Uncovering the role of VDAC in the regulation of cell life and death

Varda Shoshan-Barmatz · Nurit Keinan · Hilal Zaid

Abstract Proper cell activity requires an efficient exchange of molecules between mitochondria and cytoplasm. Lying in the outer mitochondrial membrane, VDAC assumes a crucial position in the cell, forming the main interface between the mitochondrial and the cellular metabolisms. As such, it has been recognized that VDAC plays a crucial role in regulating the metabolic and energetic functions of mitochondria. Indeed, down-regulation of VDAC1 expression by shRNA leads to a decrease in energy production and cell growth. VDAC has also been recognized as a key protein in mitochondria-mediated apoptosis through its involvement in the release of apoptotic proteins located in the inter-membranal space and as the proposed target of pro- and anti-apoptotic members of the Bcl2-family and of hexokinase. Questions, however, remain as to if and how VDAC mediates the transfer of apoptotic proteins from the inter-membranal space to the cytosol. The diameter of the VDAC pore is only about 2.5–3 nm, insufficient for the passage of a folded protein like cytochrome c. New work, however, suggests that pore formation involves the assembly of homo-oligomers of VDAC or hetero-oligomers composed of VDAC and pro-apoptotic proteins, such as Bax. Thus, VDAC appears to represent a convergence point for a variety of cell survival and cell death signals. This review provides insight into the central role of VDAC in mammalian cell life and death, emphasizing VDAC function in the regulation of mitochondria-mediated apoptosis and, as such, its potential as a rational target for new therapeutics.

Keywords Apoptosis · Cancer · Cytochrome c · Hexokinase · Mitochondria · shRNA · VDAC

Introduction

The mitochondrial voltage-dependent anion channel (VDAC) was first identified in 1976 and has been extensively studied since. The VDAC family proteins includes three isoforms, VDAC1, VDAC2, and VDAC3, all are located on the mitochondrial outer membrane (OMM). In this review, we will focus on VDAC1.

It is well established that VDAC regulates the energy balance of mitochondria and the entire cell by serving as a common pathway for metabolite exchange between mitochondria and cytoplasm (Colombini 2004; De Pinto et al. 2003; Granville and Gottlieb 2003; Lemasters and Holmuhamedov 2006; Shoshan-Barmatz and Gincel 2003; Shoshan-Barmatz and Israelson 2005; Shoshan-Barmatz et al. 2006; Tsujimoto and Shimizu 2002; Vyssokikh and Brdiczka 2003). Over the past few years, several hypotheses and mechanisms have been postulated for and against a role for VDAC in OMM permeability and the subsequent release of apoptosis-promoting factors (Abu-Hamad et al. 2006; Baines et al. 2007; Rostovtseva et al. 2005; Shoshan-Barmatz et al. 2006, 2008; Tsujimoto and Shimizu 2002; Yuan et al. 2008).

Several recent reviews have focused on VDAC as an essential player in apoptosis as well as on the contribution of VDAC to the function of mitochondria in cell life and death (Granville and Gottlieb 2003; Lemasters and

V. Shoshan-Barmatz (✉) · N. Keinan · H. Zaid
Department of Life Sciences,
Ben-Gurion University of the Negev,
Beer-Sheva 84105, Israel
e-mail: vardasb@bgu.ac.il

V. Shoshan-Barmatz · N. Keinan · H. Zaid
The National Institute for Biotechnology in the Negev,
Ben-Gurion University of the Negev,
Beer-Sheva 84105, Israel

Holmuhamedov 2006; Shoshan-Barmatz et al. 2006, 2008; Shoshan-Barmatz and Gincel 2003; Shoshan-Barmatz and Israelson 2005; Tsujimoto and Shimizu 2002). However, a review questioning some of these findings has also appeared (Rostovtseva et al. 2005). Despite the body of work pointing to VDAC as a key player in apoptosis, major unsolved questions in the field remain. The most pressing ask how mitochondrial pro-apoptotic proteins are released, how this event is regulated and what functions VDAC plays in these processes. The present review outlines current knowledge related to the role of VDAC in coordinating communication between mitochondria and cytosol and in the regulation of apoptotic cell death. Specifically, we highlight recent results related to the interaction of VDAC1 with hexokinase (HK) and the modulation of VDAC function upon such interaction. Possible therapeutic applications based upon the modulation of VDAC functions affecting cell life or death will also be considered.

VDAC is essential for energy production and cell growth

VDAC assumes an important role in energy production via controlling metabolite traffic across the OMM. As such, down-regulation of VDAC1 expression would result in decreased energy production and thus, affect cell vitality. Indeed, as we have demonstrated (Abu-Hamad et al. 2006), silencing VDAC1 expression arrests cell growth through a reduction in energy production. In these experiments, endogenous VDAC1 expression in transformed primary embryonal human kidney T-REx-293 cells was suppressed by about 70–90% when an shRNA approach was employed, in which the shRNA was designed to target human VDAC1 (hVDAC1) but not murine VDAC1 (mVDAC1). The decrease in hVDAC1 levels resulted in a dramatic decrease in cell growth, a five-fold decrease in ATP synthesis, as well as a decrease of about 50% in the cellular levels of ATP and ADP, all of which were restored by mVDAC1 expression (Abu-Hamad et al. 2006). Thus, down-expression of hVDAC1, leading to inhibition of cell proliferation, involves disrupted energy production, possibly explaining the slow growth of these cells. VDAC1 silencing by shRNA also inhibited growth of other cell lines, such as HeLa and MCF-7 (Koren and Shoshan-Barmatz unpublished results).

VDAC1 as a gatekeeper in mitochondria-mediated apoptosis

The involvement of VDAC in cytochrome c release and apoptosis had attracted interest on several fronts and has

been the focus of several recent reviews (Granville and Gottlieb 2003; Lemasters and Holmuhamedov 2006; Shoshan-Barmatz et al. 2006, 2008; Shoshan-Barmatz and Gincel 2003; Tsujimoto and Shimizu 2002; Vyssokikh and Brdiczka 2003).

