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Review

Targeting mitochondrial permeability in cancer drug development

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The past decade has seen the emergence of a new mechanistic paradigm of cancer therapeutics. Not only have mitochondria taken centre stage as key cellular organelles mediating intrinsic pathways of cell death by apoptosis, but nonapoptotic pathways have also been shown to involve mitochondrial mechanisms. Both pathways of cell death involve permeabilization of mitochondrial membranes, but the exact nature of the molecular complexes involved at the inner mitochondrial membrane (IMM) and outer mitochondrial membrane (OMM) remains uncertain in the light of recent gene knockout studies. Consequently, the boundary between mitochondrially-mediated apoptotic and nonapoptotic cell death is controversial. Here, we discuss the nature of the pore complexes involved in permeabilization of the IMM and OMM. Several compounds that interact directly with components of these pore complexes and have been shown to exhibit anticancer activity are discussed while other compounds appear to act indirectly through stress-related pathways.

Keywords: Anticancer compound / Apoptosis / Cell death / Mitochondria / Permeability

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1 Introduction

Mitochondria are active players in the development of cancer, not only from the perspective of cellular bioenergetics but also from the key role that they play in both apoptotic and some forms of nonapoptotic cell death [1]. While the role that physiological changes in mitochondrial membrane permeability (MMP) play in the regulation of cellular bioenergetics is beyond the scope of this review, both the inner mitochondrial membrane (IMM) and outer mitochondrial membrane (OMM) have been linked to apoptotic and nonapoptotic cell death by known anticancer drugs and by numerous other compounds that have the potential to be applied in cancer therapy. The primary targets of many of these compounds are not at the level of the mitochondrial membrane and consequently, mitochondrial involvement will be indirect and stress-related. However, other compounds have been shown to directly affect mitochondrial permeability in isolated mitochondria and in intact cells, at the level of both IMM and OMM, suggesting applications of these compounds in cancer treatment.

There are two main apoptotic pathways; the extrinsic pathway, which is induced by activation of specific death receptors located in the plasma membrane, and the intrinsic pathway which is stress-associated and involves changes in permeability of the mitochondrial membranes [2, 3]. This review will focus on the intrinsic apoptotic pathway and the role of different components of IMM and OMM pores that are of interest in anticancer drug development.

Permeabilization of the mitochondrial outer membrane is a key event in both apoptotic and some forms of nonapoptotic cell death [4–8]. We propose that the extent and duration of this permeabilization will determine whether a cell dies by apoptosis (programmed cell death) or nonapoptotic mechanisms such as oncosis (all swelling and osmotic lysis), and this will depend on the cell type and the nature and intensity of the stressor (see Fig. 1). A weak or intermittent stress signal (*e.g.* staurosporine, ceramide, cytostatic

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Abbreviations: ANT, adenine nucleotide translocator; **ATR,** atractyloside; **BH,** Bcl-2 homology; **BKA,** bongkrekic acid; **CsA,** cyclosporin A; **DIDS,** diisothiocyanatostilbene-2¢,2-disulphonate; **IMM,** inner mitochondrial membrane; **MAC,** mitochondrial apoptosis-induced channel; **MMP,** mitochondrial membrane permeability; **MOMP,** mitochondrial outer membrane permeabilization; **MPT,** mitochondrial permeability transition; **MPTP,** mitochondrial permeability transition pore; **OMM,** outer mitochondrial membrane; **PBDR,** peripheral benzodiazepine receptor; **TSPO,** translocator protein; **VDAC,** voltage-dependent anion-selective channel

