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Review

Multiple pathways of cytochrome *c* release from mitochondria in apoptosis

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Abstract

Release of cytochrome *c* from mitochondria is a key initiative step in the apoptotic process, although the mechanisms regulating permeabilization of the outer mitochondrial membrane and the release of intermembrane space proteins remain controversial. Here, we discuss possible scenarios of the outer membrane permeabilization. The mechanisms by which the intermembrane space proteins are released from mitochondria depend presumably on cell type and on the nature of the apoptotic stimulus. The variety of mechanisms that can lead to outer membrane permeabilization might explain diversities in the response of mitochondria to numerous apoptotic stimuli in different types of cells.

Keywords: Apoptosis; Mitochondria; Cytochrome *c*; Bcl-2 family; Permeability transition

1. Introduction

Apoptosis, an active, gene-regulated form of cell death, is involved in cell deletion during organogenesis and in the control of cell proliferation and differentiation in adult tissues, as well as in the pathogenesis of various diseases [1]. The biochemical machinery required for apoptotic cell death is constitutively present in virtually all mammalian cells and can be activated by a variety of extra- and intracellular signals.

In their seminal paper on apoptosis, Kerr et al. [1] proposed that this is a nuclear event and that mitochondria are not to be considered a part of the apoptotic process. The authors wrote that "the apoptotic body shows closely aggregated but apparently intact mitochondria of epithelial cell types". However, "when apoptotic bodies undergo a process within phagosomes, the matrix of mitochondria becomes electron-lucent and displays focal flocculent densities" [1].

Later, indirect evidence appeared pointing to a possible involvement of mitochondria in apoptosis. In one of the earliest publications, Bcl-2 protein, which blocks programmed cell death rather than affecting proliferation, was shown to be localized to the inner mitochondrial membrane, although the precise mechanism of its action was unclear [2]. Cells over-expressing Bcl-2 had a higher mitochondrial membrane potential ($\Delta\psi$) than wild-type cells. The increase in $\Delta\psi$ was suggested to be responsible for the enhanced survival of cells after TNF challenge [3]. The key role of mitochondria in apoptosis was further hypothesized by Richter [4]. According to his viewpoint, uncontrolled production of oxygen radicals, a common step in many models of apoptosis [5], stimulates Ca^{2+} release from mitochondria, followed by Ca^{2+} cycling. Subsequently, Ca^{2+} cycling causes mitochondrial uncoupling, drop of $\Delta\psi$, ATP depletion, massive disturbance of cellular Ca^{2+} homeostasis, and a direct stimulation of Ca^{2+}-dependent endonuclease(s). The importance of Ca^{2+} in apoptosis was reviewed in detail recently [6]. To explain the protective functions of Bcl-2, a model was proposed in which Bcl-2 regulates an antioxidant pathway at sites of free radical generation [7], although the detailed investigation of the location of Bcl-2 revealed that it is localized not in the inner but in the outer mitochondrial membrane [8].

Currently, it is widely accepted that mitochondria play a key role in the regulation of apoptosis [9]. Specifically, the release of different pro-apoptotic proteins that are normally present in the intermembrane space of these organelles has been observed during the early stages of apoptotic cell death [10,11]. Among these proteins is a component of the mitochondrial respiratory chain, cytochrome *c*. Once in the cytosol, cytochrome *c*

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interacts with its adaptor molecule, Apaf-1, resulting in the recruitment, processing and activation of pro-caspase-9 in the presence of dATP or ATP [12]. Caspase-9, in turn, cleaves and activates pro-caspase-3 and -7; these effector caspases are responsible for the cleavage of various proteins leading to biochemical and morphological features characteristic of apoptosis [13].

Mitochondrial outer membrane permeabilization is therefore considered a key initiative step in the apoptotic process, although the precise mechanisms regulating this event have remained elusive.

2. Mechanisms of mitochondrial outer membrane permeabilization

2.1. Induction of mitochondrial permeability transition

There are currently several mechanisms that might explain the mitochondrial outer membrane permeabilization. The first pathway, which may be engaged during both necrotic and apoptotic cell death, involves the induction of mitochondrial permeability transition (MPT) due to the opening of non-specific pores in the mitochondrial inner membrane followed by osmotic swelling of the mitochondrial matrix, mitochondrial uncoupling, rupture of the mitochondrial outer membrane, and the release of intermembrane space proteins including cytochrome *c* [14,15] (Fig. 1, upper left corner).

