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Current Medicinal Chemistry, **2017**, *24*, **1-12**

REVIEW ARTICLE

VDAC1 as a Player in Mitochondria-Mediated Apoptosis and Target for Modulating Apoptosis

Varda Shoshan-Barmatz[1,*], Yakov Krelin[1] and Quan Chen[2]

[1]*Department of Life Sciences and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel;* [2]*The State Key Laboratory of Membrane Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China*

ARTICLE HISTORY

Received: January 26, 2017
Revised: February 02, 2017
Accepted: February 16, 2017

DOI:
10.2174/0929867324666170616105200

Abstract: ***Background:*** The voltage-dependent anion channel 1 (VDAC1), an outer mitochondria membrane protein, functions as a mitochondrial governor, controlling transport of metabolites in and out of the mitochondria and energy production, while also coordinating glycolysis and oxidative phosphorylation. VDAC1 plays a key role in mitochondria-mediated apoptosis by functioning in the release of apoptotic proteins located in the inter-membranal space and due to its association with pro- and anti-apoptotic proteins. Thus, VDAC1 is considered as a promising target for controlling apoptosis.

Methods: We reviewed published data presenting accumulated evidence suggesting that VDAC1 oligomerization represents an important step in the intrinsic mitochondria-mediated apoptosis pathway.

Results: The published data support the proposal that VDAC1 oligomerization leads to the formation of a large pore that allows the release of pro-apoptotic proteins to the cytosol, thereby, activation of apoptosis. Evidence for the relationship between VDAC1 expression levels and induction of apoptosis are presented. This includes the finding that almost all apoptosis stimuli induce VDAC1 over-expression shifting VDAC1 from a monomeric to an oligomeric assembly, corresponding to the Cyto c release channel. Copounds or conditions inducing VDAC1 over-expression, VDAC1 oligomerization and apoptosis are presented. Likewise, VDAC1-interacting molecules, that inhibit both VDAC1 oligomerization and apoptosis are also presented.

Conclusion: This review highlights the findings about VDAC1 oligomerization as a potential target for controlling apoptosis, specifically using drugs to induce apoptotic cell death in cancer and inhibit apoptosis in neurodegenerative diseases, as well as possible VDAC1-based therapeutic applications.

Keywords: Apoptosis, mitochondria, oligomerization, VDAC1.

1. INTRODUCTION: OVERVIEW

Programmed cell death, or apoptosis, is the biological process by which a cell rapidly proceeds towards death upon receiving specific stimuli. The function of the mitochondria in apoptosis involves transduction of an apoptotic signal release of apoptogenic proteins, such as cytochrome *c* (Cyto *c*), apoptosis-inducing factor (AIF), and SMAC/Diablo, from the mitochondria inter-membrane space (IMS) into the cytosol [1, 2]. These proteins participate in complex processes, including the binding of Cyto *c* to apoptotic protease-activating factor-1 (Apaf-1) or pro-caspase-9, resulting in its activation [3]. Activated caspase-9 activates caspases-3 and -7, leading to protein and DNA degradation and cell death [2]. Several models were proposed to explain how apoptotic initiators cross the outer mitochondrial membrane (OMM) and are released into the cytosol. These models include swelling of the mitochondrial matrix that leads to release

*Address correspondence to this author at the Department of Life Sciences and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel;
E-mail: vardasb@bgu.ac.il

0929-8673/17 $58.00+.00

through the ruptured OMM, and formation of large channels that allow the passage of Cyto *c* and other proteins into the cytosol without affecting OMM integrity [2]. In this respect, EM study of mitochondrial ultrastructure upon apoptosis induction showed no OMM rupture or mitochondrial swelling [4]. The voltage-dependent anion channel 1 (VDAC1), found in the OMM, offers such a route. VDAC1 serves as a controller of mitochondrial metabolism and apoptosis in normal and cancerous cells [5-8].

2. VDAC1 ISOFORMS AND STRUCTURE

In mammals, three different isoforms of VDAC (VDAC1, VDAC2 and VDAC3) have been identified [9], with each proposed to serve different physiological roles [5, 6]. Mice lacking VDAC1 or VDAC2 showed reduced respiratory capacity [10] and VDAC3 knockout male were sterile, while the knockout of both VDAC1 and VDAC3 resulted in growth retardation [11] and deficits in learning behavior and synaptic plasticity [12]. Partial embryonic lethality of *VDAC1*$^{-/-}$ mice (inbred C57BL/6 background) was proposed, as the knockout mice were born in less than expected numbers according to the Mendelian ratio. The importance of VDAC1 as a transporter of metabolites across the OMM was confirmed by studies using VDAC1$^{-/-}$ [13]. Here, we focus on the VDAC1 isoform.

The structure of VDAC1 at atomic resolution was resolved and showed that VDAC1 is composed of 19 transmembrane β-strands connected by flexible loops to form a β-barrel, along with a 25-residue-long N-terminal region that lies inside the pore [14-16]. The N-terminal region is proposed to move in the open space [17] but can translocate out of the internal pore and become exposed to the cytosol, where it can interact with anti-VDAC1 antibodies, as well as with proteins associated with apoptosis, such as members of the Bcl2 family (i.e., Bax, Bcl2, and Bcl-xL) [18-22] and hexokinase (HK) [18, 23]. The involvement of N-terminal domain mobility in channel gating, interaction with anti-apoptotic proteins and VDAC1 dimer formation has been proposed [22].