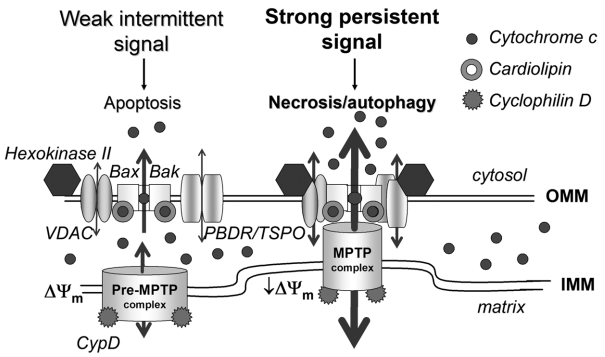

Figure 1. Model of stress-induced MMP leading to apoptotic and nonapoptotic cell death. Weak intermittent stress responses lead to well-regulated, reversible changes in ANT/cyclophilin D-containing pore complexes at the IMM that control OMM permeability *via* VDAC, Bax/Bak and PBDR/TSPO channels, associated regulatory proteins like hexokinase and cardiolipin. Depending on the stressor, OMM channels may open independently of the ANT complex to release cytochrome *c* and other proapoptotic proteins and therefore will be CsA resistant. Strong persistent stress signals result in the formation of active ANT/cyclophilin D-containing MPTP complexes that associate closely with multiple OMM channel proteins leading to irreversible channel opening and to MPT. An important feature of this model is its inherent pore complexity and multilayered redundancy.

drugs, weak oxidants or natural physiological stressors such as mild hypoxia, altered pH and nutrient stress) will result in the controlled release of proapoptotic factors, including cytochrome *c*, SMAC/Diablo, endonuclease G and apoptosis-inducing factor (AIF) from the intermembrane space. This will lead to amplification of apoptotic cascades involving a protein complex called the apoptosome [9, 10]. In these situations, the integrity of the IMM remains tightly controlled to maintain osmolarity, to facilitate adenine nucleotide transport and to preserve the mitochondrial membrane potential, $\Delta\Psi_m$ [11] which is required for ATP production. In contrast, a strong stress signal (*e.g.* high concentrations of cytotoxic drugs, high dose irradiation and strong oxidants that irreversibly damage membrane lipids, DNA and proteins) will activate the mitochondrial permeability transition pore (MPTP), which is thought to involve both mitochondrial membranes. This results in depolarization of the inner membrane, swelling of the mitochondrial matrix, and in some situations, rupture of the OMM with consequent nonspecific spillage of mitochondrial contents into the surrounding cytoplasm [12, 13]. Although apoptotic activators may be released in this process, there is now some question as to whether this type of cell death should be classified as apoptosis. Indeed, in a clinical meta-analysis supported by preclinical experimental studies, the role of apoptosis in cancer chemotherapy and radiation therapy has been questioned for malignancies other than those of haemopoietic origin [14]. As stress signals vary in type and intensity, there is likely to be a continuum of involvement of IMM and OMM permeabilization. This may explain much of the present confusion in the literature over the nature of apoptotic and nonapoptotic cell death [15] which we propose will depend on the strength and duration of the inducing stimulus as well as the cell type, its tissue of origin and physiological context [16]. In normal tissues, apoptosis will be a consequence of normal physiological stressors such as nutrient stress, hypoxia, the pH of the extracellular environment and redox status of the cell. These stressors will act on cells of which some will have sustained irrepairable DNA, protein or lipid damage and some will be aging or senescent. In cancer, the strength and multiplicity of these stressors will be greatly amplified in the rapidly growing tumour, and additionally, at later stages, cytostatic and cytotoxic drugs and radiation therapy may come into play.

2 Mitochondrial membrane permeability (MMP) in apoptotic and nonapoptotic cell death

2.1 Mitochondrial outer membrane permeabilization (MOMP)

Permeabilization of the OMM has long been recognized as a major event in the induction of the mitochondrial pathway of apoptosis [6, 9, 17]. MOMP releases a range of proapoptotic proteins from the mitochondrial intermembrane space into the cytosol, including cytochrome *c* [18], AIF [19], Smac/Diablo [20], the serine protease HtrA2/Omi [21] and endonuclease G [21]. The nature of the pore that releases these proteins is still uncertain and the identity of the proteins involved in its formation is highly controversial [8, 22]. Two channels have been suggested as being responsible for the release of cytochrome *c* and other proapoptotic proteins in mammalian cells; the MPTP and the mitochondrial apoptosis-induced channel (MAC) [8]. The finding that apoptosis can be suppressed by MPTP inhibitors like bongkrekic acid (BKA) and cyclosporin A (CsA) [23] led to the idea that MPTP might be involved in mammalian cytochrome *c* release. This hypothesis; however, has been challenged by reports showing that MPTP inhibition has no effect on cytochrome *c* release in some systems [22, 24]. The mechanisms linking MPTP opening to MOMP are also controversial. The most commonly suggested mechanisms propose either the MPTP-dependent formation of a specific releasing pore in the OMM, or OMM rupture and cytochrome *c* release as a result of MPTP-dependent mitochondrial swelling [25]. The latter hypothesis; however, does not account for the fact that in many situations only a subset of specific proapoptotic proteins is released from the intermembrane space into the cytosol. The second proposed cytochrome *c* releasing pore, MAC, appears to contain at least Bax or Bak, as Bax/Bak double knockout murine embryonic fibroblasts (MEFs) are resistant to many apop-