For a long time, MPT was regarded as the prime mechanism responsible for the permeabilization of the mitochondrial outer membrane. However, opening of pores in the inner membrane would lead to harmful consequences for the cell. If permeability transition and subsequent uncoupling of mitochondria would occur in a large subpopulation of the organelles, the mitochondria would start to actively hydrolyze cytosolic ATP (uncoupling-stimulated ATPase activity). As a result, the ATP content would drop causing a perturbation of cytosolic Ca^{2+} homeostasis and activation of various catabolic enzymes (proteases, phospholipases, etc). Hence, this model of mitochondrial outer membrane permeabilization may be most relevant during ischemia–reperfusion injury, or in response to cytotoxic stimuli resulting in localized mitochondrial Ca^{2+} overload (for recent review see [16]). However, transient pore opening might also occur whereby a small fraction of mitochondria would have open pores at a given time [17]; in this case, mitochondrial protein release would occur without observable large-amplitude swelling or drop in membrane potential of the entire organelle population. This process can be observed also under normal physiological conditions, especially in mitochondria located in close proximity to calcium "hot spots", microdomains, in which the local concentration of ionized calcium far exceeds the average concentration measured throughout the cytosol [18]. This local Ca^{2+} concentration might be high enough to induce Ca^{2+} overload and subsequent pore opening. Therefore, under the influence of apoptotic stimuli the frequency of such spontaneous pore opening and closure might increase, contributing to translocation of intermembrane space proteins into the cytosol.

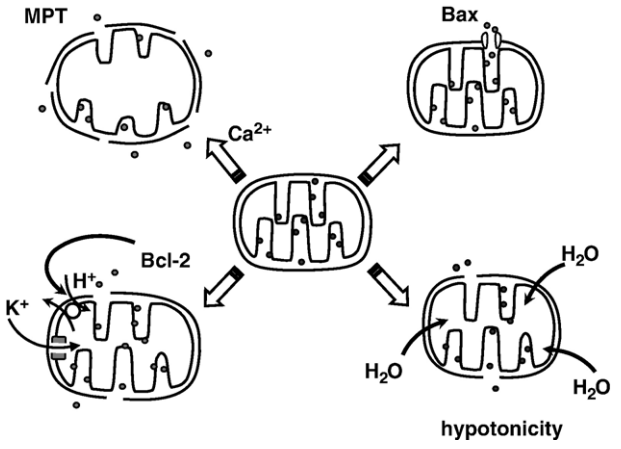

Fig. 1. Permeabilization of the outer mitochondrial membrane induced by mitochondrial permeability transition (upper left corner), pro-apoptotic Bcl-2 family proteins (upper right corner), modulation of ionic fluxes (lower left corner) and hypotonicity (lower right corner).

Recent observations have questioned the importance of MPT for the release of cytochrome *c* from the mitochondria under apoptotic conditions. Thus, overexpression of cyclophilin-D, a component of the MPT pore complex, had opposite effects on apoptosis and necrosis; whereas NO-induced necrosis was promoted, NO- and staurosporine-induced apoptosis was inhibited. These findings suggest that MPT leads to cell necrosis, but argue against its involvement in apoptosis [19]. Similarly, cyclophilin-D-deficient cells died normally in response to various apoptotic stimuli, but were resistant to necrotic cell death induced by reactive oxygen species and Ca^{2+} overload. In addition, cyclophilin-D-deficient mice showed resistance to ischemia/reperfusion-induced cardiac injury. These results suggest that the cyclophilin-D-dependent MPT regulates some forms of necrotic, but not apoptotic cell death [20,21].

2.2. Bcl-2 family proteins and mitochondrial outer membrane permeabilization

Another mechanism of outer membrane permeabilization involves members of the Bcl-2 family proteins (Fig. 1, upper right corner). The Bcl-2 family consists of more than 30 proteins, which can be divided into three subgroups: Bcl-2-like survival factors, Bax-like death factors, and BH3-only death factors. Residues from BH1, 2 and 3 form a hydrophobic groove, with which BH3-only death factors interact through their BH3 domain, whereas the N-terminal BH4 domain stabilizes this pocket (for a recent review see [22]).

Early indications of the importance of these proteins for the release of cytochrome *c* were obtained in 1997, when two groups independently showed that overexpression of Bcl-2 prevented the efflux of cytochrome *c* from the mitochondria in apoptotic cells as well as the initiation of apoptosis [23,24]. It was concluded that one possible mechanism by which Bcl-2 can prevent apoptosis is to block cytochrome *c* release from mitochondria.

The same year, the ability of Bax to stimulate cytochrome *c* release was demonstrated in yeast overexpressing Bax [25] (Fig. 1, upper right corner). It was found that Bax-induced growth