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Mitochondrial potassium channels in cell death

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ABSTRACT

Mitochondria are intracellular organelles involved in several processes from bioenergetics to cell death. In the latest years, ion channels are arising as new possible targets in controlling several cellular functions. The discovery that several plasma membrane located ion channels have intracellular counterparts, has now implemented this consideration and the number of studies enforcing the understanding of their role in different metabolic pathways. In this review, we will discuss the recent updates in the field, focusing our attention on the involvement of potassium channels during mitochondrial mediated apoptotic cell death. Since mitochondria are one of the key organelles involved in this process, it is not surprising that potassium channels located in their inner membrane could be involved in modulating mitochondrial membrane potential, ROS production, and respiratory chain complexes functions. Eventually, these events lead to changes in the mitochondrial fitness that prelude to the cytochrome c release and apoptosis. In this scenario, both the inhibition and the activation of mitochondrial potassium channels could cause cell death, and their targeting could be a novel pharmacological way to treat different human diseases.

1. Introduction

Mitochondria are intracellular semiautonomous organelles present in eukaryotic cells and originated from symbiotic bacteria, which are involved in several cellular processes, such as respiration, regulation of cell signaling, differentiation, apoptosis, cell growth or cell cycle. They are constituted by two membranes: the outer (OMM) and the inner (IMM) membrane, which delimit two areas, the inter membrane space (IMS) between the two membranes and the matrix, enclosed by the IMM. The involvement of mitochondria in cellular processes seems to depend largely on their metabolic products, especially ATP and reactive oxygen species (ROS) production. Respiratory chain complexes reside in the IMM and are able to mediate the reactions necessary to synthesize ATP. Furthermore, mitochondria are the main site of ROS production: complex I and III are the main actors in this process, but also evidences on complex II are arising in the latest years. Increased ROS production has been associated with different diseases, like cancer, diabetes, atherosclerosis, stroke, arthrosis, amyotrophic lateral sclerosis and neurodegenerative disorders such as Parkinson's and Alzheimer's diseases [1]. Mitochondria participate in many other metabolic pathways, including fatty acid synthesis, gluconeogenesis, steroidogenesis, synthesis of heme, urea cycle and apoptosis.

The concept of programmed cell death emerged by the seminal observations of Glücksmann in the 1951 [2]. Subsequently, Kerr, Willie and Curry experimentally proved and defined the “apoptosis”, an active cellular process important to physiologically eliminate redundant or pathological cells [3]. Furthermore, apoptosis has been related to the development of organs and organisms [4]. Cell shrinkage, membrane blebbing, chromatin condensation and nuclear fragmentation, followed by the formation of apoptotic bodies that are digested by phagocytosis by neighboring cells or macrophages, characterize apoptosis [3]. Mitochondria are crucially involved in programmed cell death and all different apoptotic pathways, both caspase-dependent and independent, recruit this ATP-producing organelle. Indeed, extrinsic pathway starts with the binding of the corresponding ligand to the death receptor (e.g. CD95, TNFR1) and the formation of the death-inducible signaling complex (DISC), which in turn induces the activation of procaspase-8 to caspase-8. This is one of the members

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of a cysteine-dependent aspartate-specific protease family that can cleave Bid into its truncated form (tBid). tBid can translocate to mitochondria and leads to mitochondrial membrane permeabilization by activation of Bax and/or Bak. Bax and Bak on the OMM facilitate the release of cytochrome c, Smac and Omi, favoring the assembly of the apoptosome together with procaspase-9 and Apaf-1 (apoptosis activating factor 1). Apoptosome can turn on caspase-9, which triggers the activation of the executioner death cascade, mediating the cleavage and activation of caspase-3 [5]. Similar events occurring after mitochondrial membrane permeabilization are induced also by death signals in the intrinsic pathway that acts directly on the organelles. On the contrary, caspase-independent mechanism induces endonuclease G and the apoptotic inducing factor (AIF) translocation from the mitochondria to the nucleus, where they act on nuclear DNA and they trigger cell death [5].

Furthermore, mitochondrial ROS have been associated with initiation of the mitochondrial apoptotic cascade. Indeed, at lower doses, ROS have been linked to induction of cell survival responses, whereas higher doses activate cell death processes such as apoptosis [6]. It has been shown that ROS can cause cytochrome c release from mitochondria and induction of apoptosis through the mitochondrial pathway [7]. For example, H_2O_2 can trigger mitochondrial membrane hyperpolarization, which induces the collapse of the mitochondrial membrane potential, leading to Bak and Bad translocation to the OMM and the release of cytochrome c, which in turn continues to support ROS production due to the disruption of the respiratory chain activity [1]. Moreover, ROS can also favor cytochrome c release by increasing its free fraction in the IMS via oxidation of cardiolipin, which, under normal (non-oxidized) conditions, anchors cytochrome c to the outer surface of the IMM cristae [8].