totic triggers [26]. These cells do not release cytochrome *c* in response to the truncated proapoptotic Bcl-2 homology (BH)3-only Bid protein (tBid), which rapidly triggers cytochrome *c* release in wild type cells. MAC is not inhibited by BKA or CsA, but can be inhibited by the amphiphilic cations propranolol, dibucaine and trifluoperazine [27]. In contrast to their involvement in cytochrome *c* release, de Marchi *et al*. [28] showed that Bax and Bak do not directly participate in the Ca^{2+}-induced permeability transition in isolated mitochondria from a colon carcinoma cell line. Previous studies with rat liver mitochondria had shown that Bax and Bak induced cytochrome *c* release and the mitochondrial permeability transition (MPT) [29] whereas the BH3-only proteins, Bid and Bik, induced cytochrome *c* release without affecting MPT [30]. Thus, the role of Bcl-2 family proteins in the MPT may depend on the particular family member involved and the transformed status of the cell, and results obtained with isolated mitochondrial will need to be validated in intact cells.

Involvement of Bcl-2 family members in MMP raises questions of whether expression of Bax, Bak, Bid and other apoptosis-associated Bcl-2 family proteins change in cancer. In non small cell lung carcinoma, significant changes in the expression of apoptosis-related Bcl-2 family proteins were observed between individual tumours and between histologic subtypes [31]. Recently, proapoptotic Bnip3, which is downregulated in pancreatic cancer, was shown to mediate mitochondrial dysfunction through activation of Bax and Bak independently of MPTP opening [32].

2.2 MPTP complex

The efficiency of mitochondrial energy production is dependent on maintaining $\Delta\Psi_m$ which is generated by metabolically-driven proton pumps of the mitochondrial electron transport chain in the IMM. This membrane potential is regulated by uncoupling proteins [33] and by the MPTP complex that, according to current models, spans the IMM and OMM linking the mitochondrial matrix directly with the cytosol. The role of the MPTP complex in necrotic and apoptotic cell death and its structure are currently topics of intense debate. Recently, a succession of gene knockout studies [34–37] have severely undermined the prevailing current model of the MPTP complex [4, 15, 38–42] demanding a radical revision of its structure and function. Thus, liver-specific inactivation of genes encoding the adenine nucleotide translocator (ANT) isoforms, ANT1 and ANT2 [35], which traverse the IMM and are responsible for ADP influx and ATP efflux from the mitochondrial matrix, failed to affect FCCP-induced permeability transition and cytochrome *c* release in isolated liver mitochondria or the ability of oxidants to induce the MPT. Although CsA-sensitivity of the permeability transition was retained, calcium dependence of pore opening was altered in ANT-deficient mitochondria, and regulation by the ANT ligands, atractyloside (ATR) and ADP was lost. These results indicate that these ANT isoforms are involved in regulation of the permeability transition rather than being key components of the pore structure, and indicate that ANT ligands act to modulate this control. It is of interest to note that the mitochondrial uncoupling protein, UCP2, was downregulated to undetectable levels in ANT-deficient mitochondria, linking regulation of the permeability transition with maintenance of the mitochondrial membrane potential. Reduced UCP2 expression would act as a buffer against loss of $\Delta\Psi_m$ that would occur in cells with reduced ability to transport adenine nucleotides. More recently, mitochondria from voltage-dependent anion-selective channel (VDAC)-1, VDAC-3 and VDAC-1, -2 and -3-deficient mice were shown to be indistinguishable from wild-type mitochondria in their ability to undergo permeability transition in response to calcium and oxidants [34, 36], and cell death was unaffected or even exacerbated in embryonic fibroblasts lacking all three VDAC isoforms. Thus, in these studies, VDAC isotypes were not required for MPT or apoptosis driven by oxidants, staurosporine, ionomycin or proapoptotic Bcl-2 family members. Oncotic cell death, induced by erastin, a drug that kills tumour cells expressing the RasV12 and small ST oncoproteins, has however been shown to involve VDAC-2 and -3, with VDAC-2 or VDAC-3 knock down cells exhibiting resistance to erastin-induced cancer cell death [43]. This is explained by erastin binding directly to VDAC-2 and -3 and affecting MAC pore function. In other studies, cyclophilin D knockout mice were used to show that this peptidyl–prolyl *cis*/*trans* isomerase was required for calcium-induced mitochondrial swelling, apoptosis induced by oxidative stress and for reperfusion injury but not for cell death induced by a wide range of other chemical and extrinsic receptor-mediated apoptotic stimuli [37, 44, 45]. Together, these results negate a simple role for ANT isoforms 1 and 2 and VDACs 1–3 in the basic structure of the MPTP complex and its core function, and provide support for more subtle roles of VDAC, ANT and cyclophilin D in regulating pore function. Similar results have recently been published in the yeast, *Saccharomyces cerevisiae* [46], where neither deletion of the major VDAC isoform, porin 1, or the yeast mitochondrial cyclophilin, CPR3, had a consistent inhibitory effect on cytochrome *c* release and apoptosis-like cell death triggered by acetic acid, H_2O_2 or diamide [47]. Cytochrome *c* release induced by acetic acid and diamide was strongly inhibited in yeast lacking ACC1 to 3, the yeast strain lacking porin 1 showed enhanced apoptosis, whereas cell death in the cyclophilin yeast knockout was unaffected, a result that differs from that observed in mammalian systems. Thus, the standard model of MPTP complexes involving ANT, VDAC and cyclophilin D, and other associated proteins at contact points between the IMM and OMM is no longer tenable and we must go back to the drawing board and reconstruct models that are supported by the evidence (see Fig. 1). What constitutes the core structure of the pore complex responsible for

