Targeting the Mitochondrial Permeability Transition: Cardiac Ischemia-Reperfusion Versus Carcinogenesis

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Key Words

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Abstract

Cardiovascular diseases and cancer continue to be major causes of death worldwide, and despite intensive research only modest progress has been reached in reducing the morbidity and mortality of these awful diseases. Mitochondria are broadly accepted as the key organelles that play a crucial role in cell life and death. They provide cells with ATP produced via oxidative phosphorylation under physiological conditions, and initiate cell death through both apoptosis and necrosis in response to severe stress. Oxidative stress accompanied by calcium overload and ATP depletion induces the mitochondrial permeability transition (mPT) with formation of pathological, non-specific mPT pores (mPTP) in the mitochondrial inner membrane. Opening of the mPTP with a high conductance results in matrix swelling ultimately inducing rupture of the mitochondrial outer membrane and releasing pro-apoptotic proteins into the cytoplasm. The ATP level is the determining factor in deciding whether cells die through apoptosis

or necrosis. Cardiac cells undergoing ischemia followed by reperfusion (IR) possess exactly the same conditions mentioned above to induce mPTP opening. Due to its critical role in cell death, inhibition of mPTP opening has been accepted as a major therapeutic approach to protect the heart against IR. In contrast to cardiac IR, cancer cells exhibit less sensitivity to pore opening which can be in part explained by increased expression of mPTP compounds/modulators and metabolic remodeling. Since the main goal of chemotherapy is to provoke apoptosis, mPT induction may represent an attractive approach for the development of new cancer therapeutics to induce mitochondria-mediated cell death and prevent cell differentiation in carcinogenesis. This review focuses on the role of the mPTP in cardiac IR and cancer, and pharmacological agents to prevent or initiate mPT-mediated cell death, respectively in these diseases.

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Introduction

Mitochondria play a central role in cell life by regulating both survival and death signalling pathways.

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On the one hand, mitochondria are the powerhouse providing over 90% of ATP through oxidative phosphorylation for the survival of the cell. On the other hand, mitochondria are the main regulators of cell death through apoptosis, necrosis and autophagy. Severe oxidative stress accompanied by calcium overload in the mitochondrial matrix causes an increase in the permeability of the mitochondrial membranes with formation of the pathological and non-specific mPTPs [1-3]. Opening of the mPTP with high conductance induces depolarization of the mitochondrial inner membrane (MIM) leading to ATP depletion and further reactive oxygen species (ROS) production. The sudden increase in MIM permeability enhances colloidal osmotic pressure in the mitochondrial matrix ultimately leading to matrix swelling and rupture of the mitochondrial outer membrane (MOM). Rupture of the MOM results in release of pro-apoptotic proteins from the mitochondrial intermembrane space to the cytoplasm, and thereby, initiating both caspase-dependent and caspase-independent apoptosis. Permeabilization of the MOM may also occur due to formation of non-selective channels induced by translocation of pro-apoptotic Bcl-2 family proteins to the mitochondria. The roles of Bcl-2 family members in MOM permeabilization and mitochondria-induced apoptosis are beyond the scope of this review and have been discussed elsewhere [4, 5]. Due to its central role in lethal functions, the mPTP represents a potential therapeutic target for both cell survival and death interventions. Studies over 20 years have demonstrated that acute cardiac ischemia-reperfusion (IR) which is caused by interruption of substrates and oxygen (ischemia) followed by reoxygenation and substrate supply (reperfusion) is associated with opening of mPTP [1-3]. Furthermore, pharmacological and conditional inhibition of mPTP formation significantly improved cardiac function reducing ischemic injury and myocardial infarction size in animal models [6-8] and patients [9, 10]. On the other hand, a growing number of studies on a variety of animal tumor models and cancer cell lines demonstrate that induction of mPTP opening by pharmacological interventions initiates apoptotic cell death and prevents cell differentiation in tumorigenesis which is a chronic process with tissue-specific metabolic reprogramming [11-14]. This review will focus on a comparative discussion of molecular mechanisms regulating the mPTP in acute cardiac IR and tumorigenesis. These two pathological states develop distinct functional and structural abnormalities due to various pathogenetic mechanisms and etiological factors

involved in these diseases. Overproduction of ROS and Ca^{2+} -overload of the mitochondrial matrix together with ATP depletion and phosphate accumulation are the main metabolic alterations in cardiac IR that favor mPTP formation whereas metabolic remodeling associated with acidosis and overexpression of the major structural and regulatory proteins of the mPTP complex significantly reduces pore formation and mitochondria-mediated cell death in cancer cells. We will also discuss pharmacological targeting of the mPTP as a therapeutic approach for the treatment of cardiac IR and carcinogenesis through inhibition or induction, respectively, of pore opening.

Structure of the mPTP complex

The concept of the mPT was proposed by Hunter et al. when Ca^{2+} caused a non-specific increase in the permeability of the MIM leading to matrix swelling and the transition [15]. Mitochondrial permeability transition is accompanied by formation of non-selective high conductance channels or megachannels currently accepted as the mPTPs which were initially discovered in electrophysiological studies on mitoplasts [16], and in patch-clamp experiments [17]. Existence of mPTPs and their crucial role in cell death induced by a number of pathological conditions, mostly IR have been intensively studied in the heart, brain and liver [1, 18, 19]. However, despite these studies, the actual molecular composition of the mPTP complex remains unclear. Until recently, three proteins had been accepted as key structural components of the mPTP: adenine nucleotide translocase (ANT) in the MIM, mitochondrial cyclophilin D (CyP-D) in the matrix and the voltage-dependent anion channel (VDAC) in the MOM. However, recent studies from different groups have questioned the molecular identity of the mPTP. A number of genetic studies have excluded VDAC [20, 21] and ANT [22] as essential structural components of the mPTP complex. A new model of the mPTP consisting of phosphate carrier and ANT has been proposed recently where Ca^{2+} sensitivity of the pore is regulated by CyP-D binding to a phosphate carrier [1, 23]. Many studies have provided strong evidence that CyP-D plays a major regulatory role in mPTP formation [24-26]. Although cytoplasmic cyclophilins such as CyP-A are involved in cell signalling and protein folding as chaperones [27] the role of mitochondrial CyP-D under normal conditions remains unclear. One of the functions of CyP-D may be formation of the mPTP with a low conductance to regulate the ion homeostasis in the

mitochondrial matrix under physiological conditions. Mitochondria isolated from CyP-D knockout mice were desensitized to onset of the mPT, and required much higher concentrations of Ca^{2+} to induce pore opening compared to wild type animals which is consistent with the role of CyP-D to regulate Ca^{2+} -mPTP interactions [24, 26]. High matrix phosphate levels that compete for the nucleotide binding site of ANT favor pore opening. The conformational state of the ANT is affected by specific ligands of the exchanger such as carboxyatractyloside (CAT) and bongkreikic acid (BKA) that, respectively decrease and increase matrix ADP binding affinity, thereby leading to the opening and closing of the mPTP. mPTP opening is inhibited by H^+ and divalent cations (Mg^{2+} , Mn^{2+} , Sr^{2+}) that antagonize Ca^{2+} binding to ANT [28]. The conformational state and pore forming activity of mPTP compounds are also regulated by hexokinase (HK), the peripheral benzodiazepine receptor (PBR) and Bcl2 proteins on the MOM where they bind to VDAC, and creatine kinase (CK) in the intermembrane space interacting with ANT.

Thus, the molecular identity of the mPTP remains unknown. Recent studies on transgenic mice questioned the role of ANT and VDAC as essential components of the mPTP showing their regulatory rather than structural role in pore formation along with CyP-D. Despite this, most existing studies on the formation and regulation of the mPTP complex suggest that VDAC, ANT and CyP-D are the key players in pore formation. This is why we discuss these three proteins as the main mPTP components implicating their regulatory but not a structural roles in pore formation.

mPTP formation in cardiac IR and cancer

Opening of mPTPs allows water and solutes (<1500 Da) to enter the mitochondrial matrix causing swelling due to increased colloidal osmotic pressure. Extensive matrix swelling induces rupture of the MOM and release of mitochondrial pro-apoptotic proteins. Concurrent opening of multiple mPTPs accompanied by rigorous release of apoptotic proteins can initiate the mitochondria-mediated apoptotic pathway leading to cell death through apoptosis. Most likely, apoptosis occurs in response to relatively moderate stress when mPTPs are able to reseal and mitochondria still maintain ATP production. However, severe oxidative stress accompanied by extensive mPTP opening can induce cell death through necrosis rather than apoptosis even though mitochondrial apoptotic proteins

are present in the cytosol. This is explained by insufficient levels of ATP to initiate and carry out the apoptotic cascade in the cell. The extent of oxidative stress (ROS level) presumably controls the balance between life and death through mPTP opening which determines whether cells die by apoptosis or necrosis. The release of pro-apoptotic proteins due to mPTP opening in one group of mitochondria can enhance ROS generation and Ca^{2+} overload and can induce further structural and functional disruptions in other groups of undamaged mitochondria thus amplifying cell death. Indeed, subsarcolemmal and intermyofibrillar mitochondria isolated from the rat skeletal muscle demonstrated differential susceptibility to oxidative stress, and intermyofibrillar mitochondria had greater rates of mPTP opening compared with subsarcolemmal mitochondria [29].

