Review

Redundant cell death mechanisms as relics and backups

P Golstein*,1 and G Kroemer2

1 Centre d'Immunologie de Marseille-Luminy, CNRS-INSERM-Université de la
  Mediterranée, Parc Scientifique de Luminy, Case 906, 13288 Marseille cedex
  9, France
2 Centre National de la Recherche Scientifique, UMR8125, Institut Gustave
  Roussy, 39 rue Calmette Desmoulins, F-94805 Villejuif, France
* Corresponding author: P Golstein, Centre d'Immunologie de Marseille-Luminy,
  CNRS-INSERM-Université de la Mediterranée, Parc Scientifique de Luminy,
  Case 906, 13288 Marseille cedex 9, France.
  Tel: + 33 4 91 26 94 68; Fax: + 33 4 91 26 94 30;
  E-mail: golstein@ciml.univ-mrs.fr

Abstract

**Here we review recent observations indicating the existence
of redundant cell death mechanisms. We speculate that this
redundancy reflects a particular evolutionary history for
cellular demise. Autophagic or apoptotic elements might have
been added to a primordial death mechanism, initially
improving cell dismantling and later acquiring the ability to
act themselves as death effectors. The resulting redundancy
of cell death mechanisms has pathophysiological implica-
tions.**

Introduction

While specific cell death pathways have usually been studied
as isolated phenomena (as exemplified by the extensive
research on caspase-dependent apoptosis), cell death can be
considered as a network of elementary mechanisms, acting in
series or in parallel, each of which can be variably expressed
in different eukaryotic species and, within the same species,
among distinct cell types. In the course of evolution, an
ancestral cell death mechanism, which may be extant in the
form of necrosis, may have become embellished with
elements of apoptosis and/or of autophagy, as both represent
a selective advantage since they contribute to the elimination
of cell corpses. These processes may in turn have become
autonomous cell death mechanisms, and in some cases have
even gained exclusivity. This evolutionary accumulation of cell
death mechanisms may have resulted in the availability of
more than one death effector mechanisms within each extant
cell, with important implications for the detection and
therapeutic manipulation of cell death.

Cell Death Types, Mechanisms and Stages

Different types of cell death were defined in purely morpho-
logical terms,[1–4] with limited reference to the underlying
biochemical changes. Mammalian cells can die from 'apop-
tosis' (showing nuclear condensation and fragmentation, DNA
fragmentation and cytoplasmic condensation), 'necrosis'
(generalized swelling of cells and organelles with limited or
no chromatin condensation), and 'autophagic cell death'
(death with accumulation of autophagosomes and subse-
quently vacuoles). Several intermediate or mixed cell death
types have been reported, such as 'apoptosis-like' cell death
with a less pronounced, subapoptotic chromatin condensa-
tion,[5] paraptosis,[6] oncosis,[3] necrapoptosis with cytoplasmic
vacuolization and pronounced mitochondrial swelling not
seen in apoptosis,[7] and apoptosis with autophagic vacuoliza-
tion.[8,9] Importantly, different types of cell death can be
induced in a context-dependent fashion by the very same
initiating stimulus, for instance by binding of specific ligands to
so-called death receptors anchored in the plasma membrane
(Figure 1).

A schematic and simplified view of the molecular mechan-
isms underlying the main cell death types — in mammalian
cells — may be as follows. Apoptosis involves the near-to-
obligatory activation of caspases, a family of cysteine
proteases. Autophagic cell death shows autophagy, namely
the sequestration of cytoplasmic organelles in autophago-
somes, followed by their fusion with lysosomes, a process that
results in the digestion of the luminal content within
autophagolysosomes, and requires a series of well-charac-
terized autophagy gene products.[10] Necrosis is likely to result,
at least in part, from severe ATP depletion.[11]