the permeability transition phenomenon? Are other ANT isoforms such as ANT3 [48, 49] and AAC4 [50], not targeted by Kokoszka *et al*. [35], key components of the pore complex, or are other carrier or shuttle proteins involved? What implications does this have for the numerous small molecules, including anticancer drugs, which affect the MPT and result in mitochondrial membrane permeabilization and subsequent cell death? Suggestions that cyclophilin D may interact with other members of the ANT family [51], or act as a nonspecific 'organizing centre' for various mitochondrial inner membrane solute transport proteins, and so orchestrate the permeability transition [52] represent alternative models of MPTP that need to be tested.

3 Drugs that affect mitochondrial membrane permeability (MMP)

One of the hallmarks of cancer cells is their ability to suppress proapoptotic pathways and/or to activate anti-apoptotic mechanisms [53]. In contrast, many anticancer drugs have been shown to activate apoptosis and/or suppress anti-apoptotic pathways [54, 55]. While a few of these drugs may directly target mitochondrial processes as part of their anticancer function, *e.g.* arsenic trioxide, mitomycin *c*, paclitaxel and cisplatin [56, 57], the vast majority of apoptosis-inducing anticancer drugs generate stress responses which indirectly activate the intrinsic pathway to apoptosis by inducing changes in MMP at the level of the IMM or OMM. Many other potential anticancer agents affect MMP, and some of these (*e.g.* BH3 mimetics, lipophilic cations, betulinic acid and complex II inhibitors that generate intramitochondrial oxidative stress) will be covered in more detail in other review articles in this series.

Compounds that affect MMP can be broadly divided into (i) those in which the primary target is associated with the putative MPTP complex in the IMM, (ii) those where the target is associated with the OMM and (iii) those where the primary target is nonmitochondrial. Each of these groups of compounds can be further subdivided into permeability activators and inhibitors. The activity of many MPTP modulators has been demonstrated in isolated mitochondria, but some have been investigated in cancer cells and a few such as CsA and its nonimmunosuppressive analogues [58] and arsenites have antitumour activity. Arsenic trioxide, which has pleiotropic effects that are difficult to separate mechanistically, is currently in clinical use in combination therapy with all-*trans* retinoic acid for acute promyelocytic leukaemia and other haemopoietic malignancies [59, 60].