Regulation of the mPTP by a variety of signalling molecules and cellular metabolites and ions is a complex process, and mPTP formation ultimately depends on the balance between factors favouring and inhibiting pore opening. The precise metabolic role of mPTP formation under physiological conditions is still being debated; reverse pore opening with a low conductance may be attributed to regulation of mitochondrial Ca^{2+} . Low conductive mPT-induced matrix swelling and increased mitochondrial Ca^{2+} can regulate ATP synthesis through the tricarboxylic acid cycle, electron transport chain and oxidative phosphorylation. Induction of mPT in response to pathological stresses can also be regulated with the signalling protein kinases PKA, PKC ϵ and GSK-3 β which interact with VDAC [30-32]. Expression of the major components (ANT, VDAC, CyP-D) and regulatory proteins (HK, PBR, Bcl2, CK) of the mPTP complex are presumed to play, along with metabolic and signalling factors, a key role under continuous pathological conditions such as carcinogenesis. Furthermore, unlike cardiac IR, induction of mPT can differ among various cancer diseases in a tissue/organ/cell-specific manner (Fig.1).

Cardiac ischemia/reperfusion

mPTP opening has been examined extensively in cardiac pathological conditions, mostly in IR [1-3]. Acute IR does not affect the expression of mPTP compounds due to its short duration although it induces conformational changes in essential mPTP proteins, modifying their interactions with the pore effectors in the cytoplasm and mitochondrial matrix. The ischemic heart contains all factors necessary for pore opening including ATP depletion, Ca^{2+} overload, as well as increased phosphate and ROS levels. However, a direct measurement of mPT

Fig. 1. Metabolic inducers and inhibitors of mPTP opening in cardiac ischemia-reperfusion and tumorigenesis. ANT, adenine nucleotide translocase; CK, creatin kinase; CyP-D, cyclophilin D; HK, hexokinase; MIM, mitochondrial inner membrane; MOM, mitochondrial outer membrane; P_i , inorganic phosphate; PBR, peripheral benzodiazepine receptor; SOD, superoxide dismutase; VDAC, voltage dependent anion channel.

in the intact heart utilizing the [3 H]-2-deoxy-glucose entrapment method demonstrated that pore opening occurs during reperfusion rather than ischemia [33]. This is explained, in part, by a low pH_i induced by lactate and other acidic intermediates that accumulate during ischemia due to the absence of oxygen and coronary flow in the heart. H^+ is known to be a strong inhibitor of pore opening due to its competition with Ca^{2+} at the trigger site of ANT. Post-ischemic recovery mostly depends on the ability of the ischemic mitochondria to supply cardiac cells with ATP. A significant reduction in ATP levels after severe ischemia induces Ca^{2+} -overload due to activation of reverse mode Na^+/Ca^{2+} exchanger (NCE), and reduction of the sarcoplasmic reticular Ca^{2+} -ATPase activity. Increased [Ca^{2+}] $_i$ with a burst of ROS generation provokes mPTP opening. Notably, the extent of pore formation rapidly increases immediately after pH_i returns to normal values during the first minutes of reperfusion [34]. We and others demonstrated that delaying pH_i recovery at reperfusion has a beneficial effect on post-ischemic recovery of cardiac function in association with improved mitochondrial function and less mPTP opening [35, 36]. In this regard, inhibition of Na^+/H^+ exchanger 1 (NHE-1, which exchanges intracellular H^+ for extracellular Na^+ thereby regulating the pH_i) is a promising therapeutic strategy for the treatment of cardiac IR [37, 38] (see Cardiac ischemia/reperfusion). Pore opening causes mitochondrial uncoupling which causes the F_0F_1 -ATPase to work in reverse mode hydrolyzing rather than producing ATP. Severe ATP depletion destroys the structural and functional integrity of cells ultimately leading

to their death. In addition to IR, mPTP opening was also found increased in Ca^{2+} -induced [39] and diabetic cardiomyopathy [40], heart failure following myocardial infarction [41] and intracoronary microembolizations [42].

Cancer

A growing number of studies provide controversial evidence on the roles of ROS and Ca^{2+} , the most efficient inducers of mPT in cancer cells [12, 13, 43]. Tumor cells have been shown to generate excessive amounts of ROS [44], however drug-resistant tumor cells possess upregulated anti-oxidant systems with increased expressions of superoxide dismutase, thioredoxin reductase and glutathione [45-48]. They detoxify ROS and therefore, partially reduce the efficiency of oxidative stress to induce pore opening. Likewise, drug-resistant tumor cells exhibit a well-developed capacity for cytosolic calcium handling [49, 50]. They are more resistant to Ca^{2+} stress due to their ability to efficiently regulate the cytosolic [Ca^{2+}] by sequestering and releasing it to and from the endoplasmic reticulum. It is worth noting that although tumor cells mostly demonstrate more oxidative stress and diminished calcium handling they are less sensitive to mPTP opening which can be associated with differential expression of individual pore components and regulation of their activity in carcinogenesis [12].

The majority of studies into distinct cancer cell lines and tumor models have found the main mPTP proteins overexpressed [12, 13]. Expression of the three VDAC isoforms was significantly higher in malignant tumor cells than those from normal cells [51]. In addition, of the 4 ANT isoforms [52, 53], ANT1 (expressed in heart and skeletal muscle) and ANT3 (expressed ubiquitously) are pro-apoptotic whereas ANT2 (expressed in malignant cells and proliferating myoblasts) is anti-apoptotic; ANT4 demonstrates only low expression in human tissues [53-56]. The ATP/ADP antiporter activity of ANT1 is inhibited or activated, respectively by its interactions with pro-apoptotic (Bax) and anti-apoptotic (Bcl2) proteins [57, 58]. The ANT2 isoform is required for cancer cell glycolysis [59] and its overexpression does not induce mPT [54]. Increased expression of ANT2 was observed in renal tumors [60] and in liver biopsies with abnormal "oncocytic" hepatocytes [61]. ANT2 overexpression may have resulted from metabolic reprogramming and diminished energy metabolism in cancer cells where it can be implicated to play a role different from its "primary" function. Unlike cardiac IR, CyP-D has an opposite effect inhibiting mPT-mediated apoptosis and necrosis in tumor cells [62, 63]. Although the mechanisms

underlying this effect remain unclear, they have been associated with binding of HKII to mitochondria [64]. CyP-D has been found upregulated in human tumors of the breast, ovary, and uterus [63] where it specifically repressed ANT-1-induced apoptosis. These studies suggest that upregulation of CyP-D in carcinogenesis inhibits opening of the mPTP.

Both cancer cell lines and tumor tissues exhibit overexpression of PBR which correlates positively with tumorigenesis, and specific ligands of the receptor induce apoptosis in cancer cells [65, 66]. Anti-apoptotic effects of Bcl-2 proteins such as Bcl-X_L and MCL1 were reversed in human cholangiocarcinoma cells with the PBR antagonist PK11195 [67]. However, PBR ligands (RO5-4864, PK11195 and diazepam) induced apoptosis and significantly increased the growth inhibition induced by chemotherapeutic agents in human small cell lung cancers xenografted into nude mice, suggesting that PBR activators can act as chemosensitizing agents for the treatment of tumorigenesis [68]. Since PBR is accepted as an anti-apoptotic protein in normal cells, the relationship between PBR overexpression and mPTP inhibition in cancer cells remains to be elucidated. PBR ligands may have receptor-independent anti-cancer effects in tumour cells. Creatine kinase, which inhibits the mPTP, has also been found overexpressed in many tumors [26, 69]. Likewise, various tumors exhibit overexpression of Bcl-2 proteins [70, 71] that are known to modulate the activity of the mPTP complex. Bax protein was found mutated in various tumors such as gastric cancers [72] and colon carcinomas [73].