Such cell death pathways can be divided into distinct stages
(Figure 1). During the initial *signaling* stage, cells integrate
metabolic stress signals, sense DNA damage, or receive
critical information from the extracellular space or from
neighboring cells, often through specific cell surface receptors.
The subsequent *effector* stage is marked by the first
irreversible biochemical event (also designated as point-of-
no-return), after which the removal of the death-inducing
stimulus can no longer prevent the cell's predetermined
course to death. This point-of-no-return remains a hypothe-
tical, empirically defined entity (the point from which cells
cannot recover), which lacks a clearcut biochemical definition.
The final *dismantling* stage then contributes to the disposal/
degradation of the cell corpse. The importance of this final
stage especially in caspase-dependent apoptosis is empha-
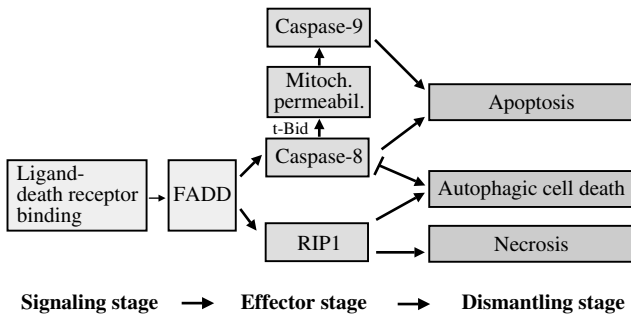sized by the existence of specialized pathways for dismantling

Figure 1 Death receptor-mediated activation of multiple cell death types. Upon engagement by their ligands, cell death receptors such as TNFR or CD95/Fas, transduce a signal that may involve the clustering of FADD. This frequently leads to apoptosis through activation of caspase-8. Upon caspase inhibition or in certain cell types, another pathway, which relies on RIP1, can lead to necrosis. In yet other circumstances, inhibition of caspase-8 can trigger autophagic cell death through RIP1. Different death receptors may vary in their mode of connexion to caspase-8 and RIP1.[73,74]. For this and other figures, see main text for other references and details

cell structures, such as the pathways leading to DNA fragmentation[12] or to cell surface blebbing.[13] At least in the animal kingdom, this systematic and structure-specific dismantling, moreover followed by rapid engulfment of dying cells,[14,15] strongly suggests that there is a strict requirement for swift and complete disappearance of dead cells. Autophagy may ensure dismantling for some cell deaths both inside and outside of the animal kingdom, and in the latter kingdom recruitment of caspases as an extra tool for dismantling may have represented a further evolutionary advantage. The selective benefit of efficient dismantling will be considered again below, when discussing the possible phylogeny of cell death types.

Inhibition/Replacement of Cell Death Types

In some model organisms (such as *Caenorhabditis elegans*), inactivation of cell death-associated hydrolases (e.g. CED-3, the *C. elegans* ortholog of mammalian caspase-3 and -9) or their activators (e.g. CED-4, the *C. elegans* ortholog of mammalian Apaf-1) can abolish somatic cell death during development.[16] However, in many other examples, the removal of such molecules does not prevent cell death as such and rather suppresses the manifestation of a particular cell death mechanism, revealing another one. Cells still die, but differently.

An example of such a situation is the death receptor pathway, in which ligand engagement of death receptors (for instance TNF-R1 or CD95/Fas) can trigger distinct lethal mechanisms. Engagement of the death receptor leads to the recruitment of the adaptor protein FADD, which in turn can recruit and activate caspase-8 (then leading to apoptosis) or RIP1 (Figure 1). Depending on cells, conditions and stimuli, inhibition of caspase-8 can truly interrupt lethal signaling, favor a switch from apoptotic to necrotic cell death,[17–19] which depends on RIP1,[20,21] or induce (again RIP1-dependent) autophagic cell death.[22] Caspase inhibition has also been shown to lead to a switch from apoptosis to necrosis in, for