3.1 Modulators of MMP and the MPT at the IMM

MPT refers to the abrupt change in permeability of the IMM that occurs when isolated mitochondria are exposed to high concentrations of calcium or reagents that increase oxidative stress [61]. This is often measured *in vitro* as mitochondrial swelling, *e.g.* turbidity, or by increased conductance. The initial proposal that the permeability transition occurred *via* a discrete pore in the IMM that allowed free passage of molecules <1 kDa led to a model in which the basic structure of the pore complex was suggested to include VDAC, ANT and cyclophilin D [7, 41, 62] at intermembrane junctional complexes [63]. The discovery that CsA inhibited pore opening [64–66] further supported this pore complex model and this became a defining pore property. Numerous inducers and inhibitors of the permeability transition in isolated mitochondria have been described and classified [6, 15, 41, 42, 67]. A few compounds with known and potential anticancer activity will be discussed here.

3.2 MPT activation

Activation of the MPT has been the main focus of attention regarding known anticancer drugs and compounds with potential anticancer activity.

ANT: although neither ANT1 nor ANT2 are essential for MPT in liver mitochondria [35], other ANT homologues may be involved in pore formation in liver and other tissues, including cancer cells. That ANT isoforms are actually involved in regulating the permeability transition is indicated by the fact that ANT ligands like ATR, BKA and ADP modulate pore activity and compromise mitochondrial function [68, 69]. Recently, random mutagenesis studies showed that ANT3 was involved in TNF-α and stress-induced apoptosis in MCF-7 breast carcinoma cells [49]. However, ANT3 was not required for cell death caused by other inducers of apoptosis. These results suggest cell-type and stimulus-related roles for ANT family members in apoptosis.

Several small molecules that induce cancer cell death also activate the MPT in isolated mitochondria. CsA reversal of these effects is often taken as an indicator of ANT involvement, since CsA binds to and inhibits cyclophilin D, which associates with ANT at the interface of the IMM and the mitochondirial matrix [70]. Such compounds include the steroid-like triterpene, betulinic acid [71], the novel retinoid, CD437 [72] and the anticancer drugs, lonidamine [73, 74] and arsenic trioxide [75], although the exact mechanism of action of these compounds appears to differ [38, 75]. In addition, low concentrations of mastoparan, a 14-amino acid amphipathic peptide from wasp venom was shown to activate the permeability transition in a Ca^{2+}-dependent and CsA-inhibitable manner [76] also suggesting effects at the level of the ANT. Lytic effects of micromolar concentrations of mastoparan on erythrocytes [77] and on pancreatic β cells [78] preclude the clinical use of this compound.

Other cytotoxic compounds that activate the MPT in a CsA-dependent manner and therefore may work at the level of ANT include the antidiabetic drug, metformin [79], the

active component of a traditional Chinese herb, honokiol [80, 81], the marine alkaloid, lamellarin D [82], the flavenoids genistein [83] and sophoranone [84], novel pyridothiopyranopyrimidine compounds [85], 1,4-anthancenediones [86] and the C-terminal peptide of the HIV-1 regulatory protein, Vpr [87].

Although CsA inhibition has been widely used to infer involvement of the MPT in cell death processes *via* its effect on cyclophilin D which associates with ANT, this assumption should be treated with caution. Both apoptotic and nonapoptotic cell death can be involved depending on the cell type under investigation and the nature and duration of the stress or cytotoxin involved, and mitochondrial and cellular studies should give concordant results. It should also be remembered that CsA is a lipophilic molecule that is not specific for cyclophilin D but binds to other members of the cyclophilin family and to other proteins. Therefore, care must be exercised in interpreting inhibitor results on MPT and cell death, particularly in the light of the present uncertainty about the essential structure of the MPTP complex. The minimal requirement for claiming involvement of CsA-inhibitable MPTP should be inhibition with one of the nonsuppressive analogues that inhibit cyclophilins that will not result in calcineurin inhibition.

Other: recently, several novel oleanane triterpenoids have been shown to induce apoptosis in normal and malignant B cells *via* CsA-insensitive MPT pores that remain 'constitutively' open [88]. These open channels are associated with the generation of mitochondrial superoxide.