The majority of malignant cells demonstrate a compensatory increase in glycolytic ATP production due to dysfunctional mitochondrial oxidative phosphorylation. Expression of HK, which is a key enzyme of glycolysis and one of the main regulators of the mPTP complex was found mostly overexpressed in cancer cells and tumors, especially those with enhanced glycolysis [74-76]. Mitochondrial dysfunction and stimulation of glycolysis is accompanied by an increase in lactate production despite the presence of oxygen in cancer cells (Warburg effect) [77]. The Warburg effect may have at least two consequences associated with mPT. First, HK inhibits VDAC activity via protein-protein interaction and blocks binding of Bax to the mPTP complex thus preventing mitochondria-mediated apoptosis [76, 78]. Second, lactate accumulation induces metabolic acidosis which can prevent pore opening by inhibiting Ca²⁺ binding to the mPTP. In addition, cancer cells of both primary origin and metastasis suffer from hypoxia due to delayed

and incomplete angiogenesis in the tumor. Hypoxia could activate mPTP formation through the hypoxia-inducible factor (HIF)-mediated upregulation of the pro-apoptotic Bcl-2 family proteins, BNIP and NIX [79, 80]. Although this pathway is activated in normal cells under hypoxic stress including cardiac IR, it is inhibited in cancer cells that exhibit better survival [81]. The mechanisms underlying the high resistance of tumor cells to the BNIP3-mPTP pathway-mediated cell death are still unknown; however it can be partially explained by the diminished expression and post-translational activation of pro-apoptotic factors (e.g. BNIP) under hypoxic conditions.

Thus, differential expression of mPTP compounds in carcinogenesis can reduce pore formation through modulations of their interaction with pore inducers/antagonists in mitochondria and cytoplasm. Furthermore, overexpression of the main components and regulators of the mPTP complex can exert an opposite effect on pore formation.

Targeting mPTP in cardiac IR and cancer

Cardiac ischemia/reperfusion

mPTP formation in cardiac IR can be inhibited through a direct targeting of main pore components or indirectly, through reducing the levels of mPTP inducers such as ROS, Ca²⁺ and pH_i (Table 1). CyP-D and ANT are the main mPTP components that have been extensively examined to target pore opening in the ischemic heart. Inhibition of the ANT has less clinical prospect since the ANT inhibitors also block the catalytic activity of the carrier leading to diminished ADP-ATP exchange in mitochondria. Bongkreikic acid and atractyloside (the main inhibitors of ANT) have been shown to have opposite effects inhibiting and provoking mPTP opening, respectively. Bongkreikic acid inhibited Ca²⁺-induced mPTP opening in isolated mitochondria [82], and prevented oxidative stress- and BNIP3-induced mPT and cell death in isolated cardiomyocytes [83, 84]. In contrast, atractyloside sensitized the mPTP to Ca²⁺ [85, 86], and blocked the protective effect of nitric oxide on Ca²⁺-induced mitochondrial swelling which is a marker of mPTP opening [87]. The divergent effects of bongkreikic acid and atractyloside are the result of their interaction with the ANT on its matrix and cytoplasmic sides inducing “m” or “c” conformations, respectively [52, 88]. The “c” conformation provokes opening whereas “m” conformation is associated with inhibition of the mPTP.

Inhibition of CyP-D with the immunosuppressive drug, cyclosporine A (CsA), has been shown to protect the heart against IR in various animal models [6-8]. CsA blocks CyP-D binding to the mPTP thus antagonizing pore formation. The protective effect of CsA could also be mediated via inhibition of calcineurin activity. However, studies using CsA derivatives, and non-immunosuppressant sanglifehrin A (SfA) which do not possess the ability to inhibit calcineurin, as well as experiments on isolated mitochondria, provided strong evidence that inhibition of CyP-D exerts the beneficial effect on the ischemic heart [8, 33]. SfA blocks mPTP opening due to inhibition of the peptidyl-prolyl *cis-trans* isomerase activity of CyP-D, thus preventing the conformational modification of the ANT required for pore formation [89]. Likewise, genetic ablation of the *Ppif* gene encoding CyP-D in mice demonstrated reduced infarct size and less cell damage in both heart and brain infarction [25, 26, 90]. Furthermore, mitochondria of CyP-D knockout animals were more resistant to Ca^{2+} - or oxidative stress-induced mPTP opening compared to wild-type mice [25]. The two most promising compounds CsA and SfA have been recently used on human cardiac tissue, and are in initial trials in patients. The drugs improved contractile function and cell survival against *in vitro* hypoxia-reoxygenation injury in atrial tissue samples harvested from patients undergoing cardiac surgery [9]. CsA induced a significant reduction in infarct size in patients with acute myocardial infarction [10].

Along with IR, a number of studies performed on other cardiac diseases that involve insufficient substrate and oxygen supply and diminished Ca^{2+} handling, demonstrated protection in response to CsA through inhibition of mPTP opening. Genetic ablation [39] and pharmacological inhibition of CyP-D [91] improved cell survival in doxorubicin-induced cardiomyopathy. Furthermore, CyP-D knockout mice exhibited significantly less cell death and cardiac fibrosis, delaying the onset of heart failure induced by Ca^{2+} -channel overexpression [39]. CsA improved contractility and mitochondrial function in cardiomyocytes isolated from dogs with heart failure [42], and attenuated phenylephrine-induced cardiomyocyte hypertrophy associated with reduced mPTP opening and mitochondrial membrane depolarization [92].

In addition to the direct effects, a number of pharmacological agents have been found to prevent mPTP opening indirectly by reducing mitochondrial accumulation of ROS and Ca^{2+} or by enhancing the levels of pore inhibitors such as H^+ . Prevention of ROS

Agent	Model	Mechanism	Reference
<i>Cardiac ischemia/reperfusion</i>			
Bongkrekic acid	<i>In vitro</i>	Inhibits ANT	[52]
CsA and its analogues	<i>In vivo</i> and <i>in vitro</i> ; Clinical trial on limited number of patients with myocardial infarction	Inhibit CyP-D binding to ANT	[6, 10, 33]
SfA	<i>In vivo</i> and <i>in vitro</i>	Inhibits PPIase activity of CyP-D	[7, 8, 89]
NHE-1 inhibitors (cariporide and its derivatives), pyruvate	<i>In vivo</i> and <i>in vitro</i>	Reduce pH_i and prevents Ca^{2+} -overload	[34, 35, 98]
ROS scavengers (e.g. propofol, MCI-186)	<i>In vivo</i> and <i>in vitro</i>	Prevent ROS-induced oxidation of thiol groups on the ANT	[94, 95]
Ubiquinone analogues (e.g. UQ0, Ro 68-3400)	<i>In vivo</i> and <i>in vitro</i>	May act via complex I or bind to ANT and phosphate carrier	[20, 23]
<i>Cancer</i>			
GSAO	<i>In vivo</i> and <i>in vitro</i>	Inhibits ANT and VDAC, acts as a thiol-oxidizing agent	[103-105]
CD437	<i>In vivo</i> and <i>in vitro</i>	Inhibits ANT	[111-115]
Lonidamine	Phase II (Glioblastoma; Solid tumours); Phase III (Breast cancer)	Inhibits ANT and mitochondrially-bound HK	[115-117]
PK11195	<i>In vivo</i> and <i>in vitro</i>	PBR ligand	[67, 68]
Betulinic acid	Phase II/III (Dysplastic nevus syndrome)	mPT inducer	[109, 110]
Clodronate	Phase III (Breast cancer)	Inhibits ANT	[108]
L-buthionine sulphoxamine	Phase I (Melanoma; Neuroblastoma)	Enhances ROS production	[121]
Imexon	Phase I (Prostate cancer; Advanced malignancy)	Reduce GSH levels	[120]
β -phenylethyl isothiocyanate	<i>In vivo</i> and <i>in vitro</i>	Enhances ROS production	[122, 123]
Mangafodipir	Phase II (Colon cancer)	Enhance H_2O_2 levels	[124]
Motexafin gadolinium	Phase I (Glioma); Phase III (Lung cancer)	Inhibits thioredoxin reductase and induces ROS accumulation	[48, 125]