VDAC1 was demonstrated to form oligomers, specifically dimers, trimers, tetramers, hexamers, and higher-order moieties [24-33]. Studies using chemical crosslinking and VDAC1 purified from rat liver [24], brain mitochondria [31] or recombinant human protein [32] showed that both when purified in solution or embedded in the membrane, the protein can assemble into dimers, trimers, tetramers and higher oligomeric states in a dynamic process. VDAC1 oligomerization has been shown following its reconstitution into membranes and by applied FRET technology [24]. For other VDAC isoforms, structural analysis of zebrafish VDAC2 revealed a crystallographic dimer, whereas in lipid micelles, a higher population of dimeric and higher order oligomers species was observed [34]. No direct demonstration of VDAC3 oligomerization has been reported.

The contact sites between VDAC1 molecules in dimers and higher oligomers were identified using several approaches, such as structural- and computational-based analysis and site-directed mutagenesis, together with cysteine replacement and chemical cross-linking [35]. The contact site in dimeric VDAC1 involves β-strands 1, 2, and 19. Moreover, the results suggested that upon apoptosis induction, VDAC1 undergoes conformational changes leading to its oligomerization into higher ordered states, with contact sites also involving β-strands 8 and 16 [35]. The proposed function of VDAC1 oligomerization, in addition to mediating the release of apoptotic proteins, is a contributing to the stabilization of the protein [32]. These oligomers serve as a docking site for interacting proteins, such as HK [24] and creatine kinase [36-38], and finally, in mediating Cyto *c* release and the binding of apoptosis-regulating proteins [18, 24, 25] (see below).

3. VDAC1, A MULTI-FUNCTIONAL CHANNEL CONTROLLING CELL METABOLISM

Located at the OMM, VDAC1 is a dynamic regulator of global mitochondrial function in both health and disease. VDAC1 controls cellular energy production and metabolism and functions in apoptosis [6, 8, 39]. VDAC1 mediates the fluxes of nucleotides, metabolites (e.g. pyruvate, malate, succinate, and NADH/NAD), ions, including Ca^{2+}, hemes and cholesterol across the OMM [6, 39, 40]. VDAC1 is also considered as a hub protein, interacting with over 150 proteins that regulate the integration of mitochondrial activities with other cellular functions [41]. Thus, VDAC1 functions as a junction point to allow cross-talk between a variety of cell survival and death signals, proceeding *via* VDAC1 interactions with ligands and proteins. In the 1920's Otto Warburg demonstrated increased lactic acid production resulting from high glycolysis in tumors, as compared to non-proliferating cells, and presented cancer as a metabolic disease. However, over the years, this view of cancer was later replaced by the somatic mutation theory [42]. Today, cancer is once again being seen as a metabolic disease, primarily associated with impaired mitochondrial function and metabolism

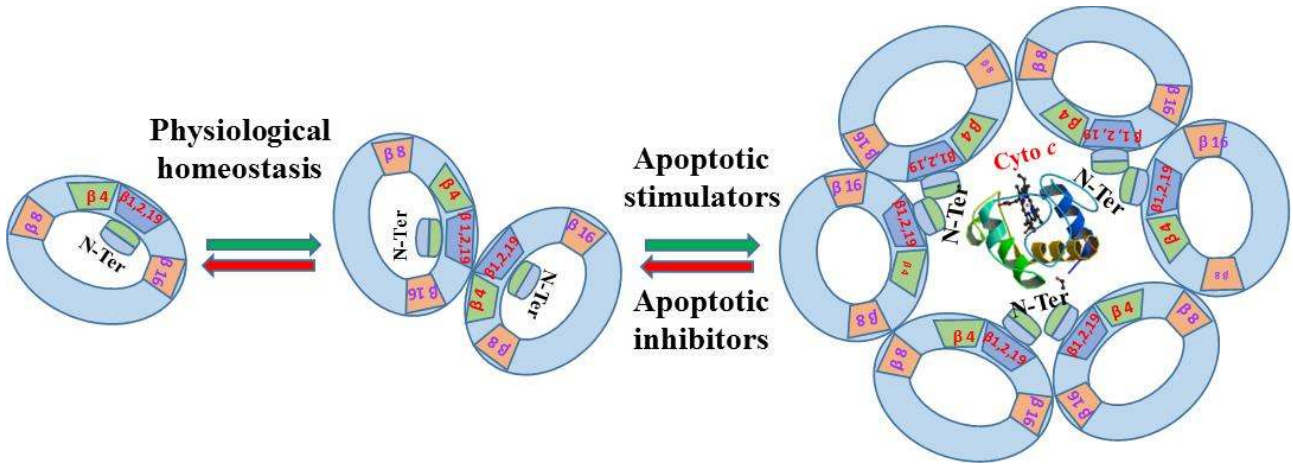

Fig. (1). VDAC1 oligomerization, structures, interacting sites, and modulation

A schematic presentation of apoptotic signal-induced VDAC1 oligomerization associated with the formation of a Cyto *c*-conducting channel. Under physiological conditions, VDAC1 exists in equilibrium between the monomeric and dimeric states and is mainly involved in metabolite and ion exchange activities. Upon induction of apoptosis, VDAC1 monomers or dimers form a large oligomer with an internal pore. VDAC1 oligomerization is accompanied by translocation of the amphipathic N-terminal domain (N-Ter) of the protein from the channel pore to the inside of the newly formed pore, situated according to its amphipathic nature, coating the hydrophobic oligomer pore to form an hydrophilic surface capable of allowing Cyto *c* and other apoptotgenic proteins to traverse the pore and be released to the cytosol, where they activate apoptosis. Furthermore, the N-Ter region interacts with the closest unstable hydrophobic region of VDAC1 (β-strands 1, 2, 19) and confers stability to the oligomer.

