Review

Mitochondrial membrane permeability transition and cell death

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Abstract

Mitochondria are important organelles for energy production, Ca^{2+} homeostasis, and cell death. In recent years, the role of the mitochondria in both apoptotic and necrotic cell death has received much attention. In apoptotic and necrotic death, an increase of mitochondrial membrane permeability is considered to be one of the key events, although the detailed mechanism remains to be elucidated. The mitochondrial membrane permeability transition (MPT) is a Ca^{2+}-dependent increase in the permeability of the mitochondrial membrane that leads to loss of $\Delta\psi$, mitochondrial swelling, and rupture of the outer mitochondrial membrane. The MPT is thought to occur after the opening of a channel, which is termed the permeability transition pore (PTP) and putatively consists of the voltage-dependent anion channel (VDAC), the adenine nucleotide translocator (ANT), cyclophilin D (Cyp D: a mitochondrial peptidyl prolyl-*cis*, *trans*-isomerase), and other molecule(s). Our studies of mice lacking Cyp D have revealed that it is essential for occurrence of the MPT and that the Cyp D-dependent MPT regulates some forms of necrotic cell death, but not apoptotic death. We have also shown that two anti-apoptotic proteins, Bcl-2 and Bcl-x$_L$, block the MPT by directly inhibition of VDAC activity. Here we summarize a role of the MPT in cell death.
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Keywords: Mitochondria; Permeability transition; Cell death

1. Introduction

Apoptosis is the best-characterized form of programmed cell death and an outline of its molecular basis is now well understood. Mammalian cells possess two major apoptotic signaling pathways, which are known as the intrinsic pathway and the extrinsic pathway [1]. Mitochondria play a crucial role in the intrinsic pathway: an increase of outer membrane permeability leads to release of proteins from the intermembrane space into the cytoplasm, including apoptogenic molecules such as cytochrome *c*, Smac/Diablo, HtrA2 (Omi), AIF, and DNaseG [1,2]. In the presence of ATP (dATP), cytochrome *c* binds to Apaf-1 and triggers its oligomerization, after which pro-caspase-9 is recruited and undergoes autoactivation. Thus, an increase in the permeability of the outer mitochondrial membrane is central to apoptosis [3,4], and membrane permeability is directly regulated by the Bcl-2 family of proteins [4,5] (see Fig. 1). However, the detailed mechanisms controlling outer mitochondrial membrane permeability during apoptosis and the exact role of Bc1–2 family members are still to be determined. The initial model used to explain the apoptotic increase of mitochondrial membrane permeability was the "mitochondrial membrane permeability transition" (MPT) [6], which has been known for some time among investigators of the mitochondria.

2. MPT

Under various conditions, such as in the presence of Ca^{2+} together with inorganic phosphate, isolated mitochondria undergo the MPT. This process is characterized by a Ca^{2+}-dependent increase in the permeability of the inner mitochondrial membrane, resulting in the loss of $\Delta\psi$, mitochondrial swelling, and rupture of the outer mitochondrial membrane [7,8] (see Fig. 1). The MPT is thought to occur after the opening of a putative channel complex, which has been termed the permeability transition pore (PTP), and consists of the voltage-dependent anion channel (VDAC: outer membrane channel), the adenine nucleotide translocator (ANT: inner membrane channel), cyclophilin D (Cyp D), and other molecule(s) [9] (see Fig. 2). The exact nature of this complex is still to be determined. A role of ANT in the MPT is supported by inhibition or activation of the MPT by bonkrekic acid and