Table 1. Pharmacological agents acting on mitochondria in cardiac ischemia/reperfusion and cancer, inhibiting or inducing, respectively mPTP formation

accumulation (which can induce pore opening even in the absence of Ca^{2+} excess) [93] exerts a protective effect against IR. Therefore, mitochondria-targeted ROS scavengers apparently have a higher likelihood to provide mPTP-mediated cardioprotection. ROS scavengers such as the anaesthetic drug propofol [94], MCI-186 [95], pyruvate [34] and others [96] have demonstrated protective effects against IR and oxidative stress in the intact heart or cardiomyocytes, and were associated with inhibition of mPTP opening. Although the role of mPTP in the cardioprotective action of Ca^{2+} antagonists has not been extensively examined, a specific inhibitor of the Ca^{2+} uniport in mitochondria, Ru360, inhibited mPTP opening, reduced mitochondrial Ca^{2+} overload, and significantly improved cardiac function after ischemia [97].

mPTP formation can be prevented by low pH_i due to inhibition of Ca^{2+} binding to the trigger site of the mPTP complex [28]. Opening of mPTPs at reperfusion after ischemia depends on pH_i , and inhibition of pore opening has been shown to correlate with a delayed pH_i recovery [34]. Our [35] and others' [36] studies have demonstrated that a delayed pH_i recovery during IR due to inhibition of NHE-1 was associated with improved

postischemic cardiac function and less mPTP opening. The NHE-1 inhibitor cariporide prevented oxidative stress-induced mitochondrial Ca^{2+} overload and $\Delta\Psi_m$ loss in neonatal cardiomyocytes [98]. Since NHE-1 activity is upregulated during IR leading to Ca^{2+} overload through reverse mode Na^+ - Ca^+ exchanger, its inhibition can reduce $[\text{Ca}^{2+}]_i$ [38]. Additionally, inhibition of NHE-1 slows pH_i recovery at the onset of reperfusion, inducing acidosis which prevents mPTP opening. Thus, inhibition of NHE-1 during IR can prevent pore opening through two mechanisms including prevention of Ca^{2+} overload and delay of pH_i recovery.

Cancer

The main goal of mitochondria related chemotherapy is to activate extrinsic and intrinsic pro-apoptotic pathways to induce apoptosis in cancer cells and thereby, prevent cell differentiation [13]. Therapeutic interventions can influence mitochondria directly or indirectly compromising their ability to produce ATP, and/or affect the structural integrity of the outer mitochondrial membrane to further release pro-apoptotic proteins from the intermembrane space to the cytoplasm. These two factors determine whether tumor cells die through necrosis or apoptosis. Malignant cells are resistant to apoptosis, and this feature could be due to among others, perturbations in the expression of individual components and regulators of the mPTP that withstand pore formation and cell death in cancer cells. Indeed, low sensitivity of cancer cells to pro-apoptotic signals and suppressed apoptosis are the central problems in cancer treatment [99]. Numerous chemical agents have been shown to induce mPTP opening and cell death due to their direct interaction with the pore components, VDAC and ANT (Table 1). In addition, the compounds inducing calcium overload and ROS generation as well as depletion of high energy phosphates (ATP and creatine phosphate) can indirectly trigger mPTP formation [100, 101].

One of the main targets initiating mPTP-mediated cell death is the ANT. The transport activity of ANT is regulated by cysteine residues responsible for inter- and intra-molecular cross-linkages [102]. Indeed, ANT-mediated mPT was induced by the thiol cross-linking agent phenyl arsenoxide and its derivatives through modulation of ANT [103, 104]. A peptide trivalent arsenical 4-(N-(S-glutathionylacetyl) amino) phenylarsenoxide (GSAO), which is the derivative of phenyl arsenoxide demonstrated anti-proliferative effects in tumor endothelial cells and inhibited tumor angiogenesis in mice [105]. It triggered Ca^{2+} -dependent mPTP opening by

cross-linking Cys(160) and Cys(257) of ANT, inactivating the exchanger. However, these agents can also induce thiol cross-linkage in different cytoplasmic and mitochondrial proteins containing cysteine groups. This non-selective chemical action significantly enhances toxicity of thiol cross-linking agents which causes difficulties in using them in cancer chemotherapy.

Arsenic trioxide demonstrated anti-cancer effects in association with its direct inhibitory effect on ANT activity [106]. Arsenite, the trivalent inorganic salt formed by arsenic trioxide, induced opening of mPTPs in isolated mitochondria, presumably by acting as a thiol-oxidizing agent [107]. Arsenic trioxide is currently used as a chemotherapeutic agent for the treatment of acute promyelocytic leukemia. In addition to ANT, arsenite also inhibits VDAC although the contribution of each pathway (ANT- or VDAC-mediated) to mPTP-induced cell death remains unknown. A bisphosphonate, clodronate has been shown to inhibit ANT activity which induces dissipation of the mitochondrial membrane potential and ATP production ultimately leading to cell death [108]. Another cytotoxic agent, betulinic acid triggers apoptosis by a direct effect on mitochondria sensitizing them to mPT [109]. Betulinic acid has been shown to exert anti-tumor effects in an *in vivo* model of human melanoma xenografts and *in vitro* models of lung, ovarian, cervical and other carcinomas [110]. Although the precise mechanism(s) of action remains elusive, accumulated experimental evidence indicates that the effect is mediated through mitochondria, presumably by induction of mPTP opening.

The synthetic retinoid CD437 and other synthetic analogs of all-*trans* retinoic acid stimulate expression of retinoic acid receptors, and have been shown to exert anti-cancer effects in patients with acute promyelocytic leukemia [111]. However, studies on all-*trans* retinoic acid resistant cells [112] and other cell models [113, 114] provide strong evidence that the anti-cancer effects of CD437 are associated with ANT-mediated mPTP induction independent of its binding to retinoid nuclear receptors. Isolated liver mitochondrial suspensions demonstrated swelling in the presence of CD437 indicating induction of mPT, and CD437-induced apoptosis was associated with mitochondrial membrane depolarization ($\Delta\Psi_m$ loss) and mPTP opening in myeloma cells [113]. Furthermore, CD437 induced permeabilization in proteoliposomes containing purified ANT, suggesting a direct interaction between the drug and ANT to induce mPT, although further studies are required to assess the exact mechanism of this interaction [115].

Anti-tumor activity of lonidamine, a derivative of an

indazole-3-carboxylic acid can also be mediated through inhibition of ANT activity. Lonidamine has been found to induce pore opening in mitochondria and affect ANT activity in reconstituted proteoliposomes [115, 116]. Furthermore, it inhibits the activity of mitochondrially-bound HK and oxidative phosphorylation leading to a decrease in cellular ATP in cancer [117].

The pharmacological effectiveness of anti-tumor drugs is generally enhanced by mPTP-targeted agents. As mentioned above (see Cancer) CyP-D is over-expressed in cancer cells and has an inhibitory effect on mPTP formation through an unknown mechanism. In contrast to cardiac IR, pharmacological inhibition of CyP-D with CsA and SfA enhanced the chemotherapeutic effect of cisplatin in glioma cells [118].

Hexokinase, which binds to VDAC and prevents mPTP formation, is overexpressed in most tumor cells. Expression of VDAC1-based peptides in cells overexpressing HK-I or HK-II has been shown to prevent HK-mediated protection against staurosporine-induced release of cytochrome c and subsequent cell death [78]. Both cell-expressed and exogenously added cell-penetrating peptides were able to detach mitochondrial-bound HK. This study concluded that VDAC1-based peptides interfering with HK-mediated anti-apoptotic activity may potentiate the efficacy of conventional chemotherapeutic agents [78].

One effective chemotherapeutic strategy is to promote production of ROS, thereby provoking mPTP opening. Accumulation of ROS can be achieved due to upregulated ROS generation and/or inhibition of the anti-oxidant systems in cancer cells [12, 13, 43]. For instance, β -lapachone is a cytotoxic *o*-naphthoquinone that increases ROS generation through NAD(P)H:quinone oxidoreductase-1 and induces cell death in lung cancer cells [119]. Imexon, a pro-oxidant small molecule, inhibits the anti-oxidant system by decreasing the reduced glutathione (GSH) levels and inducing apoptosis through the accumulation of ROS; exhibiting anti-tumor activity in patients with metastatic cancer [120]. Inhibition of GSH synthesis with L-buthionine sulfoxamine enhanced ROS production, and in combination with arsenic trioxide provided effective treatment of solid tumors [121]. The glutathione antioxidant system has also been inhibited by β -phenylethyl isothiocyanate due to its interaction with redox regulatory enzymes in cancer cells [122], triggering mitochondria-mediated apoptosis in human prostate cancer cells associated with ROS overproduction [123]. Mangafodipir, a superoxide dismutase mimetic protects normal cells from apoptosis induced by oxidative stress,

however it increases H_2O_2 levels and exerts anti-tumor effects in colon cancer cells [124]. A novel redox active drug and an aromatic macrocycle, motexafin gadolinium selectively localizes in tumours, perhaps due to their metabolic perturbations [125] and affects the redox state in cancer cells, inducing ROS accumulation. Motexafin gadolinium is likely to inhibit the enzymatic activity of thioredoxin reductase that results in cellular redox stress, cytotoxicity and an increase in tumor responsiveness to a variety of treatments including both radiotherapy and chemotherapy [48, 125]. Anti-cancer effects of many other pharmacological compounds affecting the redox state of cancer cells, leading to inhibition of the anti-oxidant system and ROS overproduction appear to be mediated by mitochondria-mediated apoptosis where the role of the mPTP is obvious [48, 104, 126].