[43, 44]. VDAC1 is highly expressed in different tumors [8, 45], contributing to their metabolism *via* its activity in the transport of metabolites in and out of the mitochondria and by supporting glycolysis *via* channelling mitochondrially produced ATP directly to VDAC1-bound HK [5]. This results in mitochondria regulating glycolysis and the TCA cycle, so as to to fulfil tumor requirements for metabolites or metabolic precursors.

4. MITOCHONDRIA-MEDIATED APOPTOSIS AND VDAC1

4.1. VDAC1 Involvement in Apoptosis

Several significant observations [6-8] connect VDAC1 to apoptosis, leading to the proposal of VDAC1 being an essential player in the release of proteins associated with cell death, such as Cyto *c* and AIF, to the cytosol *via* its interaction with pro- and anti-apoptotic proteins. Firstly, the release of Cyto *c* from isolated mitochondria induced by As_2O_3 [46] or by $O_2^{\cdot}$ in permeabilized cells [47], was shown to be prevented by anti-VDAC1 antibodies, while microinjection of anti-VDAC antibodies prevented Bax-VDAC interactions and effectively prevented ethanol-induced hepatocyte apoptosis [48]. Secondly, Cyto *c* release and cell death were inhibited by HK, with both events being obtained in cells expressing native but not with E72Q- or E202Q-mutated VDAC1 [49]. In addition, the interaction of ruthenium red (RuR) with native but not mutated VDAC1 prevented Cyto *c* release and cell death [50]. siRNA-mediated down-regulation of VDAC1 likewise strongly attenuates cisplatin-induced release of Cyto *c* and AIF, cleavage of caspase 3 and the extent of cell death [51]. si-RNA against VDAC1 also reduced endostatin-induced cell death [52]. On the other hand, over-expression of VDAC1 in the absence of any apoptotic stimuli leads to apoptosis, regardless of cell type, in a manner that could be inhibited by anti-apoptotic proteins [49, 53-56]. VDAC1 was also shown to mediate Cyto *c* release from VDAC1-containing proteoliposomes that was prevented by Bcl-xL [24, 47, 57]. In addition, mitochondria isolated from yeast expressing VDAC but not lacking VDAC1 showed Bax-induced $\Delta\psi$ loss and Cyto *c* release [57, 58]. VDAC1, furthermore, was shown to directly interact with Bcl2 and Bcl-xL, and their co-expression with native but not mutated VDAC1 prevented apoptosis [21]. HK-II binding to VDAC1 inhibited Bax-induced Cyto *c* release and apoptosis, whereas HK-I and HK-II interact with VDAC1, and, when over-expressed, prevented staurosporine (STS)-induced cell death in native but not mutated VDAC1-expressing cells [23, 31, 53]. At the same time, VDAC1 channel conductance inhibitors, such as DIDS, RuR, DPC and VBIT-4, inhibited apoptosis as triggered by various inducers [53, 59-61].

In addition, cyathin-R, a cyathane-type diterpenoid from the medicinal fungus *Cyathus africanus,* was found to induce apoptosis in Bax/Bak-depleted cells but not when VDAC1 was depleted [62]. Moreover, cyathin-R effectively decreased tumor growth and stimulated cell death in Bax/Bak-deficient cells, as well as when implanted in a xenograft mouse model [62]. Hence, these findings revealed the pro-apoptotic function of oligomeric VDAC1 independent of Bax/Bak. This is of importance as Bax and Bak are believed to serve as central regulators of mitochondria-mediated apoptosis [63]. It is proposed that Bax homo-oligomers or hetero-oligomers containing Bak yield pore-like structures that mediate Cyto *c* release and thus, apoptosis [64-66]. Indeed, Bax/Bak down-regulation or inactivation represents a common mechanism for the development of resistance to apoptosis in tumor cells [67-69]. Therefore, Cyathin-R represents a potential lead for an effective anti-cancer drug inducing cell death in cancerous cells presenting inactivated Bax/Bak.

Finally, in cancer cells under hypoxic conditions, a fraction of VDAC1 is truncated at the C-terminus (VDAC1-ΔC), and this was shown to confer resistance to cell death [70]. Likewise, it has been shown that VDAC1 over-expression-induced cell death occurs only when the protein is inserted into the mitochondria and proposed that VDAC1 trafficking modulates cell death and that this is regulated by the interaction with HK [71].