VDAC and PTP Mitochondrial permeability transition (MPT) is thought to occur due to the opening of the permeability transition pore (PTP), in response to various pro-apoptotic stimuli (Halestrap et al. 2000; Juhaszova et al. 2008; Kroemer et al. 2007; Lemasters 2007; Shoshan-Barmatz and Gincel 2003; Tsujimoto and Shimizu 2007). The PTP is proposed to be a relatively non-specific channel and is composed of VDAC, the adenine nucleotide translocator (ANT) and cyclophilin D (CypD) (Tsujimoto and Shimizu 2007). The mechanism(s) responsible for PTP opening or assembly have not yet been fully resolved. In fitting with VDAC being proposed as a component of the PTP, a variety of agents that inhibit PTP activity were also shown to interact with VDAC, modify its channel activity and prevent apoptosis. These include hexokinase-I (HK-I) ruthenium red (RuR), the trivalent cation derivative of RuR, Ru360, and NADH, all of which facilitates VDAC closure (reviewed in Shoshan-Barmatz and Gincel 2003; Shoshan-Barmatz et al. 2006). We suggest that RuR inhibition of PTP opening is due to RuR interaction with VDAC since RuR inhibited the channel activity of recombinant native mVDAC1 but not of the E72Q-mVDAC1 mutant and prevented the release of cytochrome c and apoptosis in cells expressing native but not mutated VDAC1 (Israelson et al. 2007a, b; Zaid et al. 2005). Recently, however, VDAC proteins were reported to be dispensable for Ca^{2+}- and oxidative stress-induced PTP opening (Baines et al. 2007). By contrast, recent studies reported that VDAC1 is an indispensable protein for PTP opening and induction of apoptosis (Tajeddine et al. 2008; Yuan et al. 2008). It was found that reducing VDAC1 levels by siRNA attenuates endostatin-induced apoptosis in endothelial cells, and that endostatin-induced PTP opening is accompanied by an up-regulation of VDAC1 expression (Yuan et al. 2008). Similarly, siRNA-mediated depletion of VDAC1 strongly reduced cisplatin-induced cytochrome c release and apoptotic cell death (Tajeddine et al. 2008). These and other reports strongly suggest that VDAC assumes a key function in apoptosis, as discussed in the next section.

VDAC and apoptosis There is substantial evidence suggesting VDAC to be a critical player in the release of apoptogenic proteins from mammalian mitochondria (Granville and Gottlieb 2003; Lemasters and Holmuhamedov 2006; Shoshan-Barmatz et al. 2006, 2008; Tajeddine et al. 2008; Tsujimoto and Shimizu 2002; Vyssokikh and Brdiczka 2003; Yuan et al. 2008). Cytochrome c release, the interaction of

the pro-apoptotic protein Bax with VDAC and the triggering of cell death were all inhibited by anti-VDAC antibodies (Madesh and Hajnoczky 2001; Shimizu et al. 2001; Zheng et al. 2004). In addition, mitochondria from VDAC-deficient yeast were capable of releasing cytochrome c in the presence of Bax only when hVDAC was expressed (Shimizu et al. 1999). We have demonstrated inhibition of cytochrome c release and cell death by HK in cells expressing native but not mutated VDAC1 (Abu-Hamad et al. 2008). Similarly, RuR interacts with native but not mutated VDAC1 to prevent cytochrome c release and cell death (Israelson et al. 2007b). RuR was shown to protect against cell death induced by different stimuli, including curcumin in U-937 cells (Bae et al. 2003), ursolic acid in HL-60 cells (Baek et al. 1997) and microcystin in cultured rat hepatocytes (Ding et al. 2001). We proposed that such protection results from the interaction of RuR with VDAC1 (Gincel et al. 2001). The inability of RuR to protect against apoptosis in cells expressing mutated VDAC1 (Israelson et al. 2007b) suggests that RuR exert it anti-apoptotic effect via direct interaction with VDAC1.

Recently, it was demonstrated that siRNA-mediated down-expression of VDAC1 prevented cell death induced by cisplatin and strongly reduced cisplatin-induced release of cytochrome c and AIF and maturation of caspases-3 (Tajeddine et al. 2008). In addition, depletion of VDAC1 strongly suppressed the cisplatin-induced activation of Bax (Tajeddine et al. 2008). Reducing VDAC1 expression by siRNA also attenuated endostatin-induced apoptosis (Yuan et al. 2008).

VDAC1 over-expression induces cell death As discussed in previous sections, silencing of VDAC1 expression inhibits energy production and cell proliferation. On the other hand, over-expression of human, murine, yeast, *Paralichthys olivaceus* and rice VDAC was found to induce apoptotic cell death, regardless of cell type (Abu-Hamad et al. 2008; Ghosh et al. 2007; Godbole et al. 2003; Lu et al. 2007; Zaid et al. 2005). Also, HeLa cells over-expressing VDAC1 showed enhanced FNQ13-induced H_2O_2 production and cell death (Simamura et al. 2006). Recently, it has been shown that VDAC1 over-expression in endothelial cells enhanced the activation of caspase-9, elevated the production of reactive oxygen species and endostatin-induced apoptosis (Yuan et al. 2008). Taken together, these results indicate that the cellular expression level of VDAC1 is a crucial factor in the process of mitochondria-mediated apoptosis.

The mechanism underlying the induction of cell death by enhanced VDAC1 over-expression is unknown. Since cell death induced by VDAC1 over-expression is prevented by RuR (Israelson et al. 2007b; Zaid et al. 2005), Bcl2, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) (Godbole et al. 2003) or by over-expressing HK-I (Abu-Hamad et al.

