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The Mitochondrial Voltage-Dependent Anion Channel 1, Ca^{2+} Transport, Apoptosis, and Their Regulation

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In the outer mitochondrial membrane, the voltage-dependent anion channel 1 (VDAC1) functions in cellular Ca^{2+} homeostasis by mediating the transport of Ca^{2+} in and out of mitochondria. VDAC1 is highly Ca^{2+}-permeable and modulates Ca^{2+} access to the mitochondrial intermembrane space. Intramitochondrial Ca^{2+} controls energy metabolism by enhancing the rate of NADH production *via* modulating critical enzymes in the tricarboxylic acid cycle and fatty acid oxidation. Mitochondrial $[Ca^{2+}]$ is regarded as an important determinant of cell sensitivity to apoptotic stimuli and was proposed to act as a "priming signal," sensitizing the organelle and promoting the release of pro-apoptotic proteins. However, the precise mechanism by which intracellular Ca^{2+} ($[Ca^{2+}]_i$) mediates apoptosis is not known. Here, we review the roles of VDAC1 in mitochondrial Ca^{2+} homeostasis and in apoptosis. Accumulated evidence shows that apoptosis-inducing agents act by increasing $[Ca^{2+}]_i$ and that this, in turn, augments VDAC1 expression levels. Thus, a new concept of how increased $[Ca^{2+}]_i$ activates apoptosis is postulated. Specifically, increased $[Ca^{2+}]_i$ enhances VDAC1 expression levels, followed by VDAC1 oligomerization, cytochrome *c* release, and subsequently apoptosis. Evidence supporting this new model suggesting that upregulation of VDAC1 expression constitutes a major mechanism by which apoptotic stimuli induce apoptosis with VDAC1 oligomerization being a molecular focal point in apoptosis regulation is presented. A new proposed mechanism of pro-apoptotic drug action, namely Ca^{2+}-dependent enhancement of VDAC1 expression, provides a platform for developing a new class of anticancer drugs modulating VDAC1 levels *via* the promoter and for overcoming the resistance of cancer cells to chemotherapy.

Keywords: apoptosis, Ca^{2+} transporters, mitochondria, oligomerization, voltage-dependent anion channel

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OVERVIEW

Intracellular Ca^{2+}concentration ($[Ca^{2+}]_i$) regulates a number of cellular and intercellular events, such as the cell cycle, proliferation, gene transcription, and cell death pathways, as well as processes like muscle contractility and neuronal processing and transmission (1). The alteration of Ca^{2+} homeostasis is closely related with various cancer hallmarks, including proliferation, migration, angiogenesis, invasion abilities, and resistance to cell death (2).

Various systems and mechanisms have evolved to control and respond to minute changes in Ca^{2+} concentrations and localization (3). Moreover, many cellular compartments participate in the Ca^{2+} signaling network regulation. $[Ca^{2+}]_i$ is controlled *via* its transport in and out of the cell or/and in and out of intracellular organelles. Within a given compartment, $[Ca^{2+}]$ can be buffered by binding to specific proteins and other molecules, as well as existing in its free form, albeit differentially across compartments (1). The major organelles that participate in controlling Ca^{2+} dynamics include the endoplasmic reticulum (ER) and mitochondria (4). Imbalance in the control of $[Ca^{2+}]_i$ can lead to mitochondria Ca^{2+} overload and ultimately, to toxic effects. Tumor cells exhibit a well-developed capacity for modulating cytosolic Ca^{2+} levels by remodeling the cellular machinery that participates in processes that determine Ca^{2+} dynamics and homeostasis, as well as changes in sensitivity to the induction of cell death. This review is focused on the mitochondrial gatekeeper protein voltage-dependent anion channel 1 (VDAC1) and its role in Ca^{2+} transport and on Ca^{2+}-mediated apoptosis involving regulation of VDAC1 expression levels.