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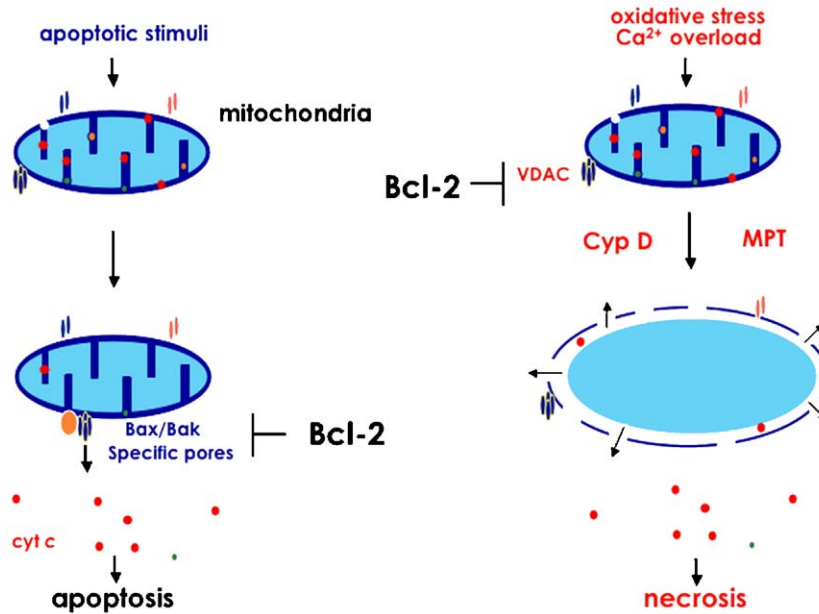 *E-mail address:* tsujimoto@gene.med.osaka-u.ac.jp (Y. Tsujimoto).

Fig. 1. Involvement of the mitochondria in apoptosis and necrosis. During apoptosis, an increase in the permeability of the outer mitochondrial membrane is crucial and is regulated by multidomain pro-apoptotic members of the Bcl-2 family (Bax and Bak), resulting in the release of several apoptogenic factors into the cytoplasm. In contrast, the Cyp D-dependent MPT (increased permeability of both the outer and inner mitochondrial membranes) is involved in necrosis induced by Ca^{2+} overload and oxidative stress. Both kinds of mitochondrial membrane permeability changes are inhibited by anti-apoptotic members of the Bcl-2 family (Bcl-2 and Bcl-x).

atractyloside, which are ligands for ANT [10]. Cyp D is a mitochondrial member of the cyclophilin family, which shows peptidyl prolyl-*cis*, *trans*-isomerase (PPIase) activity and has a crucial role in protein folding [11]. The presumed role of Cyp D in regulating the MPT is based on the observation that cyclosporin A (CsA), a specific inhibitor of the cyclophilin family, blocks the MPT [12]. Cyp D resides in the mitochondrial matrix, but associates with the inner mitochondrial membrane during the MPT. Based on the enzymatic activity of Cyp D (PPIase), it is suggested to induce a conformational change of an inner membrane channel such as ANT, leading to an increase of inner

membrane permeability. In addition to the CsA-sensitive and Ca^{2+}-dependent ("regulated") MPT, the existence of a CsA-insensitive and Ca^{2+}-independent ("unregulated") MPT has also been suggested, although its mechanism and relationship to the CsA-sensitive MPT are totally unknown [13].

Some forms of apoptosis can be inhibited by CsA, suggesting a role of the CsA-sensitive MPT in this process of cell death [9,14]. The possible role of the MPT in apoptosis is also supported by the finding that apoptosis is sometimes inhibited by bonkrekic acid [10,15], although difficulty in using bonkrekic acid as a potent inhibitor of apoptosis has often been noted by
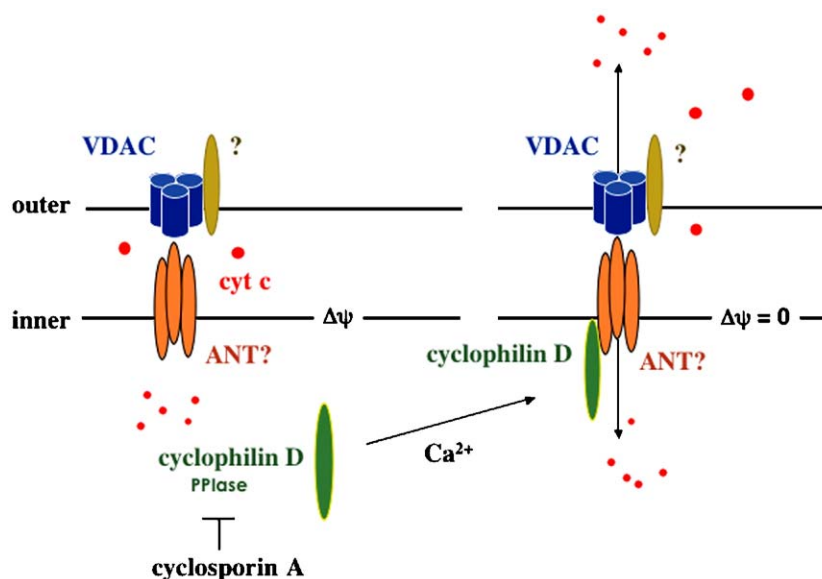