2008; Azoulay-Zohar et al. 2004; Zaid et al. 2005), shown to interact with VDAC, it seems that apoptosis induced by VDAC1 over-expression is as apoptosis induced by various stimuli. Recently, we have demonstrated that VDAC oligomerization is involved in the release of cytochrome c from mitochondria (Zalk et al. 2005). As such, VDAC1 over-expression would encourage VDAC oligomerization, and thus allow for release of pro-apoptotic proteins from the mitochondrial intermembrane space. Indeed, we have found that VDAC oligomerization is increased upon VDAC1 over-expression (Keinan and Shoshan-Barmatz unpublished results).

VDAC oligomerization and release of cytochrome c All of the apoptotic proteins known to translocate to the cyto-plasm following an apoptotic stimulus reside in the mitochondrial intermembrane space. Thus, only the perme-ability of the OMM needs to be modified for their release (Doran and Halestrap 2000; Halestrap et al. 2002; Martinou et al. 2000; Tsujimoto and Shimizu 2002). Hence, VDAC as an OMM channel could mediate cytochrome c release. Indeed, release of cytochrome c via purified VDAC reconstituted into cytochrome c-encapsulating liposomes has been demonstrated (Madesh and Hajnoczky 2001; Zalk et al. 2005). When considering models of VDAC-mediated protein release, one should consider the molecular sizes of the released proteins, ranging from 12 to 100 kDa. The diameter of the VDAC pore is 2.5–3.0 nm, sufficient to move nucleotides and small molecules but insufficient to pass a folded protein, like cytochrome c. Our finding that VDAC can exist as oligomers led us to propose that oligomeric VDAC1 mediates the release of cytochrome c (Zalk et al. 2005). Using purified rat liver (Zalk et al. 2005), brain mitochondria (Shoshan-Barmatz et al. 2004) or recombinant human VDAC (Shi et al. 2003), both purified soluble VDAC and membrane-embedded VDAC were shown to assemble into dimers, trimers and tetramers in a dynamic process. Moreover, we have found that STS-induced apoptosis was accompanied by a three–six-fold increase in VDAC oligomerization (Fig. 1). Similarly, the apoptosis-inducing effect of As_2O_3 was attributed to an induction of VDAC homo-dimerization that was prevented by over-expression of the anti-apoptotic protein, Bcl-XL (Zheng et al. 2004). It was also demonstrated that VDAC oligomerization is encouraged in the presence of cyto-chrome c (Zalk et al. 2005). The supra-molecular organi-zation of VDAC has also been demonstrated using atomic force microscopy (Goncalves et al. 2007; Hoogenboom et al. 2007) and NMR (Malia and Wagner 2007).

The protein-conducting channel could reside within a VDAC1 homo-oligomer or hetero-oligomers containing VDAC1 and pro-apoptotic proteins (Martinou et al. 2000; Shoshan-Barmatz et al. 2006). It has been shown that
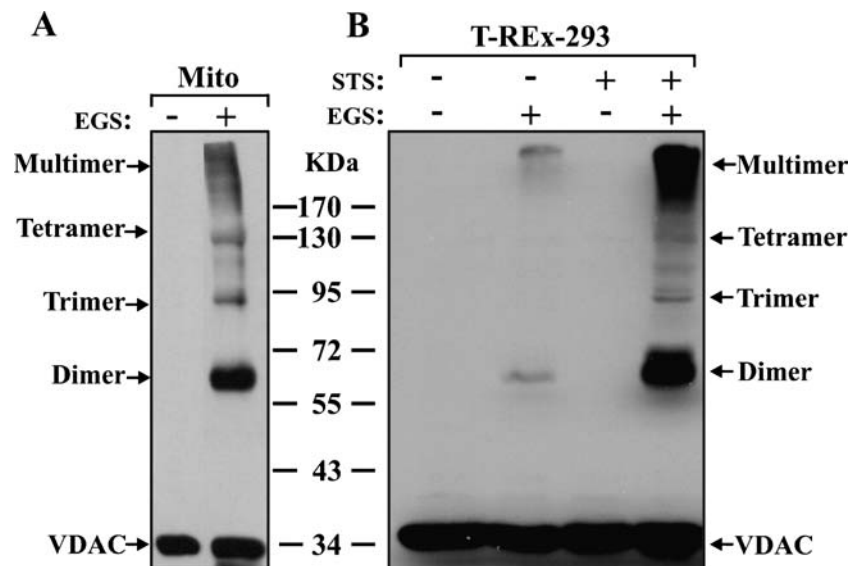
Fig. 1 VDAC oligomerization as enhanced by apoptotosis induction. A. Mitochondria (1 mg/ml) were incubated for 15 min at 30 °C in 10 mM Tricine, 150 mM NaCl pH 8.3, with or without EGS (150 μM). B. STS-induced VDAC oligomerization, as revealed by EGS-based cross-linking of T-REx-293 cells, before and after 2.5 h of incubation with STS (1.25 μM). Cells were washed with PBS and incubated with EGS at 30 °C for 20 min, followed by SDS-PAGE (9% acrylamide) and Western blotting using anti-VDAC antibodies. *VDAC* monomers, *dimers*, *trimers*, *tetramers* and *multimers* are indicated

As_2O_3 able to induce Bax and VDAC oligomerization (Yu et al. 2007). Electrophysiological studies showed that Bax and tBid increase VDAC pore size (Shimizu et al. 2000b; Banerjee and Ghosh 2004). Thus, substantial evidence for the formation of higher ordered VDAC-containing complexes and the enhancement of supra-molecular assembly of VDAC in cultured cells upon apoptosis induction (Fig. 1) supports the involvement of VDAC oligomerization in cytochrome *c* release and, thus, in apoptosis.