The majority of mitochondrial-targeted chemotherapeutic compounds or their derivatives are under clinical investigation involving Phases I-III clinical trials where they are used as monotherapy or in combination with other anti-cancer drugs. It should be pointed out that the adverse effects of mitochondrial-targeted drugs on non-tumour tissue/organs, particularly the heart must be taken into consideration in cancer therapy. For instance, doxorubicin (adriamycin), a potent and broad-spectrum antineoplastic agent, has been shown to induce cardiomyopathy associated with mitochondrial dysfunction, including induction of the mPT and inhibition of mitochondrial respiration [127]. Doxorubicin-induced cardiomyopathy was prevented in CyP-D knock-out hearts, suggesting a critical role of the mPTP-mediated pathway as the disease-inducing mechanism [39]. Development of new therapeutic strategies aimed at avoiding adverse effects of anti-cancer drugs by directly targeting tumour tissue/organs is crucial for chemotherapy.

Conclusions and future directions

Understanding of the mechanisms regulating mPTP formation have improved our knowledge on the central role of mitochondria as a target to prevent or promote cell death in cardiac IR and cancer, respectively, and to develop novel pharmacological approaches for therapeutic interventions. Developing selective mitochondria-targeting cardioprotective drugs as well as tumor-specific mitochondriotoxic agents is becoming a high priority area in the pharmaceutical industry. Notably, most of the new mitochondria targeting compounds are still in the developmental stage involving basic research and pre-clinical and clinical

(Phases I-III) trials. Design of new pharmacological agents is obscured by a lack of knowledge of the structural integrity of the mPTP complex and molecular mechanisms of the regulation of the pore opening under physiological and pathological conditions. Elucidation of the main functional, structural and metabolic differences between mitochondria in malignant and normal cells will be very useful in understanding why cancer cells are less susceptible to mPTP opening and to further develop mitochondrial-targeted chemotherapy. Adverse effects of pharmacological agents including the long-term toxic

effects and non-selectively targeting tumour cells are the main problems in drug development. Development of transporters with high efficiency may provide new perspectives for rational drug delivery to malignant cells and mitochondria.

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References

- 1 Chipuk JE, Bouchier-Hayes L, Green DR: Mitochondrial outer membrane permeabilization during apoptosis: the innocent bystander scenario. *Cell Death Differ* 2006;13:1396-1402.
- 2 Lucken-Ardjomande S, Montessuit S, Martinou JC: Contributions to Bax insertion and oligomerization of lipids of the mitochondrial outer membrane. *Cell Death Differ* 2008;15:929-937.
- 3 Halestrap AP, Pasdois P: The role of the mitochondrial permeability transition pore in heart disease. *Biochim Biophys Acta* 2009;1787:1402-1415.
- 4 Javadov S, Karmazyn M, Escobales N: Mitochondrial permeability transition pore opening as a promising therapeutic target in cardiac diseases. *J Pharmacol Exp Ther* 2009;330:670-678.
- 5 Baines CP: The molecular composition of the mitochondrial permeability transition pore. *J Mol Cell Cardiol* 2009;46:850-857.
- 6 Argaud L, Gateau-Roesch O, Muntean D, Chalabreysse L, Loufouat J, Robert D, Ovize M: Specific inhibition of the mitochondrial permeability transition prevents lethal reperfusion injury. *J Mol Cell Cardiol* 2005;38:367-374.
- 7 Hausenloy DJ, Yellon DM: The mitochondrial permeability transition pore: its fundamental role in mediating cell death during ischaemia and reperfusion. *J Mol Cell Cardiol* 2003;35:339-341.
- 8 Javadov SA, Clarke S, Das M, Griffiths EJ, Lim KH, Halestrap AP: Ischaemic preconditioning inhibits opening of mitochondrial permeability transition pores in the reperfused rat heart. *J Physiol* 2003;549:513-524.
- 9 Shanmuganathan S, Hausenloy DJ, Duchon MR, Yellon DM: Mitochondrial permeability transition pore as a target for cardioprotection in the human heart. *Am J Physiol Heart Circ Physiol* 2005;289:H237-242.
- 10 Piot C, Croisille P, Staat P, Thibault H, Rioufol G, Mewton N, Elbelghiti R, Cung TT, Bonnefoy E, Angoulvant D, Macia C, Raczk F, Sportouch C, Gahide G, Finet G, Andre-Fouet X, Revel D, Kirkorian G, Monassier JP, Derumeaux G, Ovize M: Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *N Engl J Med* 2008;359:473-481.
- 11 Fantin VR, Leder P: Mitochondriotoxic compounds for cancer therapy. *Oncogene* 2006;25:4787-4797.
- 12 Brenner C, Grimm S: The permeability transition pore complex in cancer cell death. *Oncogene* 2006;25:4744-4756.
- 13 Fulda S, Galluzzi L, Kroemer G: Targeting mitochondria for cancer therapy. *Nat Rev Drug Discov* 2010;9:447-464.
- 14 Armstrong JS: Mitochondrial medicine: pharmacological targeting of mitochondria in disease. *Br J Pharmacol* 2007;151:1154-1165.
- 15 Hunter DR, Haworth RA, Southard JH: Relationship between configuration, function, and permeability in calcium-treated mitochondria. *J Biol Chem* 1976;251:5069-5077.
- 16 Petronilli V, Szabo I, Zoratti M: The inner mitochondrial membrane contains ion-conducting channels similar to those found in bacteria. *FEBS Lett* 1989;259:137-143.
- 17 Szabo I, Zoratti M: The giant channel of the inner mitochondrial membrane is inhibited by cyclosporin A. *J Biol Chem* 1991;266:3376-3379.
- 18 Bernardi P, Krauskopf A, Basso E, Petronilli V, Blachly-Dyson E, Di Lisa F, Forte MA: The mitochondrial permeability transition from in vitro artifact to disease target. *Febs J* 2006;273:2077-2099.
- 19 Robertson CL, Scafidi S, McKenna MC, Fiskum G: Mitochondrial mechanisms of cell death and neuroprotection in pediatric ischemic and traumatic brain injury. *Exp Neurol* 2009;218:371-380.
- 20 Krauskopf A, Eriksson O, Craigen WJ, Forte MA, Bernardi P: Properties of the permeability transition in VDAC1(-/-) mitochondria. *Biochim Biophys Acta* 2006;1757:590-595.
- 21 Baines CP, Kaiser RA, Sheiko T, Craigen WJ, Molkentin JD: Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. *Nat Cell Biol* 2007;9:550-555.
- 22 Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, Jones DP, MacGregor GR, Wallace DC: The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* 2004;427:461-465.
- 23 Leung AW, Varanyuwatana P, Halestrap AP: The mitochondrial phosphate carrier interacts with cyclophilin D and may play a key role in the permeability transition. *J Biol Chem* 2008;283:26312-26323.
- 24 Basso E, Fante L, Fowlkes J, Petronilli V, Forte MA, Bernardi P: Properties of the permeability transition pore in mitochondria devoid of Cyclophilin D. *J Biol Chem* 2005;280:18558-18561.
- 25 Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA, Brunskill EW, Sayen MR, Gottlieb RA, Dorn GW, Robbins J, Molkentin JD: Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* 2005;434:658-662.
- 26 Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, Yamagata H, Inohara H, Kubo T, Tsujimoto Y: Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. *Nature* 2005;434:652-658.
- 27 Min L, Fulton DB, Andreotti AH: A case study of proline isomerization in cell signaling. *Front Biosci* 2005;10:385-397.

