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Review

Calcium and mitochondria in the regulation of cell death

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ABSTRACT

The calcium ion has long been known to play an important role in cell death regulation. Hence, necrotic cell death was early associated with intracellular Ca^{2+} overload, leading to mitochondrial permeability transition and functional collapse. Subsequent characterization of the signaling pathways in apoptosis revealed that Ca^{2+}/calpain was critically involved in the processing of the mitochondrially localized, Apoptosis Inducing Factor. More recently, the calcium ion has been demonstrated to play important regulatory roles also in other cell death modalities, notably autophagic cell death and anoikis. In this review, we summarize current knowledge about the mechanisms involved in Ca^{2+} regulation of these various modes of cell death with a focus on the importance of the mitochondria.

1. Introduction

Investigation of different modes of cell death has become an important area of biomedical research. Recently, several cell death modalities have been characterized based on both morphological and biochemical criteria. In 2009, the Nomenclature Committee on Cell Death proposed unified criteria for the definition of twelve cell death modalities [1]. Among the best characterized of these modes of cell death are apoptosis, autophagy, and necrosis. Until recently, a requirement for gene expression was documented only for apoptotic and autophagic cell death. However, accumulating evidence suggests that necrotic cell death might also be mediated by a specific set of signal transduction pathways and degradative mechanisms. The interaction between the different forms of cell death is complex and still a matter of debate, although the mitochondria have been demonstrated to play a crucial role in the effectuation of several cell death modalities and the cross-talk between them.

The Ca^{2+} ion has long been known to be critically involved in both the initiation and effectuation of cell death. Hence, necrosis was early found to be associated with a perturbation of intracellular Ca^{2+} homeostasis, and key events in the apoptotic process are known to be triggered by Ca^{2+} signals [2]. Similarly, some forms of autophagic cell death and anoikis have been shown to be Ca^{2+}-dependent. In this review, we shall discuss current knowledge about the role of Ca^{2+} in both the initiation and effectuation of cell death with a focus on the interplay between Ca^{2+} and the mitochondria.

2. Mitochondria, Ca^{2+} and necrosis

Necrosis has long been regarded as the result of an accidental and uncontrolled process, usually caused by factors external to the cell or tissue, such as infection, toxins, heat or trauma. It is characterized by disruption of the plasma membrane, cell swelling, chromatin digestion, DNA hydrolysis and, finally, cell lysis. Necrosis is often associated with local inflammation, triggered by the release of factors from dead cells that alert the innate immune system [3]. Necrosis is known to play a prominent role in many pathological conditions, including ischemia/reperfusion injury (e.g. stroke and myocardial infarction), trauma, and some forms of neurodegeneration.

The involvement of mitochondria in necrotic cell death has been known for a long time. Thus, a common cause of necrosis is the collapse of mitochondrial energy metabolism, leading to a drastic drop in ATP level. This, in turn, can result in intracellular Ca^{2+} overload and stimulation of various Ca^{2+}-dependent catabolic enzymes – phospholipases, proteases and endonucleases. Historically, the role of the Ca^{2+} ion as a death trigger dates back to Fleckenstein's observation that excess Ca^{2+} entry into cardiomyocytes underlies cardiac pathology after ischemia [4]. Subsequent studies emphasized the general importance of this finding,

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as both receptor overstimulation and many cytotoxic agents were found to cause lethal Ca^{2+} influx into cells (see Ref. [2] for review).

Many forms of toxic cell death were initially thought to be of the necrotic type and related to a perturbation of intracellular Ca^{2+} homeostasis. Hepatotoxicity caused by carbon tetrachloride, acetaminophen, or bromobenzene are classical examples thereof. Acetaminophen- and bromobenzene-induced cell death was studied intensely in the 1970's and found to be preceded by cytochrome P450-mediated formation of reactive metabolites, glutathione depletion, and disruption of Ca^{2+} homeostasis. Cytotoxicity was usually monitored by cellular leakage of lactate dehydrogenase, or uptake of trypan blue, traditional assays of the increased plasma membrane permeability associated with necrotic cell death.

2.1. Mitochondrial Ca^{2+} handling

The ability of mitochondria to accumulate Ca^{2+} was demonstrated by Vasington and Murphy [5] in the beginning of the 1960's. They postulated that mitochondrial accumulation of this ion depends on respiration and phosphorylation. Later, it was shown that mitochondria take up Ca^{2+} electrophoretically from the cytosol through a "Ca^{2+} uniporter", which could be inhibited by lanthanides or ruthenium red (RR). They can release it again via several different routes, including a calcium/sodium (excitable tissues) or a calcium/proton exchanger [6]. The uptake of Ca^{2+} is driven by the mitochondrial membrane potential, whereas its release in exchange for protons or sodium is electroneutral. The affinity for Ca^{2+} of the uniporter is low, and the size of the mitochondrial Ca^{2+} pool is small under physiological conditions. However, mitochondria can accumulate much larger amounts of Ca^{2+} under pathological conditions, when intracellular Ca^{2+} concentrations rise [7]. Hence, for many years mitochondrial Ca^{2+} uptake was regarded primarily as a safety device in situations of temporary intracellular Ca^{2+} overload. However, this view changed after the development of novel indicators, which can sense Ca^{2+} fluctuations in specific intracellular compartments [8]. Thanks to this technology, it has become apparent that mitochondrial Ca^{2+} fluxes are integrated parts of intracellular Ca^{2+} signaling. The low affinity of the mitochondrial Ca^{2+} import system is overcome by the proximity of the mitochondria to the endoplasmic reticulum (ER), where the local concentration of Ca^{2+} released from ER can reach very high levels [9]. Subsequent uptake of Ca^{2+} by the mitochondria stimulates the Ca^{2+}-sensitive matrix dehydrogenases, which provide NADH for mitochondrial respiration and ATP production.