Regulation of VDAC permeability

Accumulated evidence suggests that VDAC activities can be modulated by various physiological and non-physiological ligands (reviewed in Colombini 2004; Shoshan-Barmatz and Gincel 2003; Shoshan-Barmatz et al. 2006), such as glutamate, divalent cations, adenine nucleotides, Koenig's polyanion, RuR, DCCD (dicyclohexylcarbodiimide) and DIDS. Inhibition of PTP opening, release of cytochrome *c* or apoptosis by these reagents is often related to their interaction with VDAC.

Growing evidence also suggests that VDAC possesses regulatory binding sites for Ca^{2+} and nucleotides. While binding sites for nucleotides (Yehezkel et al. 2006, 2007) and Ca^{2+} (Israelson et al. 2007a, b) have been localized, the sites of binding of other ligands, as well as the physiological significance of such modulators, await further study.

VDAC activities are also regulated via interaction with associated proteins, most notably those of the apoptosis-regulating proteins, mainly members of the Bcl2 family (Shimizu et al. 1999, 2000a, 2001; Tsujimoto 2003) and HK (see below). VDAC possesses binding sites for glycerol kinase, HK-I, creatine kinase and forms complexes with various proteins, such as the dynein light chain, the benzodiazepine receptor, mtHSP70, the ORDIC channel, and ANT (reviewed in Colombini 2004; Shoshan-Barmatz and Gincel 2003; Shoshan-Barmatz and Israelson 2005). VDAC also interacts with glyceraldehyde 3-phosphate dehydrogenase (Shoshan-Barmatz and Israelson 2005; Tarze et al. 2007) and gelsolin (Kusano et al. 2000). In this review, we focus on the regulation of VDAC by Ca^{2+}, ATP, glutamate and HK.

Adenine nucleotides are transported by VDAC, bind to VDAC and regulate its activity The transport of adenine nucleotides via VDAC has been demonstrated using isolated mitochondria (Lee et al. 1996), VDAC reconstituted into liposomes (Bathori et al. 1993) or in a PLB (Rostovtseva et al. 2002). VDAC is also regulated by adenine nucleotides and possess one or more nucleotide-binding sites (NBS) (Florke et al. 1994; Yehezkel et al. 2006, 2007). Photo-affinity labeling with benzoyl–benzoyl-ATP (BzATP), together with mass-spectral analysis and site-directed mutation, we localized NBS(s) in VDAC. Two putative NBSs were localized to a C-terminal region (positions 271–283) and near the N-terminus (positions 19–25) of mVDAC1. The predicted N-terminal VDAC1-NBS seems to regulate OMM permeability, and, therefore, serves to couple respiration with cell survival (Yehezkel et al. 2007).

Ca^{2+} is transported by VDAC, binds to VDAC and regulates its activity Intra-mitochondrial Ca^{2+} modulates critical enzymes such as of the TCA cycle and fatty acid oxidation (Nichols and Denton 1995). Ca^{2+} overload, on the other hand, induces PTP opening (Tsujimoto and Shimizu 2007). Accordingly, mitochondria are endowed with multiple Ca^{2+} transporters for the uptake and release of Ca^{2+} across their inner membrane (Gunter et al. 1998). We have demonstrated that VDAC is permeable to Ca^{2+} and possesses Ca^{2+}-binding sites (Gincel et al. 2001). Ca^{2+} transport by VDAC was further characterized (Tan and Colombini 2007). It has also shown that the control of OMM permeability by Ca^{2+} is mediated via an interaction with VDAC (Bathori et al. 2006).

Several lines of evidence suggest that VDAC possesses Ca^{2+}-binding site(s) (Gincel et al. 2001; Shoshan-Barmatz and Gincel 2003; Israelson et al. 2007b). The finding that VDAC channel closure by RuR, a reagent shown to interact with Ca^{2+}-binding proteins, can be prevented by Ca^{2+} (Gincel et al. 2001; Israelson et al. 2007b), strongly suggests that RuR and Ca^{2+} share a common binding site(s). Recently, we used AzRu, a newly-synthesized photoactivable reagent (Israelson et al. 2005), to localize Ca^{2+}-binding site(s) in VDAC1 (Israelson et al. 2007a). We demonstrated that AzRu interacts with and closes native but not mutated VDAC1, an effect that was preventable by Ca^{2+} but not Mg^{2+}. The VDAC Ca^{2+}-binding site(s) was localized based on [^{103}Ru] AzRu binding to and inhibition of VDAC channel activity of native but not E72Q- or E202Q-mutated VDAC1. Also MALDI-TOF analysis showed AzRu bound to an E72- and E202-containing peptide (Israelson et al. 2007a). Accordingly, we suggested that the two glutamate residues, E72 and E202, located on two different cytosolic loops, form the VDAC Ca^{2+}-binding site(s), or part thereof.

Glutamate is transported by VDAC and modulates VDAC activity Glutamate is synthesized in the mitochondria. We have shown that glutamate is transported by bilayer-reconstituted VDAC and that Ca^{2+} transport by VDAC is modulated by L-glutamate (Gincel et al. 2000; Deniaud et al. 2007; Gincel and Shoshan-Barmatz 2004). Glutamate (1 to 20 mM) was found to modulate the channel activity of VDAC (Gincel et al. 2000). The effect of glutamate on channel conductance is specific, since it was observed in the presence of 1 M NaCl and not with aspartate or GABA. Finally, inhibition of PTP opening by L-glutamate is highly specific, as it is not obtained by D-glutamate, L-glutamine, L-aspartate, or L-asparagine (Gincel and Shoshan-Barmatz 2004). These results suggest that VDAC possesses a specific glutamate-binding site that modulates its activity.