- 28 Bernardi P, Vassanelli S, Veronese P, Colonna R, Szabo I, Zoratti M: Modulation of the mitochondrial permeability transition pore. Effect of protons and divalent cations. *J Biol Chem* 1992;267:2934-2939.
- 29 Adhietty PJ, Ljubicic V, Menzies KJ, Hood DA: Differential susceptibility of subsarcolemmal and intermyofibrillar mitochondria to apoptotic stimuli. *Am J Physiol Cell Physiol* 2005;289:C994-C1001.
- 30 Bera AK, Ghosh S, Das S: Mitochondrial VDAC can be phosphorylated by cyclic AMP-dependent protein kinase. *Biochem Biophys Res Commun* 1995;209:213-217.
- 31 Baines CP, Song CX, Zheng YT, Wang GW, Zhang J, Wang OL, Guo Y, Bolli R, Cardwell EM, Ping P: Protein kinase Cepsilon interacts with and inhibits the permeability transition pore in cardiac mitochondria. *Circ Res* 2003;92:873-880.
- 32 Javadov S, Rajapurohitam V, Kilic A, Zeidan A, Choi A, Karmazyn M: Anti-hypertrophic effect of NHE-1 inhibition involves GSK-3beta-dependent attenuation of mitochondrial dysfunction. *J Mol Cell Cardiol* 2009;46:998-1007.
- 33 Griffiths EJ, Halestrap AP: Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion. *Biochem J* 1995;307:93-98.
- 34 Kerr PM, Suleiman MS, Halestrap AP: Reversal of permeability transition during recovery of hearts from ischemia and its enhancement by pyruvate. *Am J Physiol* 1999;276:H496-502.
- 35 Javadov S, Choi A, Rajapurohitam V, Zeidan A, Basnakian AG, Karmazyn M: NHE-1 inhibition-induced cardioprotection against ischaemia/reperfusion is associated with attenuation of the mitochondrial permeability transition. *Cardiovasc Res* 2008;77:416-424.
- 36 Prendes MG, Torresin E, Gonzalez M, Fernandez MA, Perazzo JC, Savino EA, Varela A: Protection of ischaemic-reperfused rat heart by dimethylamiloride is associated with inhibition of mitochondrial permeability transition. *Clin Exp Pharmacol Physiol* 2008;35:201-206.
- 37 Linz WJ, Busch AE: NHE-1 inhibition: from protection during acute ischaemia/reperfusion to prevention/reversal of myocardial remodelling. *Naunyn Schmiedeberg Arch Pharmacol* 2003;368:239-246.
- 38 Karmazyn M, Sostaric JV, Gan XT: The myocardial Na⁺/H⁺ exchanger: a potential therapeutic target for the prevention of myocardial ischaemic and reperfusion injury and attenuation of postinfarction heart failure. *Drugs* 2001;61:375-389.
- 39 Nakayama H, Chen X, Baines CP, Klevitsky R, Zhang X, Zhang H, Jaleel N, Chua BH, Hewett TE, Robbins J, Houser SR, Molken JD: Ca²⁺- and mitochondrial-dependent cardiomyocyte necrosis as a primary mediator of heart failure. *J Clin Invest* 2007;117:2431-2444.
- 40 Oliveira PJ, Seica R, Coxito PM, Rolo AP, Palmeira CM, Santos MS, Moreno AJ: Enhanced permeability transition explains the reduced calcium uptake in cardiac mitochondria from streptozotocin-induced diabetic rats. *FEBS Lett* 2003;554:511-514.
- 41 Javadov S, Huang C, Kirshenbaum L, Karmazyn M: NHE-1 inhibition improves impaired mitochondrial permeability transition and respiratory function during postinfarction remodelling in the rat. *J Mol Cell Cardiol* 2005;38:135-143.
- 42 Sharov VG, Todor A, Khanal S, Imai M, Sabbah HN: Cyclosporine A attenuates mitochondrial permeability transition and improves mitochondrial respiratory function in cardiomyocytes isolated from dogs with heart failure. *J Mol Cell Cardiol* 2007;42:150-158.
- 43 Fang J, Seki T, Maeda H: Therapeutic strategies by modulating oxygen stress in cancer and inflammation. *Adv Drug Deliv Rev* 2009;61:290-302.
- 44 Pelicano H, Carney D, Huang P: ROS stress in cancer cells and therapeutic implications. *Drug Resist Updat* 2004;7:97-110.
- 45 Dvorakova K, Payne CM, Tome ME, Briehl MM, Vasquez MA, Waltmire CN, Coon A, Dorr RT: Molecular and cellular characterization of imexon-resistant RPMI8226/I myeloma cells. *Mol Cancer Ther* 2002;1:185-195.
- 46 Biaglow JE, Miller RA: The thioredoxin reductase/thioredoxin system: novel redox targets for cancer therapy. *Cancer Biol Ther* 2005;4:6-13.
- 47 Pani G, Colavitti R, Bedogni B, Fusco S, Ferraro D, Borrello S, Galeotti T: Mitochondrial superoxide dismutase: a promising target for new anticancer therapies. *Curr Med Chem* 2004;11:1299-1308.
- 48 Tonissen KF, Di Trapani G: Thioredoxin system inhibitors as mediators of apoptosis for cancer therapy. *Mol Nutr Food Res* 2009;53:87-103.
- 49 Chandra J, Mansson E, Gogvadze V, Kaufmann SH, Albertoni F, Orrenius S: Resistance of leukemic cells to 2-chlorodeoxyadenosine is due to a lack of calcium-dependent cytochrome c release. *Blood* 2002;99:655-663.
- 50 Papp B, Brouland JP, Gelebart P, Kovacs T, Chomienne C: Endoplasmic reticulum calcium transport ATPase expression during differentiation of colon cancer and leukaemia cells. *Biochem Biophys Res Commun* 2004;322:1223-1236.
- 51 Shinohara Y, Ishida T, Hino M, Yamazaki N, Baba Y, Terada H: Characterization of porin isoforms expressed in tumor cells. *Eur J Biochem* 2000;267:6067-6073.
- 52 Halestrap AP, Brenner C: The adenine nucleotide translocase: a central component of the mitochondrial permeability transition pore and key player in cell death. *Curr Med Chem* 2003;10:1507-1525.
- 53 Dolce V, Scarcia P, Iacopetta D, Palmieri F: A fourth ADP/ATP carrier isoform in man: identification, bacterial expression, functional characterization and tissue distribution. *FEBS Lett* 2005;579:633-637.
- 54 Bauer MK, Schubert A, Rocks O, Grimm S: Adenine nucleotide translocase-1, a component of the permeability transition pore, can dominantly induce apoptosis. *J Cell Biol* 1999;147:1493-1502.
- 55 Zamora M, Granell M, Mampel T, Vinas O: Adenine nucleotide translocase 3 (ANT3) overexpression induces apoptosis in cultured cells. *FEBS Lett* 2004;563:155-160.
- 56 Le Bras M, Borgne-Sanchez A, Touat Z, El Dein OS, Deniaud A, Maillier E, Lecellier G, Rebouillat D, Lemaire C, Kroemer G, Jacotot E, Brenner C: Chemosensitization by knockdown of adenine nucleotide translocase-2. *Cancer Res* 2006;66:9143-9152.
- 57 Marzo I, Brenner C, Zamzami N, Jurgensmeier JM, Susin SA, Vieira HL, Prevost MC, Xie Z, Matsuyama S, Reed JC, Kroemer G: Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis. *Science* 1998;281:2027-2031.
- 58 Belzacq AS, Vieira HL, Verrier F, Vandecasteele G, Cohen I, Prevost MC, Larquet E, Pariselli F, Petit PX, Kahn A, Rizzuto R, Brenner C, Kroemer G: Bcl-2 and Bax modulate adenine nucleotide translocase activity. *Cancer Res* 2003;63:541-546.
- 59 Chevrollier A, Loiseau D, Chabi B, Renier G, Douay O, Malthiery Y, Stepien G: ANT2 isoform required for cancer cell glycolysis. *J Bioenerg Biomembr* 2005;37:307-316.
- 60 Faure Vigny H, Heddi A, Giraud S, Chautard D, Stepien G: Expression of oxidative phosphorylation genes in renal tumors and tumoral cell lines. *Mol Carcinog* 1996;16:165-172.
- 61 Bonod-Bidaud C, Chevrollier A, Bourasseau I, Lachaux A, Mousson de Camaret B, Stepien G: Induction of ANT2 gene expression in liver of patients with mitochondrial DNA depletion. *Mitochondrion* 2001;1:217-224.

