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Intracellular ATP, a switch in the decision between apoptosis and necrosis

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Abstract

Regardless of whether apoptosis or necrosis are elicited by toxicants or by pathophysiological conditions they are considered conceptually distinct forms of cell death. Nevertheless, there is increasing evidence that classical apoptosis and necrosis represent only the extreme ends of a wide range of possible morphological and biochemical deaths. The two classical types of demise can occur simultaneously in tissues or cell cultures exposed to the same stimulus and often, the intensity of the same initial insult decides the prevalence of either apoptosis or necrosis. The execution of the death program seems to involve a relatively limited number of pathways. In many instances, their ordered execution results in characteristic morphological and biochemical changes termed apoptosis. However, some subroutines of the degradation program may not be active in all cases of cell death. Then, the morphological appearance of dying cells and some of their biochemical alterations differ from those of classical apoptosis. We have recently shown that intracellular energy levels and mitochondrial function are rapidly compromised in necrosis, but not in apoptosis of neuronal cells. Then we went on to show that pre-empting human T cells of ATP switches the type of demise caused by two classic apoptotic triggers (staurosporin and CD95 stimulation) from apoptosis to necrosis. Conditions of controlled intracellular ATP depletion, which was obtained by blocking mitochondrial and/or glycolytic ATP generation were used in combination with repletion of the cytosolic ATP pool with glucose to redirect the death program towards apoptosis or necrosis.

Keywords: Apoptosis; ATP; Proteases

1. Apoptosis and necrosis: two entirely distinct forms of cell death?

The term 'programmed cell death' has been mainly used to describe the coordinated series of events leading to genetically controlled cell demise in developing organisms (Schwartz and Osborne, 1993). Various genetically encoded programs involved in signalling or in the initiation of cell death may in fact decide the fate of individual cells or organs during development. Nevertheless, one class of death-related genes, those expressing caspases (cysteine aspartases), can execute death signals in virtually every cell (Villa et al., 1997) and caspases seem to be constitutively expressed (Weil et al., 1996). The type of cell death is frequently defined by morphological criteria (Kerr et al., 1972). Indeed, the term apoptosis (Kerr et

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al., 1972) was initially introduced to define cells that shrink and undergo a series of typical nuclear changes including chromatin margination and condensation, while in the cytoplasm, organelles appear to be intact. The concept of a death program is, however, not necessarily linked to a morphological appearance. For example, in non-vertebrate systems, programmed cell death does not always display an apoptotic-like morphology (Schwartz et al., 1993). This suggests that the shape of cell death is controlled by multiple factors. Several forms and subroutines of the death program might have evolved because of their significance for tissues and organs and may not be common to all apoptosis models. This may be particularly relevant when considering that inhibiting one subroutine of the cell death program may not result in cell survival, but rather in a different shape of cell death (Hirsch et al., 1997; Leist et al., 1997; Melino et al., 1997). Evidence that cells triggered to undergo apoptosis are instead forced to die by necrosis when energy levels are rapidly compromised has been recently provided (Leist et al., 1997). Thus, the initial death signal is propagated without leading to the shape of apoptosis. This suggests the possible existence of different execution subroutines, which become active depending, at least in part, on the intensity of the death signal, and may involve several proteolytic systems.

2. Different subroutines of cell death: the mitochondrial and non-mitochondrial pathways

It is not surprising that initially simple death programs, developed early during phylogeny, undergo complex modifications in mammalian cells. Large gene families have evolved to provide a more intricate control of cell death in higher organisms, in part perhaps to accommodate the need of individual organ differentiation. Some characteristics of the original cell death machinery that would affect predominantly the shape of death may have become more significant or predominant in some subsets of mammalian cells. A further consequence of the increased complexity may be that an increasing number of feed-back loops gives rise to multiple possibilities of initiation, control and execution. Assuming that the whole core of the death program is highly conserved as shown by the pioneering studies in *C. elegans* (Yuan and Horvitz, 1990), it seems unlikely that a single linear pathway is solely responsible for the execution. Should this be the case, viruses and transformed cells capable of completely evading or shutting-down a single program would have evolved. Also, it is difficult to conceive a single linear pathway, which the plethora of signals causing mammalian cell death would converge on. In higher organisms, additional interrelated or independent pathways may have therefore developed to regulate death.