4.2. VDAC1-Mediated Apoptosis – Proposed Mechanism of Action

It is important to characterize the VDAC1 assembly forming the Cyto *c*-conducting channel. When one considers that the inner diameter of the VDAC1 pore is 2.5-3.0 nm, or according to recent studies, 1.5 nm, it would seem that such a pore would only be large enough to permit passage of small molecules and nucleotides but that it would be too small to allow passage of proteins released from the IMS like Cyto *c* (12kDa) and AIF (67kDa). We have, therefore, proposed that VDAC1 undergoes oligomerization, creating a large pore formed by the assembled VDAC1 subunits representing a channel that permits Cyto *c* to cross the OMM [6, 7, 24, 25, 72]. The formation of a large channel for transporting completely folded proteins across membranes was demonstrated for several systems, such as for Bax/Bak-mediating Cyto *c* release [73-76], as well as for proteins crossing bacterial and thylakoid membranes [77].

Substantial support exists for dynamic VDAC1 oligomerization into dimers, trimers, tetramers, and higher oligomeric states [6, 7, 24, 25, 72]. Moreover, all apoptosis inducers tested promote VDAC1 oligomerization, regardless of their mode of action, suggesting that VDAC1 oligomerization is their common mechanism [72]. Additionally, inhibiting apoptosis prevented VDAC1 oligomerization [60, 72]. Finally, apoptosis, as induced by UV irradiation, H_2O_2, etoposide, cisplatin or selenite, was found to up-regulate VDAC1 expression levels, an event that was accompanied by VDAC1 oligomerization, Cyto *c* release and apoptosis [25, 78]. Thus, a new concept of apoptosis induction was formulated: Apoptosis inducers → increased intracellular [Ca^{2+}] → Enhanced VDAC1 expression levels → VDAC1 oligomerization → Cyto c release → Apoptosis (see Fig. **3**).

This proposed sequence of events is based on the time course showing that increased intracellular Ca^{2+} proceeds Cyto *c* release and apoptosis [78]. The use of caspase inhibitors showed inhibition of apoptosis but not VDAC1 oligomerization or Cyto *c* release [25] and finally, heterologous over-expression of VDAC induces cell death [54-56, 79].

4.2.1. Evidence for Oligomerized VDAC1 Mediating Cyto c Release

The existence of VDAC1 not only as monomers but also as oligomers has been demonstrated in several studies [7, 18, 24, 25, 72, 80]. As apoptosis induction shifts the equilibrium towards oligomeric VDAC1, we proposed that oligomeric VDAC1 contains a large pore formed at the center of the oligomer that allows Cyto *c* to cross the OMM [7, 18, 24, 25, 72, 80]. This proposal is based on the findings that apoptosis induction in cultured cells led to enhanced VDAC1 oligomerization, as revealed by chemical cross-linking or as monitored in living cells by Bioluminescence Resonance Energy Transfer (BRET) assays [25]. Apoptosis inducers, such as H_2O_2, curcumin, STS, As_2O_3, cisplatin, selenite, etoposide, TNF-α, and UV light, all induce mitochondria–mediated apoptosis and VDAC1 oligomerization, regardless of the cell type studied [25]. VDAC1 oligomerization was not, however, inhibited by caspase inhibitors, suggesting that such oligomerization occurs upstream of caspase activation, pointing to VDAC1 oligomerization as occurring an early stage of the apoptotic process [25].

Recently it was proposed that VDAC1 oligomerization is regulated by the lipid composition of the OMM

[81]. VDAC1 reconstituted into giant unilamellar vesicles, together with fluorescence cross-correlation spectroscopy, were used to follow VDAC1 oligomerization. It was found that phosphatidylglycerol (PG) significantly enhanced VDAC1 oligomerization, whereas cardiolipin (CL) disrupted the formation of VDAC1 supramolecular assemblies. It was also indicated that during apoptosis, PG levels in the mitochondria are increased, whereas CL levels are decreased. It was suggested that the specific lipid composition of the OMM could regulate the oligomeric state of VDAC1 [81]. Finally, p53 encourages VDAC1 oligomerization when added to isolated mitochondria. p53 also induced VDAC1-containing complexes (60 to 300 kDa) to assemble into even high molecular weight complexes that could no longer enter SDS-PAGE gels [82, 83].

Levels of VDAC1 oligomerization and apoptosis are highly correlated [6, 25]. Recently [60], we demonstrated that several known anion transport inhibitors, including 4,4 diisothiocyanostilbene-2,2-disulfonic acid (DIDS), 4-acetamido-4′-isothiocyanato-stilbene-2,2′-disulfonic acid (SITS), 4,4' diisothiocyanatodihydrostilbene-2,2'-disulfonic acid (H2DIDS), 4,4'-dinitrostilbene-2,2'-disulfonic acid (DNDS), and diphenylamine-2-carboxylate (DPC) (Fig. **2**), all interact with VDAC1, and inhibit release of pro-apoptotic proteins from the mitochondria, leading to apoptosis and VDAC1 oligomerization. Although these reagents are not VDAC1-specific, they directly interact with purified VDAC1, as revealed using microscale thermophoresis or VDAC1 reconstituted into a planar lipid bilayer, to show how the single channel conductance of the protein is reduced [60]. Cyathin-R was found to induce VDAC1 oligomerization and apoptosis in Bax/Bak-deficient cells, with no apoptosis being induced by Cyathin-R in cells lacking VDAC1, suggesting that this compound acts as a VDAC1-dependent apoptosis inducer [62]. Cyathin-R directly interacted with purified VDAC1 and reduced its channel conductance. However, as VDAC1 levels increased several-fold upon cell treatment with cyathin-R, as compared with other mitochondrial proteins, such as cyclophilin D, we proposed that as for other apoptosis inducers, the increase in VDAC1 levels shifts the equilibrium towards oligomerization. Furthermore, both cyathin-R-induced VDAC1 oligomerization and apoptosis were inhibited by VDAC1-interacting molecules, such as DIDS, SITS, DNDS, and DPC [62].