Ca^{2+} AND MITOCHONDRIA

Mitochondria not only play a key role in metabolism but also serve as a major hub for cellular Ca^{2+} homeostasis, regulating oxidative phosphorylation (OXPHOS) (4–7) and modulating cytosolic Ca^{2+} signals (8, 9), cell death (10), and secretion (11, 12). Enclosed by two different membranes, namely the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM), mitochondria thus present two aqueous compartments, the intermembrane space (IMS) and the matrix (M). To reach the matrix, Ca^{2+} must cross both the OMM and the IMM. Indeed, the mitochondrial matrix is one of the major cellular Ca^{2+} stores or buffers and is used to control $[Ca^{2+}]_i$ and dynamics. Within the mitochondrial matrix, Ca^{2+} is precipitated as insoluble $CaPO_4$, which exists in equilibrium with free Ca^{2+} (7, 13).

It is well established that mitochondria can rapidly sequester large and sudden increases in $[Ca^{2+}]_i$ at the expense of the membrane potential across the IMM that is generated by the electron transport chain (6). Intramitochondrial Ca^{2+} controls energy metabolism by enhancing the rate of NADH production *via* modulating critical enzymes, such as those of the tricarboxylic acid (TCA) cycle and fatty acid oxidation (14, 15), linking glycolysis to the TCA cycle (16). Indeed, matrix Ca^{2+} is an essential cofactor for several rate-limiting TCA enzymes, namely pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase.

Mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) homeostasis is important not only for energy production but also for regulating $[Ca^{2+}]_i$ and activating cell apoptotic pathways (10, 17). Several recent reviews have discussed the basic principles that govern $[Ca^{2+}]_m$ homeostasis and maintenance of Ca^{2+} dynamics within organelles (18–22). The OMM and IMM pathways allowing Ca^{2+} entry into and exit from mitochondria are presented below.

PATHWAYS MEDIATING Ca^{2+} FLUXES IN THE MITOCHONDRIA

Several mitochondria membrane proteins play central roles in Ca^{2+} signaling and/or Ca^{2+} influx and efflux in normal and disease conditions. Ca^{2+} transport across the IMM is mediated *via* several proteins, including the mitochondrial Ca^{2+} uniporter (MCU) (23, 24) and the Na^+/Ca^{2+} exchanger, NCLX, the major Ca^{2+} efflux mediator (25, 26). In the OMM, VDAC1 was shown to control Ca^{2+} permeability (27–30).

VDAC1, the Ca^{2+} Channel in the OMM

Three different isoforms of VDAC have been identified, VDAC1, VDAC2, and VDAC3. VDAC1 has been best studied, whereas only limited information regarding the cellular functions of VDAC2 and VDAC3 is available (31). Thus, we focus here on VDAC1.

VDAC1, a Multifunctional Channel, Controls Cell Metabolism

VDAC1 at the OMM controls metabolic cross talk between mitochondria and rest of the cell by allowing the entry of metabolites (pyruvate, malate, succinate, nucleotides, and NADH) into the mitochondria and the exit of newly formed molecules, such as hemes (32, 33) (**Figure 1**). VDAC1 is also involved in cholesterol transport and mediates the fluxes of ions, including Ca^{2+} (34), serves as a ROS transporter, and contributes to regulating the redox states of mitochondria and the cytosol (32–34). Moreover, VDAC1 at the OMM interacts with proteins that mediate and regulate the integration of mitochondrial functions with other cellular activities. VDAC1 forms a complex with adenine nucleotide translocase (ANT) and creatine kinase (35). The interaction of VDAC1 with hexokinase (HK) allows for coupling between OXPHOS and glycolysis, an important factor in cancer cell energy homeostasis (the Warburg effect) (36). Thus, VDAC1 appears to be a convergence point for a variety of cell survival and death signals, mediated through its association with various ligands and proteins.