example, thymocytes responding to DNA damage[23] or from apoptosis to autophagic cell death in, for example, growth factor-deprived neurons.[24] Furthermore, conditions that normally induce apoptosis through cellular stress (e.g. DNA damage or inhibition of protein kinases with staurosporine) induce necrosis when ATP supply is limited.[11] Downmodulation of the antiapoptotic protein Bcl-2[25] or upregulation of the proapoptotic Bcl-2 homolog BNIP3[26] can induce death accompanied by autophagic vacuolization. In mouse embryonic fibroblasts lacking the proapoptotic proteins Bax and Bak, alkylating DNA damage induces a necrotic type of cell death that relies on ATP depletion,[27] and etoposide induces an autophagic type of cell death dependent on autophagy proteins ATG5 and beclin,[28] while both types of damage induce apoptosis in Bax- or Bak-sufficient control cells. Conversely, inhibition of macroautophagy can provoke caspase-dependent apoptosis in starving HeLa cells.[29]

Another example is provided by mice in which the *ced-3* or *ced-4* orthologs (*casp3, casp9, apaf1*) have been knocked out. Such mice are deficient in a main caspase activation pathway, yet show relatively minor developmental defects, mostly forebrain hyperplasia.[30–33] Thus, most of the cell death associated with normal development in wild-type mice occurs also in the mutant mice, either through alternative caspase activation or through caspase-independent nonapoptotic cell death. Indeed, in *apaf1*$^{-/-}$ mice, interdigital cell death occurs through caspase-independent necrosis rather than through apoptosis[34] (Figure 2). Similarly, an histone deacetylase inhibitor triggers autophagic cell death in mouse embryonic
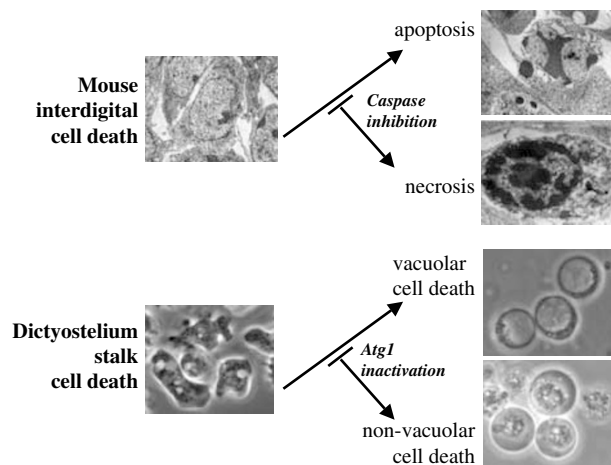

Figure 2 Two examples of inhibition/replacement of cell death types in development. The upper panel shows electron microscopy pictures of dying interdigital cells in a mouse embryo. Caspase inhibition led to a shift from apoptotic (marked apoptotic condensation, also in apoptotic bodies engulfed in adjacent cells) to necrotic (one necrotic cell is shown, with more disperse chromatin condensation and dilated mitochondria) cell death.[34] The lower panel shows light microscopy pictures of dying Dictyostelium cells, in an *in vitro* model mimicking development in this protist. Autophagy inhibition led to a shift from vacuolar (three cells are shown, each with a very large vacuole occupying most of the cell volume) to nonvacuolar (amid debris, three cells are shown, each with central organelle clustering and wih no vacuole) cell death.[42] In either example, distinct types of cell death can be triggered by the same developmental signal. Note that it is not formally demonstrated whether it is only the dismantling mechanisms, or also the effector mechanisms, which are affected by the experimental manipulation

fibroblasts from *apaf1*$^{-/-}$ mice, instead of apoptosis in wild-type control cells.[35] In some nonforebrain regions of the nervous system of *casp3*$^{-/-}$ and *casp9*$^{-/-}$ mice, the frequency and localization of dying cells was undistinguishable from wild-type controls, but the type of cell death was nonapoptotic, with marked vacuolization.[36] Similar findings were made for motoneurons in chick embryos upon caspase inhibition.[37] The differential behavior of forebrain and nonforebrain neurons in the mutant mice discussed above indicates that distinct cell types (within the same species and the same organ) can house distinct potential cell death mechanisms. Similarly, in some tissues of adult *C. elegans* necrotic cell death can occur,[38] suggesting that the nonredundancy of apoptotic developmental cell death in this organism, as analyzed by Horvitz *et al*,[39] is due to tissue-specific expression of only one of several possible cell death mechanisms. In other words, cell differentiation can reduce the spectrum of cell death mechanisms available to a given species.