The interaction of hexokinase with mitochondrial VDAC, apoptosis and cancer

VDAC as the mitochondrial target of HK—characterization of the VDAC1-binding site Of the four mammalian HK isozymes, HK-I and II are capable of binding to the OMM, more specifically to VDAC (Robey and Hay 2006; Shoshan-Barmatz et al. 2006; Wilson 2003). As a glycolytic enzyme that consumes ATP in order to phosphorylate glucose, HK binding to VDAC allows direct access to mitochondrial ATP. However, as presented below, HK binding to VDAC also protects against apoptotic cell death. Our studies, relying on purified VDAC, isolated mitochondria or cells in culture, suggest that the anti-apoptotic activity of HK-I occurs via its interaction with VDAC to modulate the mitochondrial phase of apoptosis.

We and others have provided several lines of evidence for the interaction of HK-I and HK-II with VDAC: (a) HK-I and HK-II dock onto the cytosolic surface of the OMM by binding to VDAC (Abu-Hamad et al. 2008; Azoulay-Zohar et al. 2004; Pastorino and Hoek 2003; Zaid et al. 2005). (b) HK-I and HK-II include a hydrophobic N-terminal sequence capable of targeting the enzyme to mitochondria
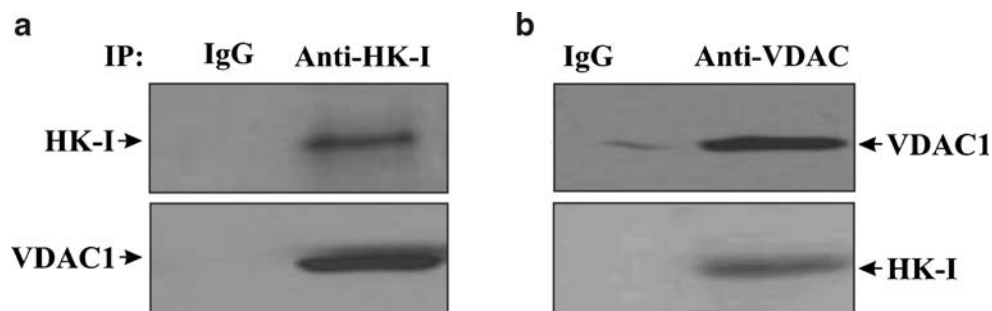

Fig. 2 Co-immunoprecipitation of HK and VDAC by anti-VDAC1 or anti-HK antibodies. The interaction between HK and VDAC in the rat brain is demonstrated by immunoprecipitation performed on total rat brain extract using anti-HK (N-19) or polyclonal anti-VDAC1 (N-18) antibodies (Santa Cruz). The precipitated proteins were detected by Western blotting using anti-HK (**a**) or monoclonal anti-VDAC (Calbiochem) antibodies (**b**)

(Sui and Wilson 1997), also required for the interaction with VDAC (Azoulay-Zohar et al. 2004). Indeed, a recent study using mutant and N-terminally-truncated HK-I and HK-II has shown that the enzyme activity as well as the mitochondrial binding of HK-I and HK-II are essential for their anti-apoptotic protective effects (Sun et al. 2007). (c) HK-I directly binds to bilayer-reconstituted VDAC and induces channel closure in a manner that is reversed by glucose-6-phosphate (Azoulay-Zohar et al. 2004). (d) Using rat brain extract, HK-I and VDAC could be co-immuno-precipitated using either anti-VDAC or anti-HK-I anti-bodies (Fig. 2). (e) HK-I bound to isolated yeast mitochondria expressing native mVDAC1 but not to E72Q-mVDAC1 (Abu-Hamad et al. 2008; Zaid et al. 2005). (f) Consistently, HK-I-GFP co-localized with Mito-Tracker, a mitochondrial marker, in T-REx cells expressing native but not E72Q-mutated mVDAC1 (Abu-Hamad et al. 2008). (g) A single mutation in VDAC1, *i.e.* E72Q, inhibited the interaction of HK-I with VDAC1 and prevented HK-I-mediated protection against apoptosis (Abu-Hamad et al. 2008; Zaid et al. 2005). Glu72 was shown to interact with the carboxyl-modifying reagent, DCCD, with such modification preventing HK binding to mitochondria (Nakashima et al. 1986). In a similar manner, HK-I protected against apoptosis induced by over-expression of native but not of certain mutated VDAC1 (Abu-Hamad et al. 2008; Zaid et al. 2005).

Although these and related studies offer strong support for a direct interaction between HK and VDAC, with this interaction being sufficient to allow cells to evade apopto-sis, the VDAC1 sequences interacting with HK-I or HK-II have only recently been identified and shown to correspond to two cytoplasmic domains (Abu-Hamad et al. 2008). Point mutations (i.e. E65Q, E72Q, K73L, D77N, E188Q or E202Q) in these domains prevented HK-I binding to mVDAC1 reconstituted into PLB. More importantly, HK-I was no longer able to prevent cytochrome *c* release and subsequently apoptosis in T-REx-293 cells over-expressing those mutants. By contrast, the G67A, N75A, K109L, K112L, K200L, or L201A mutations of VDAC1 did not prevent the protection offered by HK-I over-expression against apoptosis (Abu-Hamad et al. 2008). Thus, we proposed that the HK-I binding site is located in two cytosolic VDAC1 loops (see Fig. 3) and that the interaction of HK-I with these sites results in inhibition of cytochrome *c* release and protection against apoptosis.

