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The calcium-signaling toolkit: Updates needed☆

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ABSTRACT

Here, we review the role of Ca^{2+} in apoptosis, namely that ER Ca^{2+} depletion or a sustained elevation of cytosolic or mitochondrial Ca^{2+} concentration are sufficient to trigger apoptosis. These concepts have emerged by the use of ER stressor agents that decrease the ER Ca^{2+} pool by inhibiting SERCA pumps. However, aside from their well-known actions on Ca^{2+} homeostasis disruption leading to apoptosis, new evidence show that some ER Ca^{2+} modulators have significant implications in other Ca^{2+}-mediated or Ca^{2+}-independent pathways determining cell fate suggesting a more complex regulation of apoptosis by intracellular Ca^{2+}. Here, we discuss the crucial interplay between Ca^{2+} mediated apoptosis, the Unfold Protein Response and autophagy determining cell fate, and the molecular compounds that have been used to depict these pathways. This review of the literature clearly shows the need for new inhibitors that do not interfere concomitantly with autophagy and Ca^{2+} signaling. This article is part of a Special Issue entitled: Calcium and Cell Fate edited by Jacques Haiech, Claus Heizmann and Joachim Krebs.

1. Ca^{2+} and apoptosis

Apoptosis involves many cellular mechanisms that may either act together or independently [1,2]. In living cells, Ca^{2+} has a dual role as a survival or cell-death factor depending on the Ca^{2+} signals. In particular, it has become clear that perturbations of intracellular Ca^{2+} compartmentalization trigger either apoptotic or necrotic cell death [3]. These Ca^{2+} mediated cellular mechanisms have been studied and defined depending on cellular compartments involved: the mitochondria, the cytosol and endoplasmic reticulum (ER).

1.1. Mitochondria

Mitochondria represent a hub of Ca^{2+} regulatory signals determining cell fate [4]. A cytosolic Ca^{2+} overload, which can be triggered by various stimuli, promotes Ca^{2+} accumulation by mitochondria. Excessive accumulation of Ca^{2+} in mitochondria is a major cause of the mitochondrial permeability transition, which is partly related to the opening of the permeability transition pore (PTP). The PTP is a multi-protein complex located at the interphase of the inner and outer mitochondrial membranes. Its molecular nature was recently identified [5,6]. Opening of the PTP allows the release of mitochondrial pro-apoptotic factors such as cytochrome C (CytC) and apoptosis inducing factor (AIF) in the cytoplasm, where they in turn activate caspases [1,2]. Mitochondrial permeability and activity of the PTP can be regulated by family members of BCL-2 proteins [7].

1.2. The cytosol

ER Ca^{2+} depletions can drive apoptosis via the induction of SOCE (Store Operated Calcium Entry). This process involves two families of proteins: the ORAI family expressed at the plasma membrane and STIM family located at the ER membrane. The first one is composed of 3 isoforms, ORAI1, ORAI2, and ORAI3, which have been shown to be involved in cancerogenesis [8–11]. ORAI1 proteins form a homotetrameric Ca^{2+} channel called Store Operated Channel (SOC) activated by store depletion of the ER. STIM1 is a single transmembrane domain protein that is mostly located in the ER membrane, serving as a $[Ca^{2+}]_{ER}$ sensor through its luminal EF-hand Ca^{2+}-binding domain [12]. Following a decrease in $[Ca^{2+}]_{ER}$, STIM1 redistributes into punctae close to the plasma membrane, where it can interact with ORAI1 allowing the formation of SOC, thereby triggering its opening leading to a sustain Ca^{2+} entry into the cytosol and cell death induction through the cytosolic or mitochondrial pathways (or both) [3,13–15]. Indeed, Ca^{2+} overload in the cytosol can also induce cell death without requiring the involvement of mitochondria. This apoptotic pathway is primarily based on the activation of the calcineurin which, when dephosphorylated, can promote apoptosis by regulating the activity of a number of downstream targets, including pro-apoptotic members of the BCL-2 family [16]. It has been shown that the calcineurin was able to induce dephosphorylation of BAD, a pro-apoptotic member of the BCL-2 family genes, and allows activation of the apoptotic cascade leading to the activation of caspase 3 [17]. Other Ca^{2+}-dependent enzymes regulate

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the events leading to apoptosis: several endonucleases degrading DNA [18], as well as cysteine proteases of the calpain family [19].

1.3. The endoplasmic reticulum

The ER is an important organelle because it is the main place of intracellular Ca^{2+} store and maturation of newly synthesized proteins. Any disturbance of ER Ca^{2+} homeostasis will lead to abnormalities of synthesis and/or conformation of proteins and ultimately to the induction of apoptosis. Under sustained stress conditions, pro-apoptotic proteins BAX and BAK undergo conformational changes increasing the Ca^{2+} flux into the cytosol from the ER [20]. This Ca^{2+} increase activates the m-calpain, which in turn cleaves and activates pro-caspase 12, leading to apoptosis [21]. A second apoptotic pathway can be activated involving the CHOP protein (C/EBP-homologous protein) which will inhibit BCL-2, an anti-apoptotic factor [22]. It has also been demonstrated that BCL-2 protein located at the ER plays a crucial role in apoptosis. Indeed when expressed at the mitochondria, BCL-2 with its hydrophobic properties is capable of retaining BH3 domains of pro-apoptotic members of the BCL-2 family at the mitochondrial membrane, thus preventing the induction of cell death. At the ER, BCL-2 modulates Ca^{2+} signaling to promote proliferation, while increasing the resistance to apoptosis. BCL-2 via its N-terminal domain type BH4 binds and inhibits IP3R, a well known channel responsible for intracellular Ca^{2+} release [23,24]. We propose a scheme describing these pathways (Fig. 1).