Finally, the newly developed compounds AKOS-022 and VBIT-4 were found to directly interact with VDAC1 and inhibit VDAC1 oligomerization, along with inhibiting apoptosis as induced by various stimuli and in a variety of cancer cell lines [61]. The compounds also eliminated dissipation of the mitochondrial membrane potential and thus cell energy and metabolism, decreasing ROS production, as well as preventing disruption of intracellular Ca^{2+} levels, all apoptosis-associated mitochondria events.

The use of these apoptosis inhibitors supports the tight coupling between oligomerization of VDAC1 and induction of apoptosis. Inhibiting apoptosis at an early stage, *via* preventing VDAC1 oligomerization, may be an effective approach to prevent or reduce apoptosis in neurodegenerative disorders [84, 85] and various cardiovascular diseases, where enhanced apoptosis also occurs [86-88].

4.2.2. VDAC1 Expression Levels and Apoptosis Induction

Numerous studies [78, 89] involving silencing or over-expression of VDAC1 provide evidence suggesting that the expression level of VDAC1 controls mitochondria-mediated cell death. Over-expression of VDAC1 induces apoptosis in all tested cell type [49, 53-56]. It was further shown that the over-expression of VDAC1 is linked with VDAC1 oligomerization, changing the equilibrium to the oligomeric state, forming the channel for release of pro-apoptotic proteins, leading to apoptosis [18, 30, 78]. Moreover, it was demonstrated that VDAC1 over-expression inducing cell death is inhibited by apoptosis inhibitors, such as RuR [53, 90], DIDS or DPC [60], or upon over-expression of HK, Bcl2 or Bcl-xL [31, 49, 53, 56], molecules that directly interact with VDAC1.

Several studies [8, 72, 78] demonstrated that following apoptosis induction by pro-apoptotic drugs or conditions, VDAC1 expression levels were highly increased. Apoptosis induction by H_2O_2, etoposide, cisplatin, selenite and UV irradiation [8, 72, 78] all led to enhanced VDAC1 expression levels, which was accompanied by VDAC1 oligomerization, Cyto *c* release and apoptosis. Reactive oxygen species (ROS) were also found to induce up-regulation of VDAC1 expression levels in a manner that could be prevented by adding a ROS scavenger [91].

Elevation of both intracellular calcium ($[Ca^{2+}]i$) and VDAC1 expression levels were induced by apoptosis inducers. Direct elevation of $[Ca^{2+}]i$ by the Ca^{2+}ionphores A23187 and ionomycin and by thapsigargin, inhibiting the SERCA Ca^{2+} pump, (Fig. **2**) also led to VDAC1 over-expression, VDAC1 oligomerization and apoptosis [72, 78]. On the other hand, decreas-