VDAC1 As Ca^{2+} Transporter at OMM

Found in the OMM, VDAC1 regulates the transport of Ca^{2+} in and out of the mitochondria. VDAC1 is highly Ca^{2+}-permeable and modulates the accessibility of Ca^{2+} to the IMS (27–30) (**Figure 1**). Bilayer-reconstituted VDAC1 under voltage-clamp conditions and in the presence of different $CaCl_2$ concentration gradients showed well-defined voltage-dependent channel conductance as observed with either NaCl or KCl solution (27, 29). Bilayer-reconstituted VDAC1 showed higher permeability to Ca^{2+} in the low conductance state (29). The Ca^{2+} permeability of VDAC1 has also been established upon VDAC1 reconstitution into liposomes (27).

Various studies support the function of VDAC1 in the transport of Ca^{2+} and in cellular Ca^{2+} homeostasis. VDAC1 overexpression increases $[Ca^{2+}]_m$ concentration in HeLa cells and skeletal myotubes (37), and silencing of VDAC1 expression by

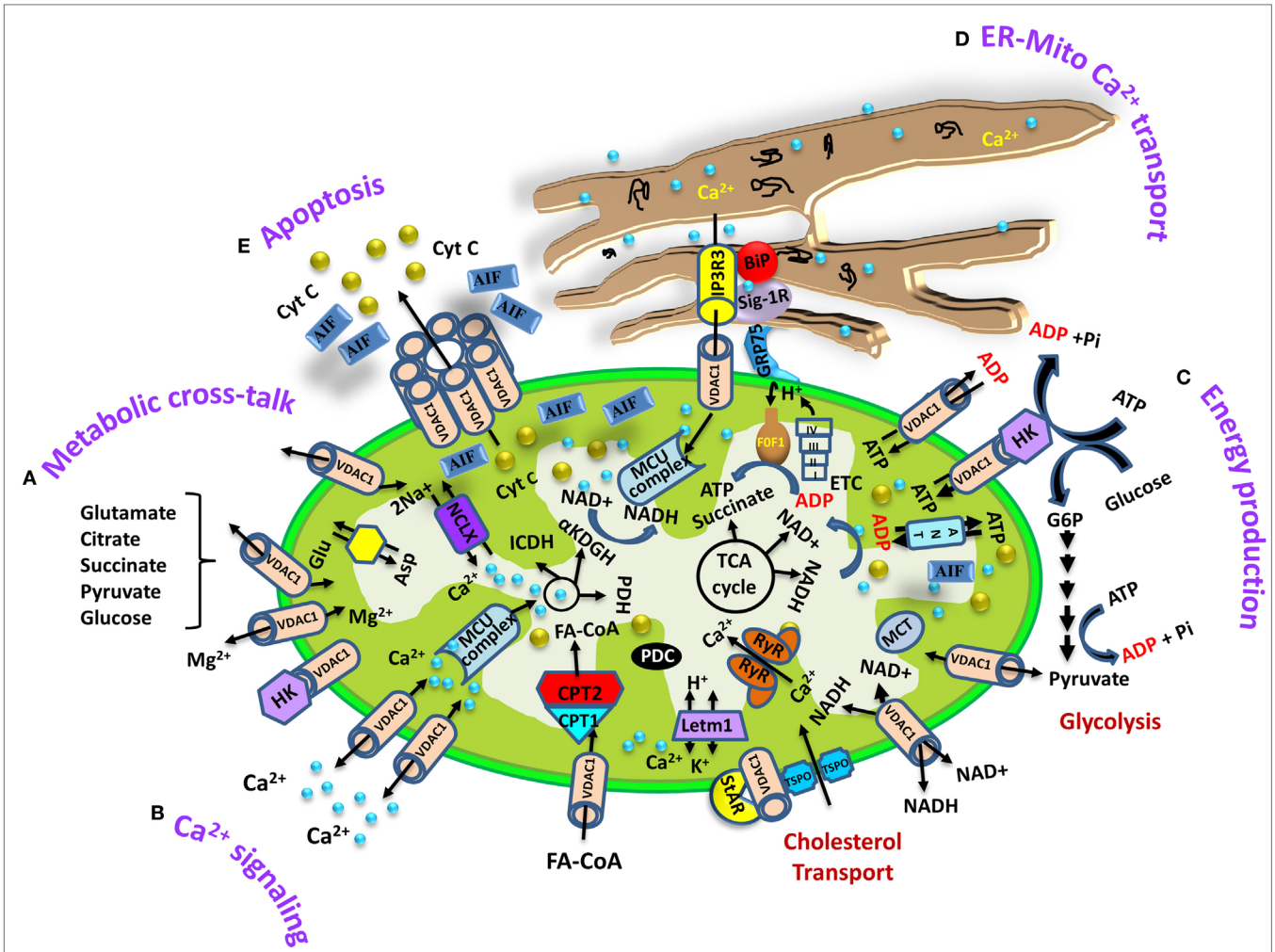

FIGURE 1 | Schematic representation of voltage-dependent anion channel 1 (VDAC1) as a multifunctional protein involved in Ca^{2+} and metabolite transport, energy production, and the structural and functional association of mitochondria with the endoplasmic reticulum (ER). The various functions of VDAC1 in cell and mitochondria functions are presented. These include **(A)** Ca^{2+} signaling by transporting Ca^{2+}; **(B)** control of metabolic cross talk between the mitochondria and the rest of the cell; **(C)** mediating cellular energy production by transporting ATP/ADP, NADH, and acyl-CoA from the cytosol to the intermembrane space and regulating glycolysis *via* the association with hexokinase (HK); **(D)** involvement in structural and functional association with the ER, mediating Ca^{2+} transport from the ER to mitochondria; **(E)** participation in apoptosis *via* its oligomerization to form a protein-conducting channel within a VDAC1 homo-oligomer, allowing Cyto *c* release and apoptotic cell death. Ca^{2+} influx and efflux transport systems in the outer mitochondrial membrane (OMM) and IMM are shown. In