For many years, the mitochondrial Ca^{2+} uniporter resisted purification and molecular characterization. However, based on early findings by Carafoli and Lehninger [6] that yeast mitochondria lack the uniporter, Perocchi et al. [10] used a comparative genomics strategy to identify human MICU1, a component of the mitochondrial uniporter complex. Soon thereafter, the pore-forming component, MCU, was identified and shown to be responsible for the RR-inhibitable Ca^{2+} uptake by mitochondria, and was also found to be capable of Ca^{2+} transfer through artificial membranes [11,12]. Using MCU as a handle, it was now possible to affinity-purify the uniporter holocomplex.

The discovery of the gene encoding the MCU protein was recently followed by the generation of knockout mice lacking the MCU [13]. Unexpectedly, these mice were found to be fully viable and showed impairment only in their ability to perform strenuous work. Mitochondria from MCU-deficient mice failed to undergo Ca^{2+}-induced permeability transition and did not respond to the permeability transition pore (PTP) inhibitor, cyclosporin A (see below). However, it is important to note that removal of MCU in zebrafish significantly influenced the formation of the notochord axis by controlling blastomere convergence and extension movements during gastrulation. Interestingly, the function of MCU in zebrafish as well as intracellular Ca^{2+} trafficking is controlled by a new Bcl-2-related multidomain apoptosis accelerator, Bcl-wav [14].

2.2. Ca^{2+}-induced mitochondrial permeability transition

Certain conditions, notably mitochondrial Ca^{2+} accumulation and oxidative stress, can trigger the opening of a high-conductance pore in the inner mitochondrial membrane (IMM) (Fig. 1). This phenomenon has been termed mitochondrial permeability transition (MPT) and is associated with drastic changes in mitochondrial morphology and functional activity [15]. Pore opening is a Ca^{2+}-dependent process, but it can be facilitated by other factors, such as inorganic phosphate, ATP depletion, low pH and oxidative stress [16]. It is followed by osmotic swelling of the mitochondria and rupture of the mitochondrial membrane, leading to the release of mitochondrial proteins including cytochrome *c* into the cytosol. Such mitochondrial collapse might occur in several forms of necrotic cell death, for example cell death caused by oxidative stress, ischemia/reperfusion, and Ca^{2+} ionophores [17,18].

According to the traditional view, the permeability transition pore (PTP) represents a multimeric protein complex composed of the voltage dependent anion channel (VDAC) located in the outer mitochondrial membrane (OMM), the adenine nucleotide translocase (ANT), an integral protein of the IMM, and the matrix protein, cyclophilin D (CyPD). VDAC and ANT form contact sites between the OMM and the IMM. ANT was thought to be critical for pore opening, since it was demonstrated that the mitochondrial ADP/ATP carrier, when incorporated into lipid membrane, could be reversibly converted into a large channel by Ca^{2+} [19]. However, it was later found that deficiency of ANT failed to block Ca^{2+}-induced permeability transition [20]. Similarly, VDAC was also found to be dispensable for Ca^{2+}-induced MPT and mitochondria-dependent cell death [21]. In contrast, experiments with down-regulation of cyclophilin D revealed it to be critical for MPT-mediated cell death [22].

In addition to the potential PTP constituents mentioned above, other proteins residing in the mitochondrial membrane have been suggested to be involved in the regulation of MPT. For example, the mitochondrial phosphate carrier (PiC) has been reported to be able to regulate MPT through interaction with cyclophilin D [23] (Fig. 1). Indeed, CypD can directly bind PiC through its N-terminus [24]. However, studies using cardiac-specific mouse strains expressing

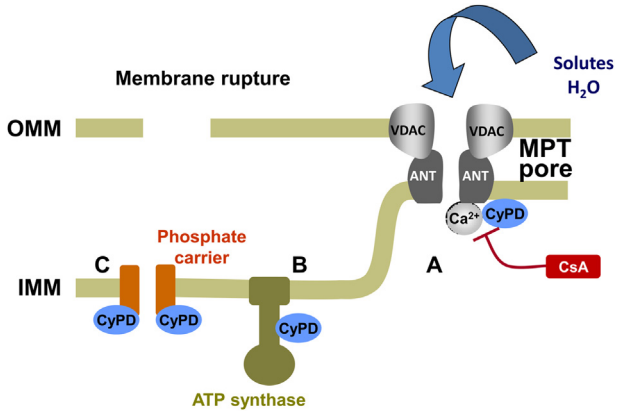

Fig. 1. Proposed models of mitochondrial permeability transition. The figure depicts the traditional view of the non-specific pore complex (A), as well as the more recently proposed involvement of the ATP synthase (B) or the phosphate carrier (C) in MPT induction.