Fig. 2. Putative protein complex mediating the MPT.

many investigators. The CsA-sensitive MPT has also been implicated in the remodeling of mitochondrial cristae and mobilization of cytochrome c stores from the cristae during apoptosis, thus promoting the complete release of cytochrome c [16]. However, the overall role of the MPT in apoptosis remains controversial because there have been a number of reports that apoptosis is not inhibited by CsA [17]. Furthermore, it has been demonstrated that $\Delta\psi$ follows cytochrome c release in at least some types of apoptosis, suggesting that the MPT is not always the cause of cytochrome c release and cell death.

3. Essential players in the MPT

It has long been considered that the VDAC, ANT, and Cyp D play an essential role in the MPT, although convincing evidence was lacking until very recently.

VDAC: Experimental evidence for a direct role of the VDAC in the MPT has been provided by studies using anti-VDAC antibodies [18] (Shimizu et al., 2001). Two polyclonal anti-VDAC antibodies were obtained that recognized different epitopes of the channel, and could inhibit VDAC activity as assessed in liposomes [18]. Both of these anti-VDAC antibodies also inhibited the Ca^{2+}-induced MPT [18], supporting a crucial role for VDAC in the MPT.

ANT: The ANTs (ANT1 and 2 in mice and ANT1, 2, and 3 in humans) have also been considered important for occurrence of the MPT. It has been demonstrated that Cyp D directly interacts with ANT, although it is not known whether CsA inhibits the interaction of Cyp D and ANT [19,20]. Regarding the role of ANT in the MPT, considerable progress was made recently: it was shown that liver mitochondria from mice lacking both ANT1 and ANT2 underwent the MPT, although the threshold for Ca^{2+} was slightly increased [21], suggesting that the ANT1/2 played only a limited role, if any, in the MPT or deficiency of ANT1/2 might be compensated by other channel(s). Other channel(s) involved in the MPT might be ANT-like channels on the inner membrane, given that the MPT is modulated by ANT ligands such as bonkrekic acid or atractyloside and that the MPT is accompanied by $\Delta\psi$ loss (increased permeability of the inner mitochondrial membrane). Identification of channel(s) in the inner mitochondrial membrane, which is directly involved in the MPT and might be a target of Cyp D, would be crucial.

Cyp D: A role of Cyp D in the MPT was initially suggested because the MPT is inhibited by CsA, which inhibits the PPIase activity of cyclophilins. This has recently been confirmed by generation of Cyp D gene (ppif)-deficient mice [22–25]: Cyp D-deficient mitochondria isolated from mouse livers do not undergo the CsA-sensitive MPT in response to a variety of MPT inducers, including Ca^{2+}, atractyloside, and H_2O_2. Because the MPT does not occur, these mitochondria accumulate a much larger amount of Ca^{2+} than control mitochondria [22,25]. However, these Cyp D-deficient mitochondria still undergo the CsA-insensitive MPT in response to high concentrations of Ca^{2+} [22,24]. In addition, the response to reagents like ubiquinone and thiol oxidants that cause the CsA-insensitive MPT is normal in Cyp D-deficient mitochondria [24]. Thus, Cyp D is specifically involved in the CsA-sensitive MPT.