the OMM, VDAC1 is presented as a Ca^{2+} channel and also functions in the transport of Mg^{2+}. In the IMM, Ca^{2+} uptake into the matrix is mediated by a Ca^{2+}-selective transporter, the mitochondrial Ca^{2+} uniporter (MCU), regulated by a calcium-sensing accessory subunit (MCU1). Ryanodine receptor (RyR) in the IMM mediates Ca^{2+} influx. Ca^{2+} efflux is mediated by NCLX, an Na^+/Ca^{2+} exchanger. High levels of matrix Ca^{2+} accumulation trigger the opening of the PTP, a fast Ca^{2+} release channel. The function of Ca^{2+} in regulation of energy production is mediated *via* tricarboxylic acid (TCA) cycle regulation. This includes activation of pyruvate dehydrogenase (PDH), isocitrate dehydrogenase (ICDH), and α-ketoglutarate dehydrogenase (αKGDH) by intramitochondrial Ca^{2+}, leading to enhanced activity of the TCA cycle. The electron transport chain (ETC) and the ATP synthase (F_oF_1) are also presented. Molecular fluxes are indicated by arrows. VDAC1 mediates the transfer of fatty acid acyl-CoAs across the OMM to the IMS, where they are converted into acylcarnitine by CPT1a for further processing by β-oxidation. VDAC1 is involved in cholesterol transport by being constituent of a multi-protein complex, the transduceosome, containing Star/TSPO/VDAC1. The ER associated with the mitochondria is presented with key proteins indicated. These include the inositol 3 phosphate receptor type 3 (IP3R3), the sigma1 receptor (Sig1R) (a reticular chaperone), binding immunoglobulin protein (BiP), the ER heat shock protein (HSP70) chaperone, and glucose-regulated protein 75 (GRP75). IP3 activates the IP3R in the ER to release Ca^{2+} that is directly transferred to the mitochondrion *via* VDAC1.

siRNA attenuates $[Ca^{2+}]_m$ uptake and cell apoptosis induced by H_2O_2 or ceramide (38). It was also proposed that the magnitude of Ca^{2+} transfer into the mitochondrial matrix is regulated by protein–protein interactions between Bcl-xL and VDAC1 or VDAC3, with this interaction promoting matrix Ca^{2+} accumulation by increasing Ca^{2+} transfer across the OMM (39).