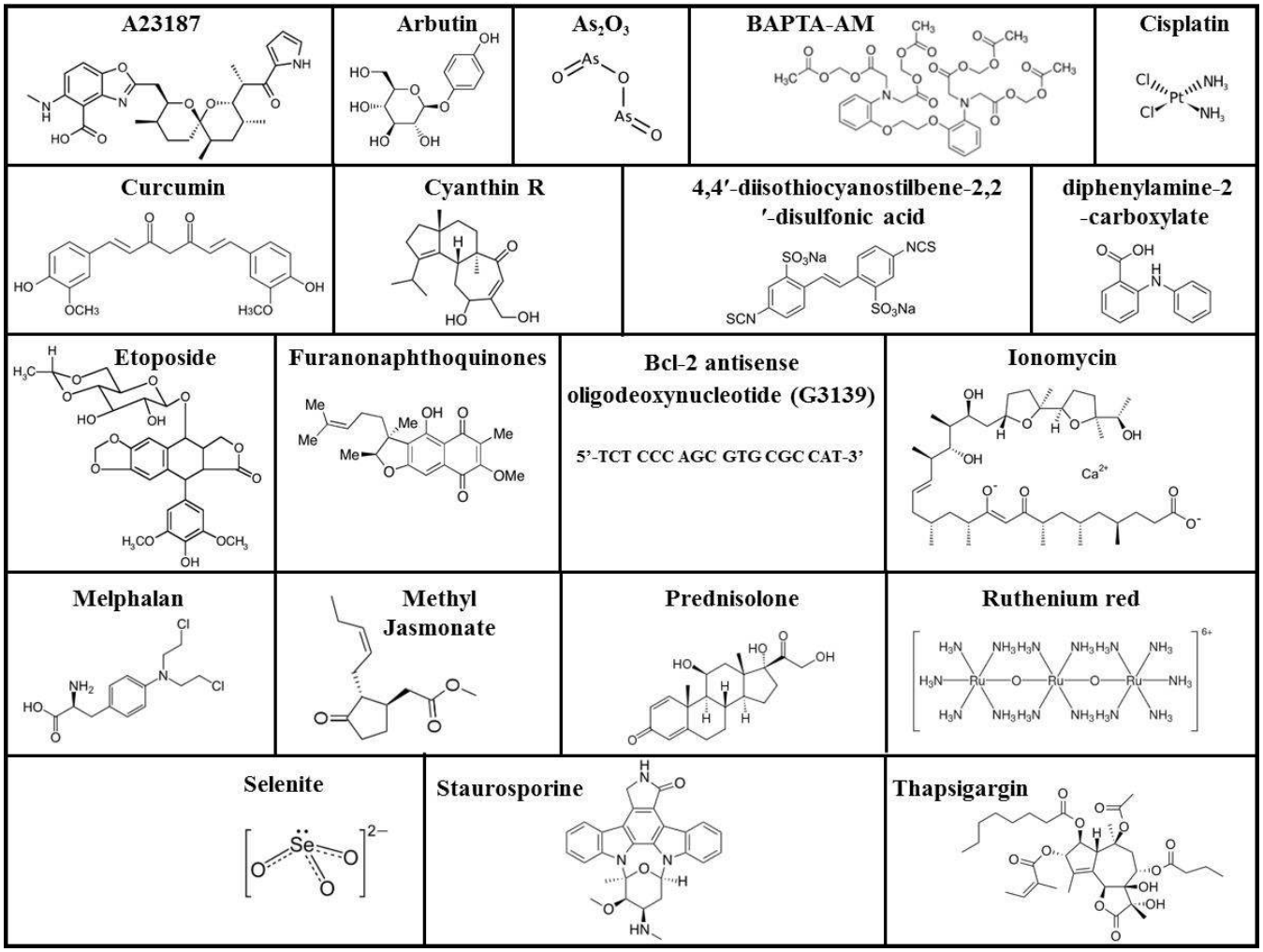

Fig. (2). Structural formulae of compounds discussed in this review.

ing $[Ca^{2+}]i$ using BAPTA-AM, a cell-permeable Ca^{2+}-chelating reagent, inhibited VDAC1 over-expression, VDAC1 oligomerization and apoptosis. Thus, the increase in cellular Ca^{2+} levels induced by apoptosis stimuli was found to be a pre-requisite for induction of VDAC1 over-expression and apoptosis [72, 78].

Together, these findings suggest that apoptosis, as induced by chemotherapy drugs and treatments, increases VDAC1 expression levels and that this may reflect the common mode of action of these agents (Fig. **3**).

4.2.3. A VDAC1 Expression Levels and Drug Sensitivity Link: Pro-Apoptotic-Agents Up-Regulate VDAC1 Expression Levels

Various studies have demonstrated the relationship between VDAC1 expression levels and the sensitivity of cells to chemotherapy drugs. For instance, increased VDAC1 expression levels were observed in three different acute lymphoblastic leukemia (ALL) cell lines following prednisolone treatment [92]. Upon treatment with cisplatin, up-regulation of VDAC1 expression levels was observed in a cisplatin-sensitive cervix squamous cell carcinoma cell line (A431), while in a cisplatin-resistant cell line (A431/Pt), it resulted in down-regulation of VDAC1 levels [93].

Several pro-apoptotic-agents were found to increase VDAC1 expression levels. For example, in human melanoma cells, arbutin ((hydroquinone-O-beta-D-glucopyranoside), a tyrosinase inhibitor, was found to induce apoptosis *via* enhancing VDAC1 expression levels [94, 95]. Somatostatin, induced increases in VDAC1 and VDAC2 expression levels in the LNCaP prostate cancer cell line [96, 97].

Several studies demonstrated the relationship between VDAC1 expression levels and sensitivity to various treatments [78]. Oblimersen sodium (G3139)-mediated induction of Cyto *c* release was found to be correlated with VDAC1 expression levels [98]. The PC3 and DU145 prostate cancer cell lines expressing