Caspases are constitutively expressed in mammals, similar to *ced-3* in *C. elegans* (Shaham and Horvitz, 1996; Weil et al., 1996). However, in mammalian cells, different sets of caspases may be recruited in different paradigms of cell death. Deletion of single caspases has only localised and partial effects on cell death (Kuida et al., 1995, 1996), and several forms of demise seem to be caspase-independent (Hirsch et al., 1997; Sarin et al., 1997; Xiang et al., 1997) or even inhibited by caspases (Vercammen et al., 1998). Indeed, other protease families have also been implicated in apoptotic cell death (Adjei et al., 1996; Grimm et al., 1996). Furthermore, in some cases, caspase inhibition does not alter the extent of cell death, but rather the shape of demise (Hirsch et al., 1997; Leist et al., 1997; Sarin et al., 1997; Xiang et al., 1997).

The existence of alternative and perhaps reverberating pathways may also warrant a more effective elimination of injured, unwanted cells. Recently, it has become clear that self-amplification of death signals requires the co-operation of mitochondrial and cytoplasmic factors. Under stressful conditions, the mitochondrial inner membrane can lose its impermeability to ions and other small molecules up to a molecular weight of approximately 2 kDa (Gunter et al., 1994). This event is defined as permeability transition (PT). Accumulating evidence supports the idea that PT is a controlled process, involving the formation of pores at contact sites between the inner and outer mitochondrial membranes. Pore opening can also occur following a selective proteolytic

step (activation of calpain or caspase-1) (Aguilar et al., 1996; Susin et al., 1997). PT may be closely related to cell death (apoptotic and necrotic) induced by a large variety of different stimuli. Extensive work performed in Dr. G. Kroemer's laboratory has shown that PT can lead to typically apoptotic nuclear alterations (Zamzami et al., 1996), possibly caused by the release of an apoptosis-inducing 50 kDa protein (AIF). PT and the release of AIF seem to be inhibited by Bcl-2, a ubiquitous negative controller of apoptosis (Zamzami et al., 1996). Alternatively, it has been proposed that mitochondria can release holocytochrome *c* (cyt-*c*) in cells undergoing apoptosis (Liu et al., 1996; Kluck et al., 1997; Yang et al., 1997). Cyt-*c* is a small protein that is, at least in part, loosely attached to the outer surface of the inner mitochondrial membrane. In contrast to the mechanisms operating after PT, cyt-*c* can also be released from energised mitochondria, i.e. when the integrity of the inner membrane is maintained. Notably, cyt-*c* release from energised mitochondria can also be prevented by Bcl-2 (Liu et al., 1996; Yang et al., 1997). Neither the mechanism of cyt-*c* release, nor the mechanism whereby PT and/or AIF/cyt-*c* release are negatively regulated have yet been elucidated. It is instead apparent that cyt-*c* in conjunction with a cytosolic protein named APAF-1 and either ATP or dATP can activate caspases (Liu et al., 1996).

3. ATP and the shape of cell death

In vivo, under pathological conditions, apoptosis and necrosis may often coexist (Leist et al., 1995) and previous work in our laboratory has shown that intracellular energy levels are rapidly dissipated in necrosis, but not in apoptosis of cultured neurons (Ankarcrona et al., 1995).

To examine the events that determine the mode of execution of cell death (apoptosis or necrosis) following exposure to a single insult, individual parts of the death program can be blocked by manipulating the intracellular ATP level. With this approach it has been possible to determine that when ATP levels were reduced, typical apoptotic stimuli caused necrosis instead (Leist et al., 1997). ATP could be either depleted or repleted to defined levels and for defined periods of time. Therefore, it has been possible to identify a defined period of time after the exposure of lymphoid cells to apoptogenic stimuli such as staurosporine or an agonistic anti-CD95 monoclonal antibody during which energy-dependent steps are required to complete the apoptotic program. If ATP concentrations are markedly reduced during this period, activation of downstream caspases and all most typical apoptotic changes are blocked. Stimulated cells die nonetheless. However, death has necrotic features. These findings provide direct evidence that the complete apoptotic program involves energy-requiring steps. More recent work suggests that one ATP requiring step may be at the level of the formation of the protein complex between Apaf-1, cyt-*c* and procaspases (Li et al., 1997). Lack of ATP at this step would prevent the resulting downstream degradative processes including caspase-3 activation, poly(ADP-ribose)-polymerase cleavage and lamin cleavage, and exposure of PS on the outer membrane.

Intracellular protein localisation or transport may also be relevant to determine the shape of cell death. Some death signals (e.g. those activated after irradiation or treatment with topoisomerase inhibitors) are predominantly generated within the nucleus. Since controllers and execution systems are believed to be located in the cytoplasm/mitochondria, then yet unknown death signals have to be transmitted from the nucleus to these compartments. Also, some caspases seem to be transported from the cytoplasm into mitochondria or the nucleus (Chandler et al., 1998). Thus, the permeability of the nuclear pore and/or the accessibility of mitochondrial sites may be relevant factors in deciding execution of individual subroutines in apoptosis (Yasuhara et al., 1997).

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