Silencing each of the VDAC isoforms in the presence of a pro-apoptotic stimulus revealed that each was differentially sensitive to H_2O_2, with VDAC1 silencing potentiating H_2O_2-induced apoptosis and impairing $[Ca^{2+}]_m$ loading, while VDAC2 silencing had the opposite effects (38). In addition, several VDAC-interacting molecules like 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid

(DIDS), 4-acetamido-4′-isothiocyanato-stilbene-2,2′-disulfonic acid, and dinitrostilbene-2,2′-disulfonic acid were shown to prevent apoptosis and also inhibit the rise in $[Ca^{2+}]$ levels associated with apoptosis induction (40). In another example, 5-aminolevulinic precluded Ca^{2+}-mediated oxidative stress and apoptosis through VDAC1 inhibition (41).

VDAC1 Possesses Ca^{2+}-Binding Sites

Several lines of evidence suggest that VDAC1 possesses divalent cation-binding site(s). $[Ca^{2+}]$, at micromolar concentrations, switched VDAC1 from a low to high conductance state (30). The trivalent ions La^{3+} and Tb^{3+}, known to bind to Ca^{2+}-binding proteins (42), reduced the channel conductance of bilayer-reconstituted VDAC (43). This and the direct demonstration of Tb^{3+} binding to purified VDAC1, as reflected in an enhanced green fluorescence, further suggest that VDAC1 possesses divalent cation-binding site(s) that its occupation by La^{3+} or Tb^{3+} lead to reduced channel conductance.

Similarly, molecules known to specifically interact with several Ca^{2+}-binding proteins like ruthenium red (RuR) (43) and ruthenium amine binuclear complex (Ru360) (44), as well as a photo-reactive analog, azido ruthenium (AzRu) (45), induced VDAC1 channel closure in a time-dependent manner and stabilized the channel in a low conducting state. These compounds also inhibited apoptosis (43–45).

The putative VDAC1 metal binding site for RuR and AzRu was analyzed by mutation of specific VDAC1 residues (43). It has been demonstrated that E72 and E202 are essential for RuR-mediated reduction of bilayer-reconstituted VDAC1 conductance and for RuR-mediated protection against VDAC1-induced cell death (43, 46). This suggests that these two glutamate residues, located in two different β-strands, may form the VDAC1 Ca^{2+}-binding site(s), or part thereof. However, their distant location and their being located in transmembrane sequences, suggest that these residues may stabilize VDAC1 in a conformation that is recognized by RuR and AzRu. Thus, these Ru-containing molecules may bind to a non-defined site in VDAC1 to induce conformation changes leading to reduced conductance and inhibited apoptosis.

The competition between Ca^{2+} and RuR (43), as well as the demonstration of VDAC gating regulation by physiological levels of Ca^{2+}, whereby Ca^{2+} increases the conductance of the VDAC1 channel (30), supports the physiological function of the VDAC1 Ca^{2+}-binding site(s).

VDAC1 Protein–Protein Interactions Regulate $[Ca^{2+}]_m$ Transport

Interactions between VDAC1 and Bcl-2 family proteins, such as Bax/Bak, Bcl-2, and Bcl-xL, mediating the regulation of apoptosis, are well documented (33, 47–55). It has been shown that interaction of Bcl-xL with VDAC1 or VDAC3 promoted $[Ca^{2+}]_m$ uptake (39). It was also reported that all three VDAC isoforms interact with regulator of microtubule dynamics protein 3 (56), a protein at the OMM involved in $[Ca^{2+}]_i$ homeostasis regulation (57, 58). VDAC1 interacts with endothelial NO synthase (eNOS), with such interaction amplifying eNOS activity in a $[Ca^{2+}]_i$-mediated manner (59). VDAC also interacts with the L-type Ca^{2+} channel, and it was suggested that impaired communication between the L-type Ca^{2+} channel and mitochondrial VDAC contributes to cardiomyopathy (60). Thus, such interactions of VDAC with proteins associated with Ca^{2+} transport or activated by Ca^{2+} point to VDAC as functioning not only in $[Ca^{2+}]_i$ homeostasis but also in many Ca^{2+}-regulated cellular activities.