However, some of these concepts have "emerged" by using natural or chemical compounds that interfere with different intracellular Ca^{2+} processes. Besides, the Ca^{2+}-apoptosis link concept was defined at a period when other important cellular processes were not taken into account: UPR and autophagy. It is now well-established that crucial crosstalk exists between UPR, autophagy and Ca^{2+} signaling. In this review, we focus on SERCA pumps that have been extensively used to induce either mitochondrial, cytosolic or endoplasmic reticulum stress.

2. Crosstalk between Ca^{2+} signaling, UPR and autophagy

A decrease in the ER Ca^{2+} concentration resulting from SERCA pump inhibition is considered as a major cellular stress signal that induces the disruption of the conformation of proteins in transit through the ER leading to an accumulation of unfolded or misfolded proteins. Under ER Ca^{2+} stress, cells activate the Unfold Protein Response (UPR). At first, the UPR aims to restore the normal function of the cell by inhibiting protein translation, degrading misfolded proteins, and activating the signaling pathways that lead to increasing the production of molecular chaperones involved in protein folding. The UPR also uses such processes as autophagy to increase the capacity of degradation of proteins [25]. Under conditions of prolonged stress and if these objectives are not achieved, the UPR commits the cell to apoptosis pathways. The precise point at which the 'apoptotic switch' is activated has not yet been determined. This apoptotic switch can be mediated through each of the three UPR receptor pathways. These receptors are expressed at the ER membrane: PERK, IRE1 α and ATF6 [26]. In non-stressful environmental conditions, PERK proteins, IRE1 α and ATF6 form stable complexes with a sensor of ER Ca^{2+} stress: an immunoglobulin (BiP or GRP78) which maintains them in an inactive state. For this, BiP binds to their respective luminal areas, preventing homodimerization of PERK and IRE1α. With regard to the binding of BiP to ATF6, it prevents the translocation of a portion of ATF6 at the Golgi. Under ER Ca^{2+} stress, the accumulation of misfolded proteins in the ER creates a binding competition causing the dissociation of BiP. Free from BiP, PERK protein dimerizes, and autophosphorylates thus allowing its kinase activity. Once PERK has been activated, it in turn phosphorylates the eukaryotic initiation factor 2α (eIF2α), which in particular leads to the inhibition of protein synthesis and the specific activation of transcription factors such as ATF4 and CHOP. ATF4 is a transcription factor that regulates pro-survival genes involved in oxidative stress, amino acid synthesis, protein folding and differentiation. Similarly, dissociation of BiP from IRE1α undergoes dimerization and self-phosphorylation, activating its endonuclease activity allowing the maturation of the mRNA encoding the binding protein 1 (XBP1). Activation of the IRE1α protein leads to

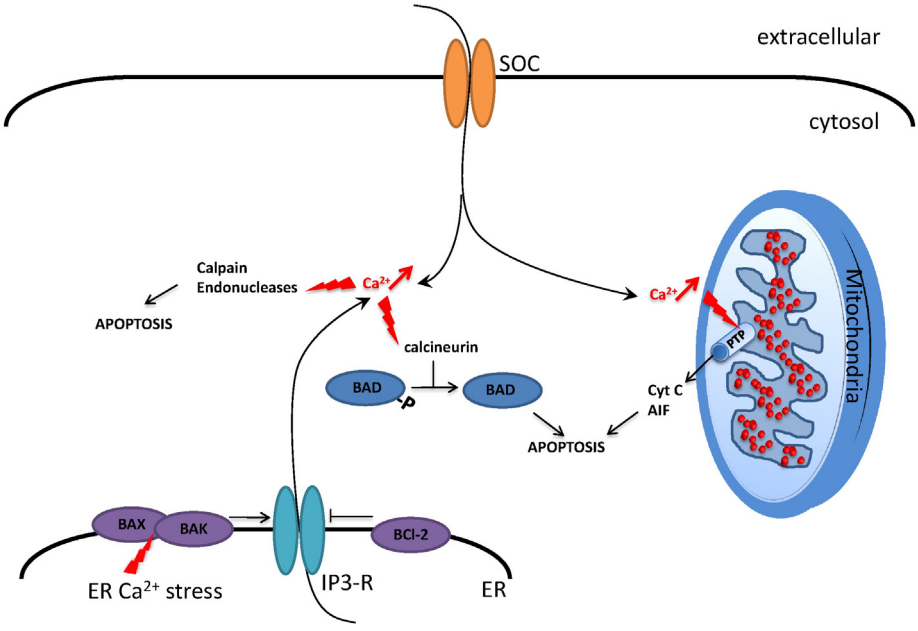

Fig. 1. Ca^{2+} signaling and apoptosis. Specific Ca^{2+} signaling pathways play an important role in apoptosis induction and/or regulation, which occurs through endoplasmic reticulum (ER) Ca^{2+} stress, cytosolic and mitochondrial Ca^{2+} overload. Store Operated Ca^{2+} Entry mediated by Store Operated Channel (SOC) is the main entry of Ca^{2+} leading to cell death. In the cytosol Ca^{2+} can directly triggers apoptosis through calpain activation. ER Ca^{2+} depletion modulates BAX/BAK interactions and ratio with BCl-2 protein at the ER membrane favoring Ca^{2+} efflux through IP3-R. This Ca^{2+} efflux through IP3-R can amplify calpain and calcineurin pathways. Calcineurin activation can lead to the dephosphorylation of BAD and its translocation to the mitochondria. Ca^{2+} can be also directly re-uptake by mitochondria leading to overload and cell death induction.

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