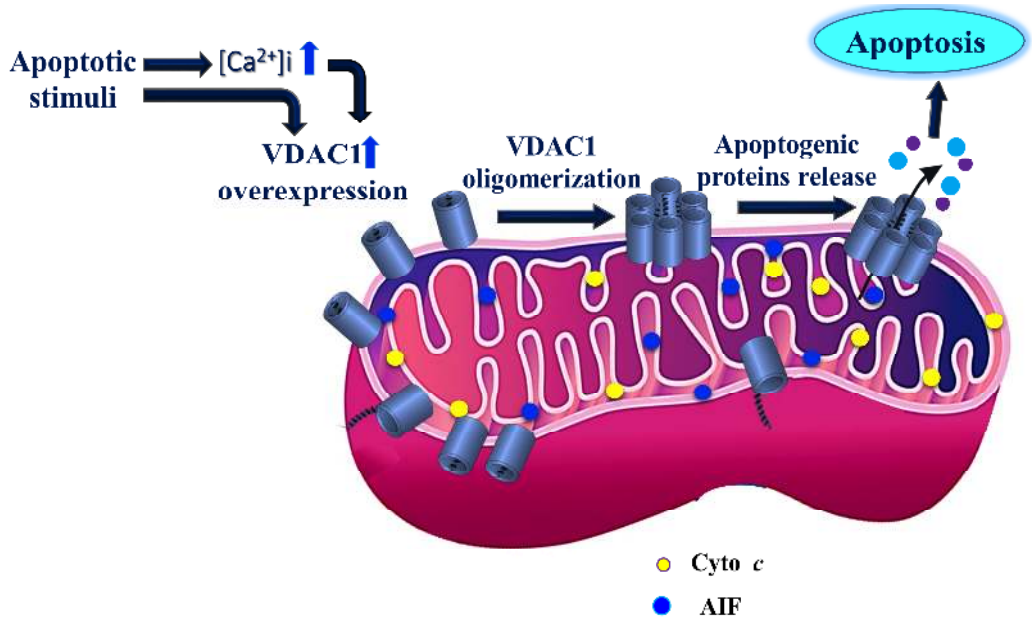

Fig. (3). Proposed model for apoptosis induction coupled to VDAC1 over-expression and oligomerization.
The model proposes that apoptosis, as induced by various agents and conditions, is associated with enhanced VDAC1 expression levels involving increases in $[Ca^{2+}]i$ levels or transcription factors leading to activation of the VDAC1 promoter. The increase in VDAC1 expression shifts the equilibrium towards the VDAC1 oligomeric state, with sequence of events as described in Fig. **1**.

low amounts of VDAC1 were relatively resistant to G3139-induced apoptosis, while LNCaP cells expressing high levels of VDAC1 underwent apoptosis in response to G3139 treatment. G3139 directly interacts with VDAC, reducing channel conductance of the bilayer-reconstituted protein by stabilizing the low conducting state of the channel [98].

Similarly, silencing VDAC1 expression by specific siRNA inhibited cisplatin-induced apoptosis and Bax activation in non-small lung cancer cells (NSCLC) [51]. The anti-cancer activity of furanonaphthoquinones (FNQs) was increased when VDAC1 expression levels were increased but decreased upon reducing VDAC1 expression levels using specific siRNA. In addition, cisplatin, mechlorethamine, and its derivative, melphalan, were found to induce apoptosis, as well as over-expression of VDAC1-sensitized cervical and colon carcinoma cells [99].

Thus, cellular sensitivity to apoptosis is related to VDAC1 expression levels. Moreover, we suggest here that the mode of action for apoptotic stimulus involves up-regulation of VDAC1 expression [78].

4.2.4. VDAC1 as a Target To Modulate Apoptosis

Due to the central role of VDAC1 in cell life and death, and its over-expression in several diseases, including cancer, Alzheimer's disease (AD), cardiovascular diseases (CVDs) and type 2 diabetes (T2D) [8, 45, 100-104], VDAC1 can be considered as an innovative target for controlling dysregulated cell metabolism and apoptosis associated with such diseases. Indeed, various approches, such as down-regulating VDAC1 expression levels by siRNA or VDAC1-based peptides and small molecules, can be considered in this capacity [8]. Another approach involving modulating VDAC1 oligomerization and thereby apoptosis using small molecules has recently been developed [60-62]. Screening for compounds that can stimulate or inhibit apoptosis was carried out using VDAC1-based BRET2 technology developed by our group [25]. Accordingly, enhancing VDAC1 oligomerization and thereby apoptosis can be used for fighting cancer, while inhibtion of VDAC1 oligomerization was associated with apoptosis in neurodegenerative disease therapy (Fig. **4**). We have recently developed new VDAC1-specific apoptosis inhibitors, AKOS-22 and VBIT-4, that inhibit apoptosis and the associated increase in ROS and $[Ca^{2+}]i$ *via* interacting with and inhibiting VDAC1 oligomerization. The VDAC1 oligomerization-based strategy, targeting specific event in mitochondria-mediated apoptosis, is highly selective to cells over-expressing VDAC1, as in cancer [8, 45] and AD [100, 101, 104]. Finally, *in vivo* study demonstrated that enhancing VDAC1 oligomerization by Cyathin-R resulted in tumor growth inhibition [62].