It is also worth noting that cardiotoxicity associated with clinical use of doxorubicin [89, 90] and gleevec [79] is, at least in part, related to the ability of these drugs to induce MPT changes in cardiac mitochondria *via* activation of the ER stress response and consequent oxidative stress.

3.3 MPT inhibition

MPT inhibitors have received much less attention than activators in the quest for novel mitochondrially-directed anticancer drugs that modulate pore activity. However, blocking MPT function should not be ignored as a potential anticancer approach. In particular, blocking ANT which is essential for nucleotide trafficking across the IMM has shown some promising results.

ANT: the 'so-called' ANT ligands ADP, ATR and BKA bind to dimeric ANT and inhibit nucleotide translocation across the IMM [68, 91, 92]. Mitochondria from cells expressing human ANT3 exhibited similar low (ADP) and high (ATR and BKA) affinity binding compared with mitochondria from mammalian cardiac mitochondria [48] which express ANT1 and 2. Another ANT inhibitor, MT-21, caused cytochrome *c* release without mitochondrial swelling [93] suggesting an MPT-independent mechanism of action similar to ATR. In general; however, loss of mitochondrial function and nonspecific toxicity considerations has largely precluded serious exploration of these inhibitors as anticancer agents.

Cyclophilin D: CsA, a potent immunosuppressant and prototypic MPT inhibitor and its nonimmunosuppressive analogues like [MeVal4]CsA, [MeIle4]CsA and [MeAla6]CsA, bind with high affinity to cyclophilin D, thereby inhibiting pore opening and suppressing nonapoptotic cell death from oxidative stress, Ca^{2+} overload and ischemic and reperfusion injury in mammalian cells [45, 94, 95].

Other: thapsigarin also inhibits MPTP opening but its effects in preventing Ca^{2+} accumulation appear to be indirect *via* a Ca^{2+}-ATPase of the ER [96].

3.4 Modulators of the MMP at the OMM

Several protein constituents of the OMM have been shown to affect MMP, MPT and apoptosis. These include the peripheral benzodiazepine receptor (PBDR), now referred to as translocator protein (TSPO) in the light of its channel-like functions [97], the voltage-dependent anion transporter (VDAC), complexes of Bcl-2 family members, Bax and Bak, that are themselves capable of forming pore structures [8], and hexokinases which associate with the OMM [98, 99], in particular with VDAC [100, 101]. Interestingly, many of these components have been shown to be differentially expressed in various tumour cells and this has been suggested to protect cancer cells against apoptosis [15].

BH3 mimetics: the main regulators of mitochondrial pathways are proteins of the Bcl-2 family that can be classified into three groups; the antiapoptotic family members (like Bcl-2 and Bcl-x_L, both containing four BH domains), the multidomain proapoptotic members (like Bax and Bak, both believed to be involved in MAC formation) and the proapoptotic BH3-only proteins (like Bad and Bim) [102]. The design of BH3 mimetics that stimulate apoptosis of cancer cells is a major focus of many pharmaceutical companies at present. One of the most exciting developments in the field is the small molecular weight inhibitor of antiapoptotic Bcl-2 family proteins, ABT-737, that induces regression of solid tumours in mice [103]. This area has been recently reviewed [104] and is covered in more detail by Hockenbery in this issue. Bax channel blockers like Bci1 and Bci2 [105] inhibit pore opening but these compounds are more relevant to degenerative diseases than to cancer.

TSPO/PBDR: the isoquinoline carboxamide, PK11195, blocks the PBDR and induces dissipation of $\Delta\Psi_m$ *via* effects on the MPT [80, 106]. The mitochondrial effects of PK11195 are associated with *de novo* generation of reactive oxygen species, are not reversed by Bcl-2 transfection [107] and potentiate the apoptosis induced by chemotherapeutic drugs [100]. In contrast, the benzodiazapine, Ro5-4864, protects tumour cells from apoptosis and this effect can be blocked by PK11195 [108]. PK11195 also inhibits drug efflux through P-glycoprotein and this may contribute

to its role in sensitizing drug-resistant AML patients to chemotherapeutic agents [109–111]. However, more recent evidence has shown that PK11195 can sensitize to apoptosis independently of MDR pumps, and in cells that lack PBDR, raising questions about the proapoptotic mechanism of this compound [112].