Also in a developmental context, but now in a protist model, *Dictyostelium discoideum* cells can be induced to die *in vitro* through starvation and addition of a differentiation factor called DIF, thus mimicking developmental induction of stalk cell death *in vivo*. Dying cells exhibit autophagy and massive vacuolization.[40,41] Disruption of the *Atg1* gene (impairing autophagy) suppresses vacuolization but not DIF-induced cell death. Atg1 mutant cells exhibit a distinct nonvacuolar morphotype when they die upon addition of DIF. Thus, impairing autophagy causes a switch from vacuolar to nonvacuolar DIF-induced cell death[42] (Figure 2). With some reserve due to uncertainties as to vacuolar cell death,[42] these results show that inhibition/replacement of cell death types can also occur outside the animal kingdom.

In animals, distinct mechanisms might intersect through shared molecules such as Beclin-1/Atg-6 (which interacts with the apoptosis regulator Bcl-2, yet is also essential for autophagy[22]) or DAP kinase (a positive regulator of autophagy and apoptotic blebbing[43]) or exert unidirectional inhibition of one on the other, as has been shown for proapoptotic caspases, which can repress necrosis[44] or antiapoptotic Bcl-XL which can facilitate autophagic cell death.[28] The latter interdeath mechanism inhibition, for which as yet there are only few examples, may well account for the masking of one mechanism by another in cells undergoing death.

In conclusion, different cell death types can substitute for each other in experimental and perhaps as discussed below in pathological circumstances. Apoptosis can be replaced by necrotic or autophagic cell death, while the latter can be replaced by apoptotic or necrotic cell death. Thus, there is no general evidence in favor of a hierarchical relationship between apoptosis and autophagic cell death. In contrast, there are relatively few reports on experimental interventions shifting necrosis to apoptosis or autophagic cell death.[21,45] Beyond the fact that the poor knowledge of the molecular mechanism governing necrosis makes it difficult to inhibit this type of death, this might point to a stratification of cell death mechanisms, perhaps reflecting their order of emergence in evolution. Apoptosis and autophagy might serve as more elaborate 'functionally dominant' mechanisms, which, when inhibited, may reveal a more primitive mode of cell death, necrosis.

Hypothetical Evolution of Cell Death Mechanisms

How did the several cell death mechanisms emerge in evolution? We hypotesize that an ancestral cell death mechanism was overlayered with added mechanisms. A popular, plausible[46] yet speculative[47] scenario states that the ancestral cell death mechanism (which may have resembled 'necrosis') might stem from the intrusion of the bacterial precursor of the mitochondrion (the endosymbiont) into the precursor of the eukaryotic cell. In favor of the mitochondrial involvement in the emergence of cell death, it appears that some of the mitochondrial proteins which can be involved in mammalian cell death (such as AIF, cytochrome *c,* and the serine protease HtrA2/Omi) and in yeast cell death (AIF1)[48] are present in the genome of bacterial ancestors of mitochondria,[46,49] as are the evolutionary ancestors of caspases, the meta-caspases.[46]