4. No role of Cyp D-dependent MPT in apoptosis

It has been controversial as to whether the MPT plays an important role in the apoptotic increase of mitochondrial membrane permeability, but recent development of Cyp D-deficient mice has finally solved this issue. Various cells isolated from Cyp D-deficient mice, such as thymocytes, MEFs, and hepatocytes, undergo apoptosis normally in response to various stimuli, including etoposide, stautosporine, and TNFα [22–25], providing the most compelling evidence that the MPT is not essential for apoptosis. These observations certainly do not exclude the possibility that some apoptosis might be mediated by the CsA-sensitive MPT, and thus may be inhibited by CsA. However, the inhibitory effect of CsA on apoptosis might need to be more carefully evaluated because CsA is normally used at relatively high concentrations that could inhibit other targets and have a secondary effect on apoptosis. It may be necessary to re-evaluate the inhibition of apoptosis by using Cyp D-deficient cells or by silencing Cyp D in cells to assess the real effect of CsA.

There have been several reports that overexpression of Cyp D protects cells against some forms of apoptosis. For example, Cyp D overexpression inhibits apoptosis induced by the over-expression of caspase-8 (but not Bax) or by exposure to arsenic trioxide [26,27]. These observations are apparently inconsistent with the findings obtained using Cyp D-deficient cells. It might be possible that apoptosis is mediated by the MPT, which is somehow affected by overexpression of Cyp D in these circumstances. However, studies of transgenic mice with myocardial expression of Cyp D have revealed that cardiac myocytes from these mice are prone to undergo mitochondrial swelling and spontaneous death [23].

5. Involvement of the MPT in necrosis

In contrast to the lack of any impact of Cyp D deficiency on apoptosis, the Cyp D-dependent MPT seems to play an important role in some forms of necrotic cell death (see Fig. 1). It has been shown that Cyp D-deficient MEFs are significantly more resistant to H_2O_2-induced necrosis [22,23], while Cyp D-deficient hepatocytes gain resistance to necrosis induced by a Ca^{2+} ionophore (A23187) or by H_2O_2 [22,23]. Interestingly, when necrosis is inhibited by Cyp D-deficiency in these cells, apoptosis does not occur as an alternate death mechanism [22], suggesting that these cells somehow block the apoptotic signaling pathway activated by H_2O_2 and Ca^{2+} overload.

6. Regulation of the MPT by Bcl-2

Although anti-apoptotic members of the Bcl-2 family (Bcl-2 and Bcl-x_L) are known to inhibit the Bax/Bak-dependent apoptotic increase of mitochondrial membrane permeability by direct interaction with pro-apoptotic members of this family, they have also been shown to inhibit the MPT [28,29](see Fig. 1). How do these proteins act to block the MPT? Since Bax/Bak is not essential for the MPT [22], Bcl-2 (Bcl-x) might directly inhibit a component of the PTP complex. This concept is supported by the

observation that Bcl-2 (Bcl-x) can block the VDAC [28], suggesting that Bcl-2 may inhibit the MPT via VDAC blockade. It has also been shown that Bcl-2 can inhibit ANT activity [30] (Marzo et al., 1998b). However, ANT might not be a major player of the MPT [21], as described above, so Bcl-2 might inhibit other unidentified channels similar to ANT that are actually involved in the MPT. Since Bcl-2 resides mainly on the outer mitochondrial membrane, it is likely to act on the MPT by inhibiting VDAC.

7. Future studies

Studies using Cyp D-deficient mice enabled us to provide convincing evidence that the Cyp D-dependent MPT does not play a role in apoptosis. However, there are still many important questions to be answered: (1) What is the molecular nature of the MPT pore complex? (2) What is the target of Cyp D and how does Cyp D induce the MPT? (3) What is the biological significance of the MPT? (4) What is the relationship between the Cyp D-dependent MPT and the unregulated MPT? (5) Does the unregulated MPT have a role in apoptosis or other forms of cell death? Further studies are needed to answer these important questions.

Acknowledgements

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