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News and Commentary

Mitochondrial permeability transition in apoptosis and necrosis

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Prior to 10 years, our group discovered that mitochondrial membrane permeabilization (MMP) generally precedes apoptotic cell death, both *in vitro* and *in vivo*.[1,2]. At that time, the only mechanism of MMP that was known was the so-called permeability transition (PT), a molecularly ill-defined phenomenon that is generally studied in isolated mitochondria. The exact nature of the PT pore has not been established until nowadays, although attempts to isolate the so-called PT pore complex (PTPC) have been launched.[3,4] PT is mostly referred to as a phenomenon involving mitochondrial matrix swelling, accompanied by a loss of the mitochondrial inner transmembrane potential ($\Delta\Psi$m), which can be inhibited by several pharmacological agents, in particular cyclosporin A (CsA) and bongkrekic acid (BA).[5,6] The mitochondrial target of CsA is cyclophilin D (gene name: *Ppif*), while that of BA is one of the isoforms of the adenine nucleotide translocase (ANT1 and 2 in mice, ANT1, 2 and 3 in humans). CsA and BA can inhibit apoptosis induction in a variety of models of apoptosis, for instance, glucocorticoid-triggered thymocyte death[2,7] or tumor necrosis factor-α (TNFα)-induced hepatocyte apoptosis.[8] Similarly, it was reported that CsA or BA protected isolated mitochondria against the MMP-inducing effects of two proapoptotic proteins from the Bcl-2 family, namely recombinant Bax[9,10] and recombinant Bid.[11,12] However, it was noticed that these inhibitory effects were partial and that they often only induced a delay in MMP,[7–12] suggesting the existence of alternative, PT-independent pathways of MMP.[13]

The core of the so-called PTPC is thought to involve the voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane, ANT in the inner membrane and cyclophilin D in the mitochondrial matrix. Direct interactions between ANT and cyclophilin D have been demonstrated, although it has been a matter of debate whether CsA inhibits the binding of cyclophilin D to ANT or not.[14,15] The ANT–cyclophilin D interaction has been suggested to be independent of the CsA-inhibited *cis-trans* peptidylprolyl isomerase (PPIase) activity of CsA.[16] While there is no doubt that CsA inhibits PT, the question as to whether cyclophilin D inhibits or induces PT has been a matter of intense debate. The overexpression of cyclophilin D can inhibit the induction of apoptosis induced by overexpression of ANT1, suggesting that cyclophilin D would be the antagonist of ANT1, which (together with ANT3, but in contrast to ANT2) would be capable of mediating PT.[17] This effect of cyclophilin D was not abrogated by point mutations that abolish its PPIase activity. In HeLa cells, cyclophilin D inhibited apoptosis induction by caspase-8 overexpression, but not that of Bax or RIP, and this inhibitory profile correlated with that of BA.[17] Moreover, cyclophilin D overexpression inhibited apoptosis induction by arsenic trioxide and to a lesser extent by TNFα plus cycloheximide in HeLa cells.[17] Cyclophilin D overexpression also delayed the collapse of the mitochondrial transmembrane potential ($\Delta\Psi$m) and cell death induced by tert-butylhydroperoxide and staurosporine in HEK293 and C6 glioma cells, but this effect was abolished by mutations affecting the PPIase activity.[16] In such cells, cyclophilin D induced a hyperpolarization of the $\Delta\Psi$m.[16] In contrast, it was reported that the transfection-enforced cyclophilin D overexpression in B50 neuronal cells caused a reduction of the $\Delta\Psi$m, coupled to a reduction of apoptosis induced by staurosporine.[18] Altogether, these data suggest that cyclophilin D can function as a cell death inhibitor. Accordingly, cyclophilin D was found to be overexpressed in breast, uterus and ovary cancers.[17]

Three recent papers published in Nature and in the Journal of Biological Chemistry report the phenotype of cyclophilin D knockout mice[19–21] and challenge the view that cyclophilin D would be a cytoprotector. *Ppif*$^{-/-}$ mice manifested no particular pathological alterations, although they exhibited changes in PT pore opening and cell death regulation. Hepatocyte mitochondria from *Ppif*$^{-/-}$ mice displayed a desensitization of the PT pore to Ca^{2+}, meaning that mitochondrial swelling required twice the Ca^{2+} load necessary to open the pore than in wild-type (WT) mitochondria.[19–21] *Ppif*$^{-/-}$ hepatocytes thus displayed a similar phenotype as cells in which both ANT isoforms (ANT1 and 2 in mice) have been knocked out.[22] Ca^{2+}-induced pore opening of *Ppif*$^{-/-}$ liver mitochondria was insensitive to CsA,[21] confirming that cyclophilin D is indeed the pharmacological target of CsA in mitochondria. However, the PT pore response to ubiquinone, depolarization, pH, adenine nucleotides and thiol oxidants was similar in mitochondria from WT and *Ppif*$^{-/-}$ mice.[21] Moreover, liver mitochondria from WT and *Ppif*$^{-/-}$ mice exhibited identical cytochrome *c* release patterns when treated with recombinant Bax or tBid protein, in conditions in which *Ppif*$^{-/-}$ mitochondria were protected from Ca^{2+}-induced cytochrome *c* release.[19,20] This indicates that the mitochondrial outer membrane permeabilization induced by proapoptotic Bcl-2 family members can occur independently from cyclophilin-D-regulated events.

