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Mitochondrial regulation of apoptotic cell death

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Abstract

Mitochondria play a decisive role in the regulation of both apoptotic and necrotic cell death. Permeabilization of the outer mitochondrial membrane and subsequent release of intermembrane space proteins are important features of both models of cell death. The mechanisms by which these proteins are released depend presumably on cell type and the nature of stimuli. Of the mechanisms involved, mitochondrial permeability transition appears to be associated mainly with necrosis, whereas the release of caspase activating proteins during early apoptosis is regulated primarily by the Bcl-2 family of proteins. However, there is increasing evidence for interaction and co-operation between these two mechanisms. The multiple mechanisms of mitochondrial permeabilization may explain diversities in the response of mitochondria to numerous apoptotic stimuli in different types of cells.

Keywords: Mitochondria; Apoptosis; Cytochrome *c*; Cardiolipin

1. Introduction

Apoptosis and necrosis are two modes of cell death with distinct morphological and biochemical features. Apoptosis is an active process characterized by cell shrinkage, nuclear and cytoplasmic condensation, chromatin fragmentation and phagocytosis of dying cells. In contrast, necrosis is a passive form of cell death associated with inflammation and certain forms of cell injury. One of the characteristic features of necrosis is cellular and organelle swelling, rupture of the plasma membrane and spilling of cellular contents into the extracellular milieu. Different toxicants may trigger either apoptotic or necrotic cell death, depending on the cell type and severity of insult. Further, completion of the apoptotic death program requires maintenance of a sufficient intracellular energy level and of a redox state compatible with caspase activation. Thus, ATP depletion or severe oxidative stress may re-direct otherwise apoptotic cell death to necrosis.

Mitochondria play a key role in the regulation of apoptotic cell death [1]. Specifically, different pro-apoptotic proteins, such as Cytochrome *c* and Smac/Diablo, which are normally present in the intermembrane space of these organelles are released during the early stages of apoptosis [2,3]. Once in the cytosol, Cytochrome *c* participates in the formation of the apoptosome complex together with its adaptor molecule, Apaf-1, resulting in the recruitment, processing and activation of pro-caspase-9 in the presence of dATP or ATP [4]. Subsequently, caspase-9 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 [5]. The release of Cytochrome *c* is therefore considered a key initiative step in the apoptotic process, although the precise mechanisms regulating this event remain unclear.

Several mechanisms have been proposed to explain the mitochondrial outer membrane permeabilization. The first pathway, which may be engaged during both

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necrotic and apoptotic cell death, involves the induction of mitochondrial permeability transition (MPT), which for a long time was regarded as the prime mechanism responsible for the permeabilization of the mitochondrial outer membrane.

1.1. Induction of mitochondrial permeability transition

This phenomenon was described some 30 years ago by Haworth and Hunter in a series of seminal papers [6–8], in which they showed that Ca^{2+} uptake stimulates drastic changes in mitochondrial morphology and functional activity due to the opening of a non-specific pore in the mitochondrial inner membrane, commonly known as the MPT pore.

The existence of a non-specific channel in the inner mitochondrial membrane was confirmed by electrophysiological experiments. A multiple conductance channel (MCC) has been identified by Kinnally et al. [9]. Zoratti and co-workers found a similar high-conductance channel in the membrane of rat liver mitoplasts (mitochondrial megachannel, MMC) [10]. Different characteristics of MMC, such as size, voltage dependence, activation by Ca^{2+} and inhibition by cyclosporin A (CSA), Mg^{2+}, Mn^{2+}, Sr^{2+}, H^+, and ADP, resembled those of the MPT pore [11]. Both channels, MCC and MMC, revealed similar conductance ranges and multiple substrates, including the "half-conductance" one. Interestingly, overexpression of Bcl-2 suppressed activation of MCC by calcium, indicating a possible involvement of this channel in apoptosis [12].

Measurements of single-channel current of excised patches with reconstituted purified mitochondrial adenine nucleotide translocase revealed the presence of a large cation-selective channel [13]. The properties of the channel were similar to the MPT pore, and resembled both MCC [9] and MMC channels [10]. The authors concluded that the adenine nucleotide translocase, when converted into a large unselective channel, is a key component of the MPT pore. The channel opening was proposed to be caused by binding of Ca^{2+} to cardiolipin, which is tightly bound to the adenine nucleotide translocase, thus releasing positive charges within the adenine nucleotide translocase to open the gate.

MPT results in osmotic swelling of the mitochondrial matrix, mitochondrial uncoupling, rupture of the mitochondrial outer membrane, and the release of intermembrane space proteins, including Cytochrome *c*, into the cytosol [14,15]. However, recent observations have questioned the importance of MPT as prime mechanism 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; while NO-induced necrosis was promoted, NO- and staurosporine-induced apoptosis was inhibited [16]. These findings suggest that MPT leads to cell necrosis, but argue against its involvement in apoptosis. Furthermore, recent genetic studies demonstrated that 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 [17,18]. In addition, cyclophilin-D-deficient mice showed resistance to ischemia/reperfusion-induced cardiac injury. These results also support the assumption that the cyclophilin-D-dependent MPT mediates some forms of necrotic cell death.

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 [19]). On the other hand, transient pore opening in a sub-fraction of mitochondria could result in the release mitochondrial proteins without observable large-amplitude swelling, or drop in membrane potential, of the entire organelle population [20]. This could occur in close proximity to calcium "hot spots" — microdomains, in which the local concentration of ionized calcium far exceeds the average cytosolic concentration [21]. This local Ca^{2+} elevation might be high enough to induce Ca^{2+} overload and subsequent pore opening in a sub-fraction of mitochondria. The frequency of such spontaneous pore opening and closure might increase under the influence of apoptotic stimuli, contributing to translocation of intermembrane space proteins into the cytosol.

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

The most important mechanism of outer membrane permeabilization under apoptotic conditions involves members of the Bcl-2 family of proteins. 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 BH1–3 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 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 overexpres-