Recent experimental evidences indicate that ion channels might regulate the chain of events taking place at the level of mitochondria during apoptosis. Potassium (K^+) channels are the most present channels in the organisms and permit K^+ transport across biological membranes. K^+ channels have a tetrameric structure and are constituted by four α subunits and distinct regulatory β and γ subunits. All these subunits are associated to form a complex arranged around the central ion-conducting pore. In general, each subunit consists of at least two transmembrane α-helices (TM1 and TM2), with a short amino-acid segment between them called P loop (pore loop). The selectivity filter is formed by a five residues signature sequence, TVGYG, within the P loop of each subunit, as defined by combination of site-directed mutagenesis with electrophysiology and the resolution of the crystal structure of the *Streptomyces lividans*' KCsA channel [9,10]. The specificity for K^+ ions is due to the presence of carbonyl oxygens in the filter that are arranged horizontally, belonging to the canonical amino acid sequence TVGYG located in the P-loop: pairs of these quartets of oxygens partially surround K^+ ions, precisely mimicking the configuration of oxygen atoms around a solvated K^+ ion [11]. Finally, the presence of a set of negatively charged amino acids in the vestibule region helps the transport of cations through the pore. Importantly, these negative residues are evolutionary conserved and can interact with a preserved positively charged amino acid present in the peptide toxins of some venoms, which can block the channel [12].

It is now clearly demonstrated that several K^+ channels have a mitochondrial counterpart [13]. These channels can mediate an inward K^+ flux from the cytosol into the mitochondrial matrix, following the electrochemical gradient for K^+ [14]. Indeed, K^+ is more concentrated in the cytosol (around 150 mM) than in the mitochondrial matrix, where it moves to also driven by the presence of a negative mitochondrial membrane potential (−180 mV). The electrochemical equilibrium is continuously maintained by the coupling of K^+ influx and efflux mediated by different transport systems [15]. The opening of K^+ channels in the IMM would induce depolarization, while their block would produce a hyperpolarization of IMM [16]. Mitochondrial swelling will occur following the continuous K^+ entry, caused by osmotic entrance of water through aquaporins, and it will trigger the blockade of mitochondrial functions, favoring release of cytochrome c and of other small molecules via OMM ruptures. To avoid osmotic volume changes and mitochondrial depolarization, this positive flux is counterbalanced by the efflux of protons (H^+), which can be excreted both by the respiratory chain complexes activities and by the presence of a K^+/H^+ antiporter [17]. This pathway represents the called K^+ "futile cycle". This pathway solves the K^+ distribution and the mitochondrial volume homeostasis, but induces energy dissipation through the IMM. For these reasons, K^+ channels are finely regulated to slow down K^+ transport inside the mitochondria, to reduce energy dissipation during its "futile cycle". To counterbalance the movement of positive charges, the respiratory chain must increase the proton transfer rate into the IMS: the K^+ "futile cycle" influences ATP synthesis and mitochondrial respiration, thus contributing to the regulation of different processes including mitochondrial volume, structural integrity and ROS production [18]. K^+ channels regulation affect mitochondrial ROS production since the inhibition of K^+ fluxes leads to IMM hyperpolarization, which can induce changes in the redox state of the respiratory chain complexes, thereby increasing single electron leakage at complexes I and III to molecular oxygen to facilitate superoxide anion production [19–21]. Furthermore, even IMM depolarization by inward K^+ transport can activate complex III-dependent ROS formation, under conditions of a highly reduced coenzyme Q pool [22]. Finally, IMM depolarization could trigger permeability transition pore (PTP) opening that, in turn, can favor mitochondrial ROS production by the ROS-induced ROS release mechanism [23].

In summary, the role of the K^+/H^+ exchanger in trying to avoid mitochondrial swelling due to K^+ entry is clear, whereas the reason for the presence and the activity of K^+ channels is less straightforward. A possible function of these latter entities could be found during mitochondrial biogenesis, since K^+ fluxes can control mitochondrial matrix volume, so affecting the respiratory chain activity and triggering an increase in fatty acid oxidation [24].

In this review, we will focus our attention on the basic insights as well as the recent updates regarding different types of mitochondrial K^+ channels (Fig. 1) and their role in regulation of apoptotic cell death (Fig. 2), considering their possible pharmacological targeting to treat human diseases.

1.1. Voltage gated K^+ (Kv) channels

Voltage gated K^+ channels (Kv) constitute the largest and most diverse family of K^+ channels, comprising 40 of the 90 human genes, divided into 12 families (Kv1-12) [25]. Generally, Kv channels are constituted by six transmembrane helices (S1–S6), with four of them preceding the pore loop and the remaining two helices

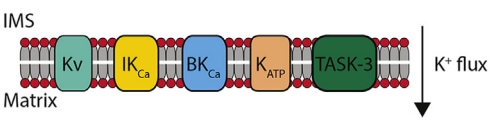

Fig. 1. Mitochondrial K^+ channels. K^+ channels are the most existing channels in the organisms and allow K^+ passage through biological membranes. Until now, mitochondrial K^+ channels that have been shown to have a role in cell death are the follow: Voltage Gated K^+ (Kv) channels (Kv1.1, Kv1.3 and Kv1.5), Ca^{2+} activated K^+ channels (IK_{Ca} and BK_{Ca}), ATP-sensitive K^+ (K_{ATP}) channels and Two-pore K^+ (K2P) channels (TASK-3).

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