Fibroblasts from *Ppif*$^{-/-}$ mice were resistant against PT pore opening induced by H_2O_2, associated with enhanced

Table 1 Examples of apoptosis induction pathways inhibited by PT pore inhibitors

Cell type	Apoptosis inducer	Cell death inhibition by PT inhibitors	Ref.
Tadpole tale (*Xenopus*)	3,5,3′-triiodothyronine, *in vivo*	CsA	29
Hepatocytes (rat)	Injection of LPS plus D-galactosamine *in vivo*	CsA	30
	Ischemia reperfusion damage	CsA, ruthenium red	31
Hepatocytes (mouse)	Injection of CD95/Fas agonistic antibody	CsA	32
	Injection of atractyloside	CsA	33
Cerebellar granule cells (rat)	H_2O_2	BA, CsA	34
Neonatal facial motorneurons (rat)	Axotomy, *in vivo*	BA, CsA	35
Cortical neurons (rat)	Transient hypoglycemia (*in vivo*)	CsA	36
Basal ganglion neurons (rat)	Focal ischemia (*in vivo*)	BA	37
T-cell lymphoma (human)	Genistein	BA	38
	TNFα	CsA	39
Ventricular heart myocytes (mouse)	Inducible BNIP3 expression	BA	40
Eosinophil granulocytes	Glucocorticoids	BA	41
B lymphoma cells (human)	Taurine chloramine	BA, CsA	42
CHO cells (hamster)	Cr(VI)	CsA, trifluoperazine	43
Vanilloid receptor-transfected cell lines	Capsaicin	BA, CsA	44

Note that in all examples listed in this table, cells undergo *bona fide* apoptosis, as indicated by the presence of chromatin condensation, nuclear DNA fragmentation and/or caspase activation.

resistance to cell death, and re-introduction of WT cyclophilin D (but not of a PPIase-deficient mutant of cyclophilin D) restored efficient cell death in *Ppif*$^{-/-}$ fibroblasts.[19] Fibroblasts from *Ppif*$^{-/-}$ mice were also resistant against cell death induced by thapsigargin (which causes Ca^{2+} release from the ER), but were not protected against cell death induced by staurosporine, TNFα, adenovirus-mediated Bax overexpression,[19] or transfection with Bax or Bid cDNA.[20] Moreover, *Ppif*$^{-/-}$ and WT thymocytes died at a similar rate in response to multiple proapoptotic stimuli, including etoposide, staurosporine, TNFα plus cycloheximide or the Ca^{2+} ionophore A23187.[20] No difference was found either for apoptosis induction in hepatocytes transfected with Bax or Bid or intestinal epithelial cells irradiated *in vivo*.[20] Hepatocytes devoid of cyclophilin D were protected against necrotic cell death induced by A23187 or H_2O_2.[20] Heart infarction induced by ischemia reperfusion was strongly reduced in *Ppif*$^{-/-}$ mice.[19,20]. Conversely, overexpression of a *Ppif* transgene under the control of a heart-specific promoter induced an elevated propensity of mitochondria to PT pore opening *in vitro* and signs of cardiomyocyte apoptosis (cytochrome *c* release, caspase-9 activation, TUNEL positivity) *in vivo*.[19] Overexpression of cyclophilin D in B50 cells can also facilitate necrosis induction by the NO donor sodium nitroprusside and sensitized isolated mitochondria from such cells to PT induced by Ca^{2+} and oxidative stress.[18]

From the data discussed above, one might draw two major conclusions. First, the overexpression and the knockout of cyclophilin D both can have cytoprotective effects, depending on the experimental context and the cell death-initiating stimulus. Cyclophilin D overexpression protects against some apoptosis inducers (such as ANT1 overexpression and staurosporine), while the knockout of cyclophilin D protects against necrosis induced by Ca^{2+} overload and oxidative stress. In many instances, however, the knockout of cyclophilin D has no effect on apoptosis induction (see above). However, it appears premature to conclude that cyclophilin D is generally required for necrotic cell death but not for apoptotic cell death, because CsA and BA can inhibit apoptotic cell death in a variety of experimental systems (Table 1). Moreover, cyclophilin D overexpression can induce cardiomyocyte apoptosis (rather than necrosis).[19]

Second, there are two different pathways leading to MMP, one that partially relies on cyclophilin D expression (and that is CsA-inhibitable) and another that is cyclophilin D-independent (and that is CsA-resistant). Thus, the CypA-negative cells and mice provide a fascinating tool to investigate the mechanisms of pathological cell death and to actively search for drugs that can inhibit cyclophilin D-independent MMP (and hence would have another range of activities than CsA) or inhibit cyclophilin D-dependent-MMP (but ideally would not mediate immunosuppression). Nonetheless, it would be premature to conclude that PT pore opening is irrelevant to cell death in those circumstances in which removal of cyclophilin D fails to modulate cellular demise. Indeed, the PT pore opens normally in response to arsenicals,[21] which act on ANT (and perhaps other similar proteins from the mitochondrial transporter protein family).[23]

It should be emphasized that the nature of the pore-forming protein(s) acting at the levels of the inner and outer mitochondrial membranes is still elusive. Thus, the cyclophilin D and the ANT1/2 knockout both decrease the Ca^{2+} sensitivity of liver mitochondria. However, ANT1/2 null mitochondria resist even higher Ca^{2+} concentration without PT when CsA is added into the system,[22] suggesting that cyclophilin D (the bona fide CsA target) can act on mitochondrial targets other than ANT (perhaps ANT-related proteins from the mitochondrial carrier protein family) and that PT pore regulation is far more complicated than it was suspected. A wealth of data have suggested highly dynamic functional physical interactions between putative PT pore constituents (such as ANT and VDAC) on one side and members of the Bcl-2 protein family on the other side.[24–28] Future research must disentangle a likewise complex network of local protein–protein and protein–lipid interactions that determine MMP and seal the cell's fate.

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