- 62 Li Y, Johnson N, Capano M, Edwards M, Crompton M: Cyclophilin-D promotes the mitochondrial permeability transition but has opposite effects on apoptosis and necrosis. *Biochem J* 2004;383:101-109.
- 63 Schubert A, Grimm S: Cyclophilin D, a component of the permeability transition-pore, is an apoptosis repressor. *Cancer Res* 2004;64:85-93.
- 64 Machida K, Ohta Y, Osada H: Suppression of apoptosis by cyclophilin D via stabilization of hexokinase II mitochondrial binding in cancer cells. *J Biol Chem* 2006;281:14314-14320.
- 65 Beinlich A, Strohmeier R, Kaufmann M, Kuhl H: Relation of cell proliferation to expression of peripheral benzodiazepine receptors in human breast cancer cell lines. *Biochem Pharmacol* 2000;60:397-402.
- 66 Maaser K, Hopfner M, Jansen A, Weisinger G, Gavish M, Kozikowski AP, Weizman A, Carayon P, Riecken EO, Zeitz M, Scherubl H: Specific ligands of the peripheral benzodiazepine receptor induce apoptosis and cell cycle arrest in human colorectal cancer cells. *Br J Cancer* 2001;85:1771-1780.
- 67 Okaro AC, Fennell DA, Corbo M, Davidson BR, Cotter FE: Pk11195, a mitochondrial benzodiazepine receptor antagonist, reduces apoptosis threshold in Bcl-X(L) and Mcl-1 expressing human cholangiocarcinoma cells. *Gut* 2002;51:556-561.
- 68 Decaudin D, Castedo M, Nemati F, Beurdeley-Thomas A, De Pinieux G, Caron A, Pouillart P, Wijdenes J, Rouillard D, Kroemer G, Poupon MF: Peripheral benzodiazepine receptor ligands reverse apoptosis resistance of cancer cells in vitro and in vivo. *Cancer Res* 2002;62:1388-1393.
- 69 Pratt R, Vallis LM, Lim CW, Chisnall WN: Mitochondrial creatine kinase in cancer patients. *Pathology* 1987;19:162-165.
- 70 Kornblau SM, Thall PF, Estrov Z, Walterscheid M, Patel S, Theriault A, Keating MJ, Kantarjian H, Estey E, Andreeff M: The prognostic impact of BCL2 protein expression in acute myelogenous leukemia varies with cytogenetics. *Clin Cancer Res* 1999;5:1758-1766.
- 71 Manion MK, Hockenbery DM: Targeting BCL-2-related proteins in cancer therapy. *Cancer Biol Ther* 2003;2:S105-114.
- 72 Ionov Y, Yamamoto H, Krajewski S, Reed JC, Perucho M: Mutational inactivation of the proapoptotic gene BAX confers selective advantage during tumor clonal evolution. *Proc Natl Acad Sci USA* 2000;97:10872-10877.
- 73 Rampino N, Yamamoto H, Ionov Y, Li Y, Sawai H, Reed JC, Perucho M: Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. *Science* 1997;275:967-969.
- 74 Rempel A, Mathupala SP, Griffin CA, Hawkins AL, Pedersen PL: Glucose catabolism in cancer cells: amplification of the gene encoding type II hexokinase. *Cancer Res* 1996;56:2468-2471.
- 75 Pedersen PL, Mathupala S, Rempel A, Geschwind JF, Ko YH: Mitochondrial bound type II hexokinase: a key player in the growth and survival of many cancers and an ideal prospect for therapeutic intervention. *Biochim Biophys Acta* 2002;1555:14-20.
- 76 Azoulay-Zohar H, Israelson A, Abu-Hamad S, Shoshan-Barmatz V: In self-defence: hexokinase promotes voltage-dependent anion channel closure and prevents mitochondria-mediated apoptotic cell death. *Biochem J* 2004;377:347-355.
- 77 Warburg O: On the origin of cancer cells. *Science* 1956;123:309-314.
- 78 Arzoin L, Zilberberg N, Ben-Romano R, Shoshan-Barmatz V: Voltage-dependent anion channel 1-based peptides interact with hexokinase to prevent its anti-apoptotic activity. *J Biol Chem* 2009;284:3946-3955.
- 79 Sowter HM, Ratcliffe PJ, Watson P, Greenberg AH, Harris AL: HIF-1-dependent regulation of hypoxic induction of the cell death factors BNIP3 and NIX in human tumors. *Cancer Res* 2001;61:6669-6673.
- 80 Kubasiak LA, Hernandez OM, Bishopric NH, Webster KA: Hypoxia and acidosis activate cardiac myocyte death through the Bcl-2 family protein BNIP3. *Proc Natl Acad Sci USA* 2002;99:12825-12830.
- 81 Harris AL: Hypoxia-a key regulatory factor in tumour growth. *Nat Rev Cancer* 2002;2:38-47.
- 82 Halestrap AP, Davidson AM: Inhibition of Ca^{2+} -induced large-amplitude swelling of liver and heart mitochondria by cyclosporin is probably caused by the inhibitor binding to mitochondrial-matrix peptidyl-prolyl cis-trans isomerase and preventing it interacting with the adenine nucleotide translocase. *Biochem J* 1990;268:153-160.
- 83 Regula KM, Ens K, Kirshenbaum LA: Inducible expression of BNIP3 provokes mitochondrial defects and hypoxia-mediated cell death of ventricular myocytes. *Circ Res* 2002;91:226-231.
- 84 Akao M, O'Rourke B, Teshima Y, Seharaseyon J, Marban E: Mechanistically distinct steps in the mitochondrial death pathway triggered by oxidative stress in cardiac myocytes. *Circ Res* 2003;92:186-194.
- 85 Haworth RA, Hunter DR: Control of the mitochondrial permeability transition pore by high-affinity ADP binding at the ADP/ATP translocase in permeabilized mitochondria. *J Bioenerg Biomembr* 2000;32:91-96.
- 86 Xu M, Wang Y, Hirai K, Ayub A, Ashraf M: Calcium preconditioning inhibits mitochondrial permeability transition and apoptosis. *Am J Physiol Heart Circ Physiol* 2001;280:H899-908.
- 87 Wang G, Liem DA, Vondriska TM, Honda HM, Korge P, Pantaleon DM, Qiao X, Wang Y, Weiss JN, Ping P: Nitric oxide donors protect murine myocardium against infarction via modulation of mitochondrial permeability transition. *Am J Physiol Heart Circ Physiol* 2005;288:H1290-1295.
- 88 de Macedo DV, Nepomuceno ME, Pereira-da-Silva L: Involvement of the ADP/ATP carrier in permeabilization processes of the inner mitochondrial membrane. *Eur J Biochem* 1993;215:595-600.
- 89 Clarke SJ, McStay GP, Halestrap AP: Sanglifehrin A acts as a potent inhibitor of the mitochondrial permeability transition and reperfusion injury of the heart by binding to cyclophilin-D at a different site from cyclosporin A. *J Biol Chem* 2002;277:34793-34799.
- 90 Schinzel AC, Takeuchi O, Huang Z, Fisher JK, Zhou Z, Rubens J, Hetz C, Danial NN, Moskowitz MA, Korsmeyer SJ: Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia. *Proc Natl Acad Sci USA* 2005;102:12005-12010.
- 91 Zhou S, Heller LJ, Wallace KB: Interference with calcium-dependent mitochondrial bioenergetics in cardiac myocytes isolated from doxorubicin-treated rats. *Toxicol Appl Pharmacol* 2001;175:60-67.
- 92 Javadov S, Baetz D, Rajapurohitam V, Zeidan A, Kirshenbaum LA, Karmazyn M: Antihypertrophic effect of Na^+/H^+ exchanger isoform 1 inhibition is mediated by reduced mitogen-activated protein kinase activation secondary to improved mitochondrial integrity and decreased generation of mitochondrial-derived reactive oxygen species. *J Pharmacol Exp Ther* 2006;317:1036-1043.
- 93 Kim JS, Jin Y, Lemasters JJ: Reactive oxygen species, but not Ca^{2+} overloading, trigger pHⁱ and mitochondrial permeability transition-dependent death of adult rat myocytes after ischemia-reperfusion. *Am J Physiol Heart Circ Physiol* 2006;290:H2024-2034.
- 94 Javadov SA, Lim KH, Kerr PM, Suleiman MS, Angelini GD, Halestrap AP: Protection of hearts from reperfusion injury by propofol is associated with inhibition of the mitochondrial permeability transition. *Cardiovasc Res* 2000;45:360-369.