As additions to such an ancestral cell death mechanism, while autophagy emerged in evolution before apoptosis and possibly initially only as a restorative pathway, both apoptosis and autophagy might have entered cell death pathways at the dismantling stage, in order to facilitate the disposal of cell corpses (Figure 3). We inferred above the probably important selective advantage of getting rid of the cell corpse, at least in the animal kingdom, as judged from the several sophisticated specialized pathways at play at the dismantling stage reinforced by further disposal of the dead cell corpse through an equally sophisticated engulfment process. Rapid degradation/clearance of dying cells may be beneficial through minimization of energy expenditure by dying cells, rapid recycling of building blocks from dead to live cells, quick liberation of the space occupied by corpses, and in vertebrates prevention of autoimmune responses. Thus, evolution may have favored the recruitment of elaborate dismantling mechanisms. Autophagy may ensure dismantling for some cell deaths in all eukaryotic kingdoms, while in the animal kingdom recruitment of caspases may have represented a further evolutionary advantage. These additional mechanisms (apoptotic caspase activation and autophagy) then may have extended their role from their original function — dismantling — to a participation in cell death effector mechanisms and thus acquired 'autonomy' and sometimes even 'exclusivity', in some species or cell types. Autonomy may have been achieved by coupling a lethal signal (or absence of a survival signal) and stress sensors to the immediate activation of caspases (e.g. CD95/Fas to caspase-8, nuclear DNA damage to caspase-2, ER stress to caspase-12), upstream of the phylogenetically ancient mitochondrial checkpoint. Exclusivity would then result from the suppression of the original checkpoint as well as that of all parallel pathways of lethal signal transduction (Figure 3). One example for an advanced degree of autonomisation is provided by a restricted fraction of cell types ('type-1 cells') in which CD95 ligation can result in massive caspase activation, overriding the mitochondrial checkpoint,[50] although such cells still can undergo mitochondrion-dependent apoptosis in response to DNA damage. An example of possible extension even to the signaling stage for the role of caspases is provided by the caspase-induced activation of
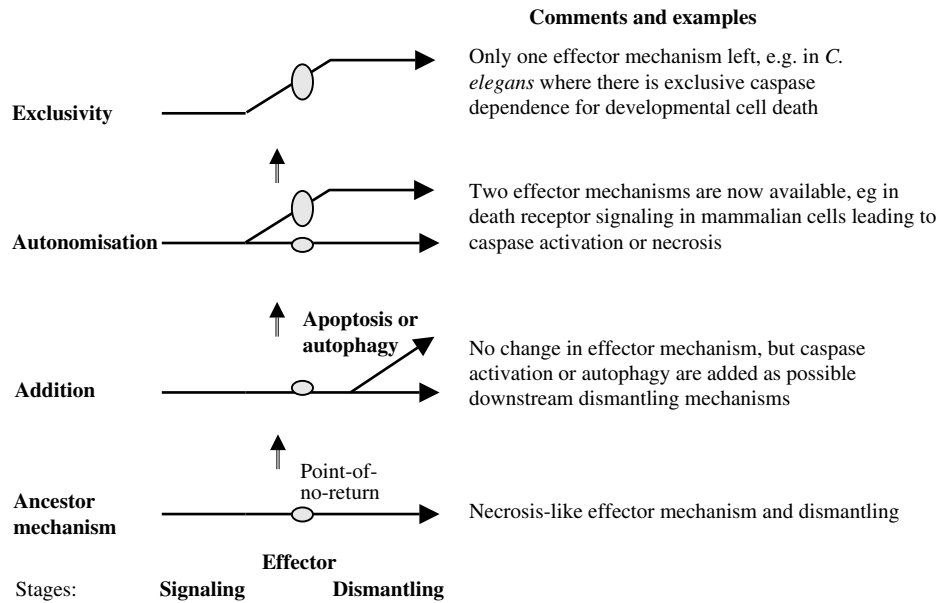
Comments and examples

Only one effector mechanism left, e.g. in *C. elegans* where there is exclusive caspase dependence for developmental cell death

Exclusivity

Autonomisation

Two effector mechanisms are now available, eg in death receptor signaling in mammalian cells leading to caspase activation or necrosis

Apoptosis or autophagy

Addition

No change in effector mechanism, but caspase activation or autophagy are added as possible downstream dismantling mechanisms