Hexokinase, VDAC, apoptosis and cancer Cancer cells are characterized by a high rate of glycolysis which serves as their primary energy generating pathway (Mathupala et al. 2006; Pedersen et al. 2002; Pedersen 2007). The molecular basis for this high rate of glycolysis involves a number of genetic and biochemical events, including the over-expression of the mitochondrial-bound HK-I and HK-II (Bryson et al. 2002; Gottlob et al. 2001; Pastorino and Hoek 2003). The elevated levels of mitochondria-bound HK in cancer cells results in apoptosis being evaded, thereby allowing cells to continue proliferating. The molecular mechanisms by which HK promotes cell survival are not yet fully understood. However, when taken together,

Fig. 3 Models showing the pro-posed HK-interacting site on VDAC1. VDAC1 is presented as a barrel with the amino acid sequences of two of the three cytosolic loops predicted according to (Colombini 2004) shown. Those amino acids that when mutated prevented HK binding, are indicated by *circles*. In **a**, the interaction of HK with loop 1 and loop 3, located on the same VDAC1 molecule is shown; while in **b** the interaction of HK with loops located on two VDAC1 molecules is proposed
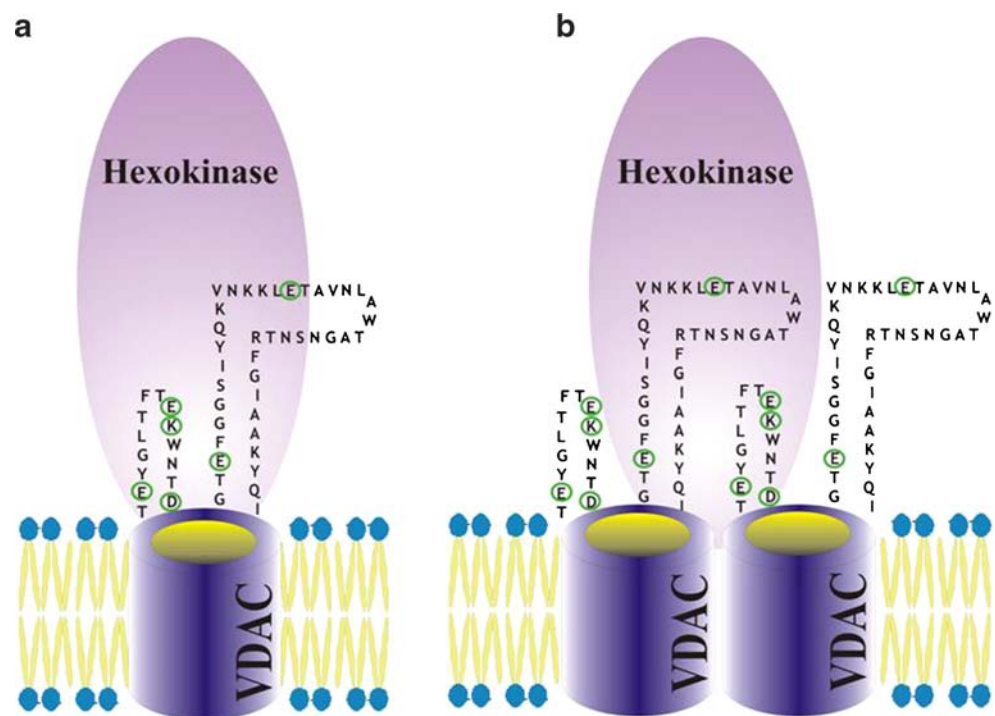
the direct binding of HK to VDAC and the involvement of VDAC in cell death, raise the possibility that the HK-VDAC interaction lies at the base of apoptosis regulation by HK. Indeed, over-expression of mitochondria-bound HK in U-937, T-REx-293 or in vascular smooth muscle cells suppressed cytochrome c release and apoptotic cell death, as induced by different stimuli (Abu-Hamad et al. 2008; Azoulay-Zohar et al. 2004; Pastorino et al. 2002; Zaid et al. 2005). A decrease in apoptosis and an increase in cell proliferation have also been reported to be induced by HK-II expression in the NIH-3T3 (Fanciulli et al. 1994) and rat 1a cell lines (Gottlob et al. 2001). In addition, over-expression of HK-II in HeLa cells inhibited Bax-induced cytochrome c release and apoptosis (Pastorino et al. 2002). As presented above, a single mutation in the VDAC1 molecule (E72Q) diminished HK-I binding to VDAC1 and prevented HK-I-mediated protection against cell death induced by VDAC1 over-expression or STS treatment (Abu-Hamad et al. 2008; Zaid et al. 2005). Collectively, these studies strongly indicate that HK and VDAC are key components in the regulation of apoptosis and that the protective effect of HK against cell death is mediated via its direct interaction with VDAC. Furthermore, these results suggest that HK over-expression in cancer cells not only assures an adequate supply of energy, but also corresponds to an anti-apoptotic defense mechanism.

Disruption of HK-VDAC interaction as an approach to cancer therapy It has been shown that a HK-VDAC1 interaction is critical for preventing induction of apoptosis in tumor-derived cells (Abu-Hamad et al. 2008; Azoulay-Zohar et al. 2004; Gelb et al. 1992; Zaid et al. 2005). Accordingly, via mutagenesis of key VDAC1 amino acids, we have disrupted the interaction of HK with VDAC and identified the HK binding site (Abu-Hamad et al. 2008; Zaid et al. 2005). Thus, promoting detachment of HK from VDAC may be a promising cancer strategy. Indeed, in a recent study (Arzoine et al. submitted), we used specific peptides corresponding to the identified HK-I-binding site of VDAC1 to interfere with the HK-VDAC1 interaction. Moreover, these VDAC1-based peptides detached HK bound to mitochondria isolated from tumor cells. Thus, interfering with HK binding to VDAC could be the basis for a novel form for cancer treatment.

Recently (Goldin et al. 2008), we have shown that a plant stress hormone of the jasmonate family, methyl jasmonate (MJ), binds in a specific manner to HK and leading to its detachment from mitochondria isolated from several cancer cell types. The susceptibility of cancer cells to jasmonates is dependent on the expression of HK. This finding, thus, provide an explanation for the selective effects of jasmonates on cancer cells. Among these lines, it should be noted that intra-arterial injection of 3-bromopyruvate, an inhibitor of mitochondrially-bound HK, into tumors implanted in rabbit liver killed up to 90% of the tumor cells without significant damage to the surrounding healthy tissue (Geschwind et al. 2002; Pastorino et al. 2005).