VDAC: VDAC was first described as a major protein of the OMM, but was later shown to be also expressed in the plasma membrane [113]. In mammals three VDAC genes have been demonstrated to encode functionally active isoforms. They share a similar organization and thus result from duplication events [114]. VDAC isoform 1 is a small, 30–35 kDa protein, originally discovered by Schein *et al*. [115]. VDAC-1 is the pore-forming protein responsible for the permeability of the OMM to negatively charged, solutes involved in mitochondrial energy metabolism [116]. Consequently, VDAC regulates mitochondrial metabolite flux [117]. Its opening stimulates oxidative phosphorylation, its closing suppresses mitochondrial function, making it a global regulator/governor of mitochondrial function [118]. Mitochondrial VDAC-1 directly associates with the members of the anti-apoptotic Bcl-2 family of proteins [119–121]. Physiologically, VDAC pore opening can be regulated by oxidative stress, NADH/NAD^+, association with other proteins, and phosophorylation/dephosphorylation [122]. Pharmacological VDAC inhibitors include diisothiocyanatostilbene-2′,2-disulphonate (DIDS) [123, 124], König's polyanion [117] and two related compounds known to bind to calcium-binding sites, ruthenium red [125] and ruthenium amine binuclear complex (Ru360) [126].

VDAC at the plasma membrane – although VDAC-1 is predominantly expressed in the OMM, several groups have also shown that VDAC can be expressed in the plasma membrane [127–134]. Consistent with these observations, patch clamp techniques have documented the presence of plasma membrane channels with physiological properties similar to VDAC-1 [135, 136]. Furthermore, immunocytochemical studies using antibodies raised against the NH_2-terminal of VDAC-1 block a high conductance anion channel found in the plasma membrane of bovine astrocytes [131]. These antibodies also appear to specifically label the plasma membrane in human B-lymphocytes. The mouse VDAC1 gene harbours two alternative first exons, which leads to the expression of VDAC1 isoforms with different N-terminal sequences. One of these isoforms carries a leader sequence that directs the protein *via* the Golgi apparatus into the secretory pathway [130]. The other VDAC-1 isoform, devoid of any cleavable presequence is targeted to the mitochondria, where it is efficiently inserted into the outer membrane [130]. How human VDACs are directed to the plasma membrane still remains a mystery. The function of VDAC at the plasma membrane is still in debate, but many functions have been suggested, including a role in ATP release [133], a function as a transplasma membrane NADH-ferricyanide reductase [128] and a receptor for plasminogen kringle 5 [132]. Whatever the function(s) of VDAC at the plasma membrane, targeting mitochondrial VDAC will also affect its function at the plasma membrane.

We and others have reported that loss of mitochondrial function, as occurs in many glycolytic cancers, can be at least partially buffered by *trans*-plasma membrane electron transport (tPMET) [137–142]. Thus, VDAC and other tPMET systems may compensate for loss of mitochondrial electron transport in glycolytic cancers by directly oxidizing NADH to facilitate glycolytic ATP production, a role traditionally assumed by lactate dehydrogenase. Inhibiting VDAC function in the OMM and at the level of the plasma membrane may be an attractive approach for anticancer drug development in the future. With plasma membrane VDAC and other PMET targets, this could be achieved by designing 'safe' drugs that act at the level of the plasma membrane.

4 Summary

In addition to their basic role in energy metabolism, mitochondria are now well-recognized as central players in mediating cell death in normal development and tissue homeostasis and in disease. Pore structures in both the IMM and OMM that are involved in these cell death pathways have become a focus of attention for anticancer drug development. Many cancer therapeutics deliver their cytotoxic effects either indirectly through stress-related pathways that involve mitochondrial membrane integrity or, in some cases, directly *via* components of mitochondrial membrane pore complexes or their associated regulatory molecules. To better exploit the potential of targeting mitochondrial permeability in the treatment of cancer, we need a much clearer understanding of the structures of these membrane pores in healthy and malignant tissues and the way in which the regulation of pore function changes during malignant transformation. Physical interaction and crosstalk between the pores needs to be better defined, and results obtained with isolated mitochondria translated into physiological conditions in cells, tissues and whole organisms as well as in a range of cancer cells. This knowledge will provide a framework for exploiting altered pore structure and regulation in cancer treatment.

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