Ancestor mechanism

Point-of-no-return

Necrosis-like effector mechanism and dismantling

Effector

Stages: **Signaling**   **Dismantling**

Figure 3 A schematic hypothetical representation of cell death mechanism evolution. From lower to upper panels, initially the ancestral mechanism includes a point-of-no-return at the effector stage, which may have a mitochondrial basis as described in the main text, and may correspond to a necrotic type of cell death. At the next step, while the point-of-no-return would still be 'necrotic', addition at the dismantling stage of autophagic (in several eukaryotic kingdoms) or apoptotic (only in the animal kingdom in its classical caspase-dependent version) elements would allow necrotic, apoptotic or autophagic cell death types to develop. Further autonomization of apoptosis or autophagy would confer effector activity to them. The upper and extreme situation would occur when the ancestral mechanism is genetically lost in the whole organism or epigenetically suppressed in some tissues, leaving full effector activity in an exclusive way to either apoptosis or autophagy. The overall result is the replacement of the ancestral effector mechanism by the incoming mechanism originally operative in dismantling only

some dependence receptors.[51] An example of exclusivity is developmental cell death of somatic cells in *C. elegans*, an apoptotic cell death whose inhibition led to no death rather than to an alternative type of cell death.[39]

These and other data indicate that caspases can either participate in the dismantling phase only, or in both the effector and the dismantling phases. Although it is often assumed that autophagy can participate in the effector phase of cell death (at or around the point-of-no-return), thus far there is no definitive proof that genetic ablation of Atg genes (which are required for autophagy) can prevent cell death, in spite of suggestive evidence that autophagy is indeed involved in the lethal process.[10,52–54] Our provisional conclusion on this point is that caspases may participate in both the effector and the dismantling phases of apoptosis, while for autophagy only a dismantling role has been formally demonstrated. More generally, the hypothesis above predicts that in any given cell the multiplicity of cell death pathways may be greater at the dismantling than at the effector stage. This is not only of academic interest. Whether a cell that can show two types of death (and thus houses two mechanisms of dismantling) possesses only a single or two effector mechanism(s) is of obvious practical importance. We often do not know enough as yet to clearly identify dismantling mechanisms, let alone effector mechanisms.

Thus, cell death mechanisms emerging later in evolution would mask more ancient mechanisms, which would however persist, not only as phylogenetic relics but also as back-up mechanisms as further discussed below. Altogether, this scenario of cell death evolution would account for the widely

observed redundancy of cell death types, implying that suppression of one particular type of cell death (for instance apoptosis) may often cause the manifestation of another type of death (for instance autophagic or necrotic cell death).

Pathophysiological Implications

The redundancy of cell death mechanisms has wide-reaching pathophysiological and medical implications. The redundancy of effector mechanisms (as opposed to dismantling mechanisms) has important consequences for therapeutic inhibition of unwarranted cell death, while redundancy of dismantling mechanisms constitutes a challenge for the diagnostic detection of cell death.

It has often been tacitly assumed that mammalian cell death is mostly mediated by apoptosis. This consensus stems, in part, from the pioneering description of apoptosis as a defined cell death modality,[1] as well as from the early studies on *C. elegans* somatic cell death,[16] which, while elegantly and importantly demonstrating genetic control of apoptotic cell death, may have led researchers to apprehend and characterize caspase-dependent apoptosis while neglecting other cell death modalities. This bias was reinforced by wide spread identification of (apoptotically) dying cells with the TUNEL staining, a method which only detects a largely caspase-dependent type of DNA fragmentation,[55] and hence ignores nonapoptotic events.

There are indeed arguments in favor of a relatively high incidence of nonapoptotic cell deaths *in vivo*, especially in

conditions in which key apoptotic effectors are inhibited. As stated above, genetic inactivation or pharmacological inhibition of caspases may have only limited effects on developmental cell death. Knockout studies often revealed unexpected effects of caspases in differentiation and activation of particular cell types, yet did not reveal general developmental cell death impairment affecting multiple cell types.[56] Similarly, pharmacological caspase inhibition did not always rescue cells from pathological death, for instance in the context of lethal TNF-induced shock,[57] or of myocardial infarction and neurodegeneration.[58] In heart failure, cardiomyocytes show autophagic rather than apoptotic cell death.[59] One of the few diseases in which caspase inhibitors may yield convincing preclinical effects is septic shock,[60] which involves the contribution of caspases, not only as cellular demolition enzymes but also for the biosynthesis of proinflammatory cytokines. Thus, although caspase inhibition can successfully prevent the phenotypic manifestation of apoptosis, it often does not prevent cell death, which is likely to be executed through different mechanisms (including apoptosis-like cell death, autophagy and necrosis)[5,23,24,34,61] and perhaps through phagocytic recognition and destruction.[62,63] As mentioned above, it is possible that different cell types present in the same organism differ in their potential to execute cell death through distinct mechanisms. In this context, the tissue distribution of various effector mechanisms and of their redundancy may warrant a more detailed exploration, since it would determine the success of therapeutic approaches to control cell death.