To conclude, interfering with HK binding to VDAC can, therefore, serve to guide development of a new selective approach for cancer therapy.

Concluding remarks

Following the identification of VDAC as the OMM channel, much has been learned about the protein's structure–function relationships and the manners in which VDAC activity is modulated. Biochemical and molecular approaches have revealed a remarkable diversity of regulatory mechanisms controlling VDAC function, ranging from Ca^{2+} and adenine nucleotides to phosphorylation and specific associated proteins. Recent studies involving both down- and over-expression clearly indicate that the expression level of VDAC is a critical element in cell life and death and that VDAC plays a key role in the regulation of mitochondria-mediated apoptosis. Thus, both targeted over-expression and knock-down expression of VDAC in living cells, as well as interfering with HK binding to VDAC, open novel avenues for cancer biotherapy. However, many interesting and important questions remain. These include further information concerning the location of VDAC modulator binding sites, characterization of the process of VDAC oligomerization-coupled apoptosis and further characterization of the interaction of anti-apoptotic proteins with VDAC and regulation of the release of cytochrome c. Finally, the identification of VDAC as playing a role at critical control points in the apoptotic pathway points to VDAC as a rational target for the development of a new generation of therapeutics.

References

Abu-Hamad S, Sivan S, Shoshan-Barmatz V (2006) Proc Natl Acad Sci U S A 103:5787–5792

Abu-Hamad S, Zaid H, Israelson A, Nahon E, Shoshan-Barmatz V (2008) J Biol Chem 283(19):13482–13490

Azoulay-Zohar H, Israelson A, Abu-Hamad S, Shoshan-Barmatz V (2004) Biochem J 377:347–355

Bae JH, Park JW, Kwon TK (2003) Biochem Biophys Res Commun 303:1073–1079

Baek JH, Lee YS, Kang CM, Kim JA, Kwon KS, Son HC, Kim KW (1997) Int J Cancer 73:725–728

Baines CP, Kaiser RA, Sheiko T, Craigen WJ, Molkentin JD (2007) Nat Cell Biol 9:550–555

Banerjee J, Ghosh S (2004) Biochem Biophys Res Commun 323:310–314

Bathori G, Sahin-Toth M, Fonyo A, Ligeti E (1993) Biochim Biophys Acta 1145:168–176

Bathori G, Csordas G, Garcia-Perez C, Davies E, Hajnoczky G (2006) J Biol Chem 281:17347–17358

Bryson JM, Coy PE, Gottlob K, Hay N, Robey RB (2002) J Biol Chem 277:11392–11400

Colombini M (2004) Mol Cell Biochem 256–257:107–115

De Pinto V, Messina A, Accardi R, Aiello R, Guarino F, Tomasello MF, Tommasino M, Tasco G, Casadio R, Benz R, De Giorgi F, Ichas F, Baker M, Lawen A (2003) Ital J Biochem 52:17–24

Deniaud A, Rossi C, Berquand A, Homand J, Campagna S, Knoll W, Brenner C, Chopineau J (2007) Langmuir 23:3898–3905

Ding WX, Shen HM, Ong CN (2001) Biochem Biophys Res Commun 285:1155–1161

Doran E, Halestrap AP (2000) Biochem J 348:343–350

Fanciulli M, Paggi MG, Bruno T, Del Carlo C, Bonetto F, Gentile FP, Floridi A (1994) Oncol Res 6:405–409

Florke H, Thinnes FP, Winkelbach H, Stadtmuller U, Paetzold G, Morys-Wortmann C, Hesse D, Sternbach H, Zimmermann B, Kaufmann-Kolle P (1994) Biol Chem Hoppe-Seyler 375:513–520

Gelb BD, Adams V, Jones SN, Griffin LD, MacGregor GR, McCabe ER (1992) Proc Natl Acad Sci U S A 89:202–206

Geschwind JF, Ko YH, Torbenson MS, Magee C, Pedersen PL (2002) Cancer Res 62:3909–3913

Ghosh T, Pandey N, Maitra A, Brahmachari SK, Pillai B (2007) PLoS ONE 2:e1170

Gincel D, Shoshan-Barmatz V (2004) J Bioenerg Biomembr 36:179–186

Gincel D, Silberberg SD, Shoshan-Barmatz V (2000) J Bioenerg Biomembr 32:571–583

Gincel D, Zaid H, Shoshan-Barmatz V (2001) Biochem J 358:147–155

Godbole A, Varghese J, Sarin A, Mathew MK (2003) Biochim Biophys Acta 1642:87–96

Goldin N, Arzoine L, Heyfets A, Israelson A, Zaslavsky Z, Bravman T, Bronner V, Notcovich A, Shoshan-Barmatz V, Flescher E (2008) Oncogene (in press)

Goncalves RP, Buzhynskyy N, Prima V, Sturgis JN, Scheuring S (2007) J Mol Biol 369:413–418

Gottlob K, Majewski N, Kennedy S, Kandel E, Robey RB, Hay N (2001) Genes Dev 15:1406–1418

Granville DJ, Gottlieb RA (2003) Curr Med Chem 10:1527–1533

Gunter TE, Buntinas L, Sparagna GC, Gunter KK (1998) Biochim Biophys Acta 1366:5–15

Halestrap AP, Doran E, Gillespie JP, O'Toole A (2000) Biochem Soc Trans 28:170–177

Halestrap AP, McStay GP, Clarke SJ (2002) Biochimie 84:153–166

Hoogenboom BW, Suda K, Engel A, Fotiadis D (2007) J Mol Biol 370:246–255

Israelson A, Arzoine L, Abu-hamad S, Khodorkovsky V, Shoshan-Barmatz V (2005) Chem Biol 12:1169–1178

Israelson A, Abu-Hamad S, Zaid H, Nahon E, Shoshan-Barmatz V (2007a) Cell Calcium 41:235–244