- 95 Rajesh KG, Sasaguri S, Suzuki R, Maeda H: Antioxidant MCI-186 inhibits mitochondrial permeability transition pore and upregulates Bcl-2 expression. *Am J Physiol Heart Circ Physiol* 2003;285:H2171-2178.
- 96 Takeda T, Akao M, Matsumoto-Ida M, Kato M, Takenaka H, Kihara Y, Kume T, Akaike A, Kita T: Serofendic acid, a novel substance extracted from fetal calf serum, protects against oxidative stress in neonatal rat cardiac myocytes. *J Am Coll Cardiol* 2006;47:1882-1890.
- 97 de Jesus Garcia-Rivas G, Guerrero-Hernandez A, Guerrero-Serna G, Rodriguez-Zavala JS, Zazueta C: Inhibition of the mitochondrial calcium uniporter by the oxo-bridged dinuclear ruthenium amine complex (Ru360) prevents from irreversible injury in postischemic rat heart. *FEBS J* 2005;272:3477-3488.
- 98 Teshima Y, Akao M, Jones SP, Marban E: Cariporide (HOE642), a selective Na⁺-H⁺ exchange inhibitor, inhibits the mitochondrial death pathway. *Circulation* 2003;108:2275-2281.
- 99 Hanahan D, Weinberg RA: The hallmarks of cancer. *Cell* 2000;100:57-70.
- 100 Fulda S, Debatin KM: Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. *Oncogene* 2006;25:4798-4811.
- 101 Kroemer G, Galluzzi L, Brenner C: Mitochondrial membrane permeabilization in cell death. *Physiol Rev* 2007;87:99-163.
- 102 Majima E, Ikawa K, Takeda M, Hashimoto M, Shinohara Y, Terada H: Translocation of loops regulates transport activity of mitochondrial ADP/ATP carrier deduced from formation of a specific intermolecular disulfide bridge catalyzed by copper-o-phenanthroline. *J Biol Chem* 1995;270:29548-29554.
- 103 McStay GP, Clarke SJ, Halestrap AP: Role of critical thiol groups on the matrix surface of the adenine nucleotide translocase in the mechanism of the mitochondrial permeability transition pore. *Biochem J* 2002;367:541-548.
- 104 Costantini P, Belzacq AS, Vieira HL, Larochette N, de Pablo MA, Zamzami N, Susin SA, Brenner C, Kroemer G: Oxidation of a critical thiol residue of the adenine nucleotide translocator enforces Bcl-2-independent permeability transition pore opening and apoptosis. *Oncogene* 2000;19:307-314.
- 105 Don AS, Kisker O, Dilda P, Donoghue N, Zhao X, Decollogne S, Creighton B, Flynn E, Folkman J, Hogg PJ: A peptide trivalent arsenical inhibits tumor angiogenesis by perturbing mitochondrial function in angiogenic endothelial cells. *Cancer Cell* 2003;3:497-509.
- 106 Kroemer G, de The H: Arsenic trioxide, a novel mitochondriotoxic anticancer agent? *J Natl Cancer Inst* 1999;91:743-745.
- 107 Costantini P, Chernyak BV, Petronilli V, Bernardi P: Modulation of the mitochondrial permeability transition pore by pyridine nucleotides and dithiol oxidation at two separate sites. *J Biol Chem* 1996;271:6746-6751.
- 108 Lehenkari PP, Kellinsalmi M, Npnkangas JP, Ylitalo KV, Mnkknen J, Rogers MJ, Azhayev A, Vnen HK, Hassinen IE: Further insight into mechanism of action of clodronate: inhibition of mitochondrial ADP/ATP translocase by a nonhydrolyzable, adenine-containing metabolite. *Mol Pharmacol* 2002;61:1255-1262.
- 109 Fulda S, Scaffidi C, Susin SA, Krammer PH, Kroemer G, Peter ME, Debatin KM: Activation of mitochondria and release of mitochondrial apoptogenic factors by betulinic acid. *J Biol Chem* 1998;273:33942-33948.
- 110 Eiznhamer DA, Xu ZQ: Betulinic acid: a promising anticancer candidate. *IDrugs* 2004;7:359-373.
- 111 Slack JL, Rusiniak ME: Current issues in the management of acute promyelocytic leukemia. *Ann Hematol* 2000;79:227-238.
- 112 Hsu CA, Rishi AK, Su-Li X, Gerald TM, Dawson MI, Schiffer C, Reichert U, Shroot B, Poirer GC, Fontana JA: Retinoid induced apoptosis in leukemia cells through a retinoic acid nuclear receptor-independent pathway. *Blood* 1997;89:4470-4479.
- 113 Marchetti P, Zamzami N, Joseph B, Schraen-Maschke S, Mereau-Richard C, Costantini P, Metivier D, Susin SA, Kroemer G, Formstecher P: The novel retinoid 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid can trigger apoptosis through a mitochondrial pathway independent of the nucleus. *Cancer Res* 1999;59:6257-6266.
- 114 Notario B, Zamora M, Vinas O, Mampel T: All-trans-retinoic acid binds to and inhibits adenine nucleotide translocase and induces mitochondrial permeability transition. *Mol Pharmacol* 2003;63:224-231.
- 115 Belzacq AS, El Hamel C, Vieira HL, Cohen I, Haouzi D, Metivier D, Marchetti P, Brenner C, Kroemer G: Adenine nucleotide translocator mediates the mitochondrial membrane permeabilization induced by lonidamine, arsenite and CD437. *Oncogene* 2001;20:7579-7587.
- 116 Ravagnan L, Marzo I, Costantini P, Susin SA, Zamzami N, Petit PX, Hirsch F, Goulbern M, Poupon MF, Miccoli L, Xie Z, Reed JC, Kroemer G: Lonidamine triggers apoptosis via a direct, Bcl-2-inhibited effect on the mitochondrial permeability transition pore. *Oncogene* 1999;18:2537-2546.
- 117 Pelicano H, Martin DS, Xu RH, Huang P: Glycolysis inhibition for anticancer treatment. *Oncogene* 2006;25:4633-4646.
- 118 Han X, Yoon SH, Ding Y, Choi TG, Choi WJ, Kim YH, Kim YJ, Huh YB, Ha J, Kim SS: Cyclosporin A and sanglifehrin A enhance chemotherapeutic effect of cisplatin in C6 glioma cells. *Oncol Rep* 2010;23:1053-1062.
- 119 Bey EA, Bente MS, Reinicke KE, Dong Y, Yang CR, Girard L, Minna JD, Bornmann WG, Gao J, Boothman DA: An NQO1- and PARP-1-mediated cell death pathway induced in non-small-cell lung cancer cells by beta-lapachone. *Proc Natl Acad Sci USA* 2007;104:11832-11837.
- 120 Dragovich T, Gordon M, Mendelson D, Wong L, Modiano M, Chow HH, Samulitis B, O'Day S, Grenier K, Hersh E, Dorr R: Phase I trial of imexon in patients with advanced malignancy. *J Clin Oncol* 2007;25:1779-1784.
- 121 Maeda H, Hori S, Ohizumi H, Segawa T, Kakehi Y, Ogawa O, Kakizuka A: Effective treatment of advanced solid tumors by the combination of arsenic trioxide and L-buthionine-sulfoximine. *Cell Death Differ* 2004;11:737-746.
- 122 Trachootham D, Zhou Y, Zhang H, Demizu Y, Chen Z, Pelicano H, Chiao PJ, Achanta G, Arlinghaus RB, Liu J, Huang P: Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by beta-phenylethyl isothiocyanate. *Cancer Cell* 2006;10:241-252.
- 123 Xiao D, Powolny AA, Moura MB, Kelley EE, Bommarreddy A, Kim SH, Hahn ER, Normolle D, Van Houten B, Singh SV: Phenethyl isothiocyanate inhibits oxidative phosphorylation to trigger reactive oxygen species-mediated death of human prostate cancer cells. *J Biol Chem* 2010;285:26558-26569.
- 124 Alexandre J, Nicco C, Chereau C, Laurent A, Weill B, Goldwasser F, Batteux F: Improvement of the therapeutic index of anticancer drugs by the superoxide dismutase mimic mangafodipir. *J Natl Cancer Inst* 2006;98:236-244.
- 125 Magda D, Miller RA: Motexafin gadolinium: a novel redox active drug for cancer therapy. *Semin Cancer Biol* 2006;16:466-476.
- 126 Palmeira CM, Wallace KB: Benzoquinone inhibits the voltage-dependent induction of the mitochondrial permeability transition caused by redox-cycling naphthoquinones. *Toxicol Appl Pharmacol* 1997;143:338-347.
- 127 Wallace KB: Adriamycin-induced interference with cardiac mitochondrial calcium homeostasis. *Cardiovasc Toxicol* 2007;7:101-107.