In the face of the multiplicity of cell death types, how should cell death be detected? Apoptosis is commonly detected by techniques that measure postcaspase events, such as the cleavage of caspase substrates or the CAD-mediated degradation of nuclear DNA. As discussed above, nonapoptotic cell death mechanisms may play a significant role in mammals in developmental situations. Apoptosis may also be inhibited by pathogens (for instance by virus-encoded caspase inhibitors), in which case other lethal mechanisms may take over.[64,65] This emphasizes the need for tools able to detect non-apoptotic cell death in pathological tissues. Unfortunately, methods for the detection of autophagy applicable to whole animals are based on transgenics.[66] A histological method for the visualization of apoptosis-like cell death (which detects AIF in the nucleus)[67] has still to be validated *in vivo*. Optimal identification of necrotic cells *in vivo* would require electron microscopy, which is impractical for routine diagnostics. Reliable and convenient markers for easy *in situ* detection of nonapoptotic cell deaths are badly needed, yet elusive.

In the face of functional redundancy of cell death mechanisms, how can cell death be prevented? Attempts to reduce unwarranted cell death in human medicine must take into account the fact that therapeutic inhibition of a given mechanism may not necessarily inhibit cell loss and may rather unmask another mechanism of cell death. Serial unmasking is conceivable, whereby a cell usually dying from apoptosis might shift to autophagic death when caspases are inhibited and to necrosis when, in addition, Atg genes are inhibited. Caspase inhibition must either be combined with additional inhibitors (for instance of autophagy) to rescue cells

from death[22,24] or cell rescue must be attempted by targeting upstream events, perhaps in particular mitochondrial events. Thus, gene therapies and pharmacological agents capable of suppressing the cell death-associated mitochondrial membrane permeabilization have been shown to have a broad cytoprotective effect, in preclinical[68] as well as clinical studies.[69] This emphasizes the probable need to inactivate the putative ancestral, yet elusive cell death mechanism for cytoprotection, and therefore to direct further efforts towards a detailed molecular definition of this process. On the other hand, the reciprocal attempts at trying to increase cell death, in particular of cancer cells, are less affected by, and may even sometimes benefit from, the redundancy of mechanisms, provided care is taken to keep cell death selective. In certain circumstances, it may be even useful to shift from one cell death pathway to another, for instance by inhibiting apoptosis of cancer cells, because necrotic tumor cells may elicit a productive anticancer immune response.[70]

Any given cell contains a specific death-governing network associating different effector and/or dismantling mechanisms. It is not a given mechanism, but all possible effector elements of this network that should be inhibited if one wishes a given cell to survive. A major task ahead, at least for the less well known nonapoptotic types of cell death, will be to shift from the identification and study of cell death types, which reflect dismantling events, to an understanding of effector mechanisms. A second task will be to clarify for each cell the degree of multiplicity of these effector mechanisms. These include nonapoptotic cell death mechanisms, further analyses of which are therefore urgent. One approach to this end might be to enlist nonconventional model organisms,[71] especially outside of the animal kingdom, where experimental accessibility of nonapoptotic cell death might be easier and molecular redundancy might be less than in the usual animal models. The assumption here is that some cell death mechanisms may be conserved across kingdoms, which is perhaps not unreasonable considering the degree of phylogenetic conservation of other major cell functions. In parallel, a search for the molecular bases of nonapoptotic cell death mechanisms in the usual animal models might greatly benefit from novel approaches such as RNAi[72] and proteomics.

Acknowledgements

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