Israelson A, Zaid H, Abu-Hamad S, Nahon E, Shoshan-Barmatz V (2007b) Cell Calcium 43:196–204

Juhaszova M, Wang S, Zorov DB, Bradley Nuss H, Gleichmann M, Mattson MP, Sollott SJ (2008) Ann N Y Acad Sci 1123:197–212

Kroemer G, Galluzzi L, Brenner C (2007) Physiol Rev 87:99–163

Kusano H, Shimizu S, Koya RC, Fujita H, Kamada S, Kuzumaki N, Tsujimoto Y (2000) Oncogene 19:4807–4814

Lee AC, Xu X, Colombini M (1996) J Biol Chem 271:26724–26731

Lemasters JJ (2007) J Gastroenterol Hepatol 22(Suppl 1):S31–S37

Lemasters JJ, Holmuhamedov E (2006) Biochim Biophys Acta 1762:181–190

Lu AJ, Dong CW, Du CS, Zhang QY (2007) Fish Shellfish Immunol 23:601–613

Madesh M, Hajnoczky G (2001) J Cell Biol 155:1003–1015

Malia TJ, Wagner G (2007) Biochemistry 46:514–525

Martinou JC, Desagher S, Antonsson B (2000) Nat Cell Biol 2:E41–E43

Mathupala SP, Ko YH, Pedersen PL (2006) Oncogene 25:4777–4786

Nakashima RA (1986) Biochemistry 25:1015–1021

Nichols BJ, Denton RM (1995) Mol Cell Biochem 149–150:203–212

Pastorino JG, Hoek JB (2003) Curr Med Chem 10:1535–1551

Pastorino JG, Shulga N, Hoek JB (2002) J Biol Chem 277:7610–7618

Pastorino JG, Hoek JB, Shulga N (2005) Cancer Res 65:10545–10554

Pedersen PL (2007) J Bioenerg Biomembr 39:211–222

Pedersen PL, Mathupala S, Rempel A, Geschwind JF, Ko YH (2002) Biochim Biophys Acta 1555:14–20

Robey RB, Hay N (2006) Oncogene 25:4683–4696

Rostovtseva TK, Komarov A, Bezrukov SM, Colombini M (2002) Biophys J 82:193–205

Rostovtseva TK, Tan W, Colombini M (2005) J Bioenerg Biomembr 37:129–142

Shi Y, Jiang C, Chen Q, Tang H (2003) Biochem Biophys Res Commun 303:475–482

Shimizu S, Narita M, Tsujimoto Y (1999) Nature 399:483–487

Shimizu S, Shinohara Y, Tsujimoto Y (2000a) Oncogene 19:4309–4318

Shimizu S, Ide T, Yanagida T, Tsujimoto Y (2000b) J Biol Chem 275:12321–12325

Shimizu S, Matsuoka Y, Shinohara Y, Yoneda Y, Tsujimoto Y (2001) J Cell Biol 152:237–250

Shoshan-Barmatz V, Gincel D (2003) Cell Biochem Biophys 39:279–292

Shoshan-Barmatz V, Israelson A (2005) J Membr Biol 204:57–66

Shoshan-Barmatz V, Zalk R, Gincel D, Vardi N (2004) Biochim Biophys Acta 1657:105–114

Shoshan-Barmatz V, Israelson A, Brdiczka D, Sheu SS (2006) Curr Pharm Des 12:2249–2270

Shoshan-Barmatz S, Arbel N, Arzoine L (2008) Cell Sci 4:74–118

Simamura E, Hirai K, Shimada H, Koyama J, Niwa Y, Shimizu S (2006) Cancer Biol Ther 5:1523–1529

Sui D, Wilson JE (1997) Arch Biochem Biophys 345:111–125

Sun L, Shukair S, Naik TJ, Moazed F, Ardehali H (2007) Mol Cell Biol 28(3):1007–1017

Tajeddine N, Galluzzi L, Kepp O, Hangen E, Morselli E, Senovilla L, Araujo N, Pinna G, Larochette N, Zamzami N, Modjtahedi N, Harel-Bellan A, Kroemer G (2008) Oncogene (in press)

Tan W, Colombini M (2007) Biochim Biophys Acta 1768(10):2510–2515

Tarze A, Deniaud A, Le Bras M, Maillier E, Molle D, Larochette N, Zamzami N, Jan G, Kroemer G, Brenner C (2007) Oncogene 26:2606–2620

Tsujimoto Y (2003) J Cell Physiol 195:158–167

Tsujimoto Y, Shimizu S (2002) Biochimie 84:187–193

Tsujimoto Y, Shimizu S (2007) Apoptosis 12:835–840

Vyssokikh MY, Brdiczka D (2003) Acta Biochim Pol 50:389–404

Wilson JE (2003) J Exp Biol 206:2049–2057

Yehezkel G, Hadad N, Zaid H, Sivan S, Shoshan-Barmatz V (2006) J Biol Chem 281:5938–5946

Yehezkel G, Abu-Hamad S, Shoshan-Barmatz V (2007) J Cell Physiol 212:551–561

Yu J, Qian H, Li Y, Wang Y, Zhang X, Liang X, Fu M, Lin C (2007) Cancer Biol Ther 6:580–586

Yuan S, Fu Y, Wang X, Shi H, Huang Y, Song X, Li L, Song N, Luo Y (2008) FASEB J (in press)

Zaid H, Abu-Hamad S, Israelson A, Nathan I, Shoshan-Barmatz V (2005) Cell Death Differ 12:751–760

Zalk R, Israelson A, Garty ES, Azoulay-Zohar H, Shoshan-Barmatz V (2005) Biochem J 386:73–83

Zheng Y, Shi Y, Tian C, Jiang C, Jin H, Chen J, Almasan A, Tang H, Chen Q (2004) Oncogene 23:1239–1247