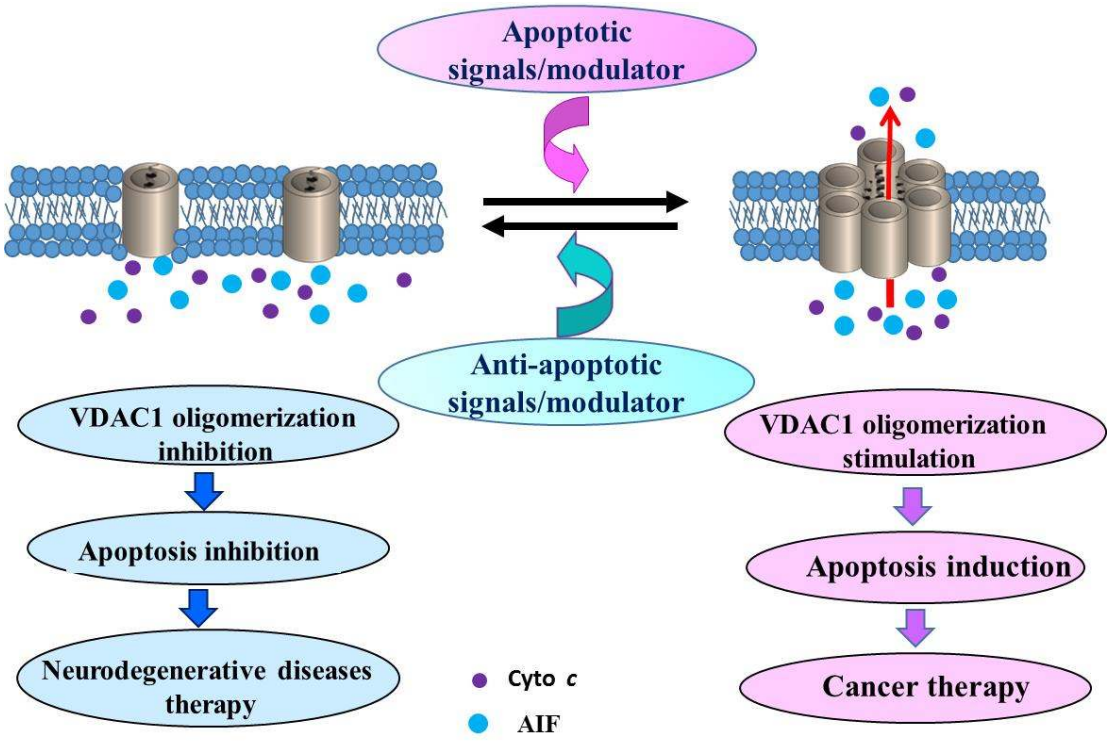

Fig. (4). Modulation of VDAC1 oligomerization as a strategy to control apoptosis in cancer and neurodegenerative disease.

Controlling VDAC1 oligomerization, and hence apoptosis, offers a VDAC1-based innovative conceptual framework as new therapeutic paradigms for several diseases, with expected impact in the treatment of cancer and neurodegeneration in which VDAC1 is over-expressed, where in cancer there is resistance to apoptosis yet in neurodegenerative diseases, apoptotic cell death is activated.

Thus, modulating VDAC1 oligomerization, a common feature of mitochondria-mediated apoptosis, would be activated in cells over-expressing VDAC1, such as in cancer, and is expected to transpire even in tumors resistant to chemotherapy.

CONCLUDING REMARKS

The results summarized and discussed here and depicted in (Figs. **3,4**) support the notion of VDAC1 involvement in apoptosis. Cellular expression levels of VDAC1 are important elements in apoptosis induction and sensitivity to drugs and point to a role for VDAC1 in apoptosis. These observations, along with the link between the efficacy of a drug and the expression level of VDAC1, all indicate that various anti-cancer drugs and treatments act *via* regulating VDAC1 expression levels and the subsequent VDAC1 oligomerization and Cyto *c* release that lead to apoptosis. The enhanced VDAC1 over-expression observed in response to various apoptosis inducers offers support for a new mechanism behind the mode of action of pro-apoptotic drugs, namely enhancement of VDAC1 expression, leading to VDAC1 oligomerization and apoptosis. Still, it should be noted that different cell death mechanisms might operate in a sequential or parallel manner, in the same cell or in different cells, depending on the apoptosis-inducing signal. Further study of the novel mechanism proposed (Fig. **3**) may thus provide a new therapeutic strategy for treating different diseases associated with inhibited apoptosis, such as cancer, or enhanced apoptosis, such as neurodegenerative diseases, pointing to VDAC1-based strategies as new therapeutic paradigms for several diseases.

ABBREVIATIONS

AIF	=	apoptosis-inducing factor
arbutin	=	hydroquinone-O-beta-D-glucopyranoside
Bak	=	Bcl2 homologous antagonist/killer
BAPTA-AM	=	1,2-Bis(2-Aminophenoxy)ethane-N,N,N,N-tetra acetic acid-tetra (actoxymethyl ester)
Bax	=	Bcl2-associated X protein

Bcl2	=	B-cell lymphoma 2
Bcl-xL	=	B-cell lymphoma-extra-large
BRET2	=	bioluminescence resonance energy transfer
CK	=	creatine kinase
Cyto *c*	=	cytochrome c
DIDS	=	4,4-diisothiocyanostilbene-2,2-disulfonic acid
DMSO	=	dimethyl sulfoxide
DNDS	=	4,4-dinitrostilbene-2,2-disulfonic acid
DPC	=	diphenylamine-2-carboxylate
H2DIDS	=	4,4'-diisothiocyanatodihydrostilbene-2,2'-disulfonic acid
EGS	=	ethylene glycol bis [succinimidylsuccinate]
FNQs	=	furanonaphthoquinones
FRET	=	fluorescence resonance energy transfer
G3139	=	oblimersen sodium
HK	=	hexokinase
NSCLC	=	non-small lung cancer cells
OMM	=	outer mitochondrial membrane
PLB	=	planar lipid bilayer
ROS	=	reactive oxygen species
RuR	=	Ruthenium red
SITS	=	4-acetamido-4′-isothiocyanato-stilbene-2,2′-disulfonic acid
SMAC/Diablo	=	second mitochondria-derived activator of caspases
siRNA	=	small interfering RNA
STS	=	staurosporine
TNF-α	=	tumor necrosis factors alpha
UV	=	ultraviolet
VBIT	=	VDAC-based inhibitor therapy
VDAC	=	voltage-dependent anion channel.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGMENTS

This research was supported by a grant (307/13) from the Israel Science Foundation. Support from Phil and Sima Needleman is highly acknowledged.

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PMID: 28618997