudt D, Cubre A, Orenstein J, Chan FK *et al.* Competitive control of independent programs of tumor necrosis factor receptor-induced cell death by TRADD and RIP1. *Mol Cell Biol* 2006; **26**: 3505–3513.
121. Bender LM, Morgan MJ, Thomas LR, Liu ZG, Thorburn A. The adaptor protein TRADD activates distinct mechanisms of apoptosis from the nucleus and the cytoplasm. *Cell Death Differ* 2005; **12**: 473–481.
122. Janssens S, Tinel A, Lippens S, Tschopp J. PIDD mediates NF-kappaB activation in response to DNA damage. *Cell* 2005; **123**: 1079–1092.
123. Tinel A, Janssens S, Lippens S, Cuenin S, Logette E, Jaccard B *et al.* Autoproteolysis of PIDD marks the bifurcation between pro-death caspase-2 and pro-survival NF-kappaB pathway. *EMBO J* 2007; **26**: 197–208.
124. Cuenin S, Tinel A, Janssens S, Tschopp J. p53-induced protein with a death domain (PIDD) isoforms differentially activate nuclear factor-kappaB and caspase-2 in response to genotoxic stress. *Oncogene* 2008; **27**: 387–396.
125. Felmer R, Horvat S, Clinton M, Clark AJ. Overexpression of Raidd cDNA inhibits differentiation of mouse preadipocytes. *Cell Prolif* 2003; **36**: 45–54.
126. Motaln H, McWhir J, Horvat S. In situ analysis of Raidd-beta-galactosidase fusion gene expression in transgenic mouse midgestation embryos. *Transgenic Res* 2005; **14**: 27–40.
127. Zamzami N, Larochette N, Kroemer G. Mitochondrial permeability transition in apoptosis and necrosis. *Cell Death Differ* 2005; **12** (Suppl 2): 1478–1480.
128. Brenner C, Grimm S. The permeability transition pore complex in cancer cell death. *Oncogene* 2006; **25**: 4744–4756.
129. Vieira HL, Belzacq AS, Haouzi D, Bernassola F, Cohen I, Jacotot E *et al.* The adenine nucleotide translocator: a target of nitric oxide, peroxynitrite, and 4-hydroxynonenal. *Oncogene* 2001; **20**: 4305–4316.
130. Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, Yamagata H *et al.* Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. *Nature* 2005; **434**: 652–658.
131. Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA *et al.* Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* 2005; **434**: 658–662.
132. Galluzzi L, Kroemer G. Mitochondrial apoptosis without VDAC. *Nat Cell Biol* 2007; **9**: 487–489.
133. Baines CP, Kaiser RA, Sheiko T, Craigen WJ, Molkentin JD. Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. *Nat Cell Biol* 2007; **9**: 550–555.
134. Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, Jones DP *et al.* The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* 2004; **427**: 461–465.
135. Belzacq AS, Brenner C. The adenine nucleotide translocator: a new potential chemotherapeutic target. *Curr Drug Targets* 2003; **4**: 517–524.
136. Colombini M. Voltage gating in the mitochondrial channel, VDAC. *J Membr Biol* 1989; **111**: 103–111.
137. Gothel SF, Marahiel MA. Peptidyl-prolyl *cis–trans* isomerases, a superfamily of ubiquitous folding catalysts. *Cell Mol Life Sci* 1999; **55**: 423–436.
138. Schlattner U, Tokarska-Schlattner M, Wallimann T. Mitochondrial creatine kinase in human health and disease. *Biochim Biophys Acta* 2006; **1762**: 164–180.
139. Papadopoulo V. Peripheral benzodiazepine receptor: structure and function in health and disease. *Ann Pharm Fr* 2003; **61**: 30–50.
140. Wilson JE. Isozymes of mammalian hexokinase: structure, subcellular localization and metabolic function. *J Exp Biol* 2003; **206**: 2049–2057.
141. Delivani P, Adrain C, Taylor RC, Duriez PJ, Martin SJ. Role for CED-9 and Egl-1 as regulators of mitochondrial fission and fusion dynamics. *Mol Cell* 2006; **21**: 761–773.
142. Wissing S, Ludovico P, Herker E, Buttner S, Engelhardt SM, Decker T *et al.* An AIF orthologue regulates apoptosis in yeast. *J Cell Biol* 2004; **166**: 969–974.
143. Madeo F, Herker E, Maldener C, Wissing S, Lachelt S, Herlan M *et al.* A caspase-related protease regulates apoptosis in yeast. *Mol Cell* 2002; **9**: 911–917.
144. Lim HW, Kim SJ, Park EH, Lim CJ. Overexpression of a metacaspase gene stimulates cell growth and stress response in Schizosaccharomyces pombe. *Can J Microbiol* 2007; **53**: 1016–1023.
145. Ambit A, Fasel N, Coombs GH, Mottram JC. An essential role for the Leishmania major metacaspase in cell cycle progression. *Cell Death Differ* 2008; **15**: 113–122.
146. Siegel RM. Caspases at the crossroads of immune-cell life and death. *Nat Rev Immunol* 2006; **6**: 308–317.