VDAC1 Function in Mitochondria—ER/Sarcoplasmic Reticulum (SR) Ca^{2+}-Cross Talk

The participation of VDAC1 in supramolecular complexes and intracellular communication, including Ca^{2+} signal delivery between the ER and mitochondria, was postulated over a decade ago (28, 61, 62). The components involved in ER–mitochondria interaction include the IP_3 receptor and grp75 on the ER as tethering components and VDAC1 on the OMM (63). VDAC1 (but not VDAC2 or VDAC3) was found to provide the route for Ca^{2+} entry into mitochondria upon apoptotic stimulus, representing a fundamental factor in mitochondria physiology (38). It was, moreover, proposed that the magnitude of Ca^{2+} transfer from the ER into the mitochondrial matrix is regulated by Bcl-xL (39, 51). ER–mitochondria cross talk regulates not only Ca^{2+} transfer but also different processes, such as mitochondrial fission, autophagy, and inflammation (64). Finally, Ca^{2+} dynamics are greatly enhanced where there is close apposition of the ER with mitochondria, as compared to the bulk cytosol. Such changes in Ca^{2+} signal profiles were modified by ROS, as monitored with genetically encoded redox indicators (65).

MCU and Auxiliary Subunits Form a Selective Ca^{2+} Transporter in the IMM

Ca^{2+} transport across the IMM and into the matrix is mediated *via* the MCU (23, 24), with the driving force being the steep mitochondrial membrane potential (8, 66, 67) (**Figure 1**). Such delivery is inhibited by RuR and its derivative, Ru360 (68).

The major channel-forming subunit of the MCU complex (CCDC109A) consists of two transmembrane and the N-terminal domains and forms a complex in the IMM with many gatekeeper membrane proteins (23, 24, 69–71). The calcium-sensing accessory subunits MICU1, MICU2, and MCUb are proposed to serve as negative regulators, while mitochondrial Ca^{2+} uniporter regulator 1 (MCUR1), essential MCU regulator, and SLC25A23 are essential for MCU activity (72–76). MCUR1 may, however, also play other roles, such as in cytochrome *c* oxidase assembly (77), as a cytosolic Ca^{2+}-buffering agent (78), or in ROS generation (79).

The functional role of MCU under physiological conditions was extensively studied using several silencing techniques (80–85). Interestingly, MCU knockout mice did not exhibit obvious defects in mitochondrial number or morphology and any physiological function (82, 86, 87). MCU deletion was found to be lethal for C57BL/6 mice, whereas knockout mice with an outbred CD1 background were viable, albeit with reduced numbers (88). Basal organ functions were maintained, and impairment was only observed in the physiological adaptation of skeletal muscle to exercise (82). In cardiac-specific conditional MCU-deficient mice, the heart displayed increased resistance to ischemia–reperfusion injury (87, 89).

Na^+/Ca^{2+} Exchanger Function in Ca^{2+} Efflux and Its Regulation

Mitochondrial Ca^{2+} is mainly determined by the balance between influx through the MCU and efflux *via* NCLX (90). To restore resting $[Ca^{2+}]_m$ levels, Ca^{2+} efflux across IMM is mediated by the Na/Ca/Li exchanger (NCLX) or Na^+/Ca^{2+} exchanger (25, 26, 91), and possibly by Letm1, under certain conditions, which functions as a Ca^{2+}/H^+ anti-porter in addition to being a H^+/K^+ anti-porter (see Other Proteins Proposed as Participating in or Mediating Ca^{2+} Efflux from Mitochondria) (92). NCLX mediates efflux of Ca^{2+} from the mitochondrial matrix to the IMS (20, 25, 93–95). In contrast to the plasma membrane Na^+/Ca^{2+} exchanger, NCLX transports Li^+ ions in addition to Na^+ and Ca^{2+} (96). NCLX has also been proposed to regulate Ca^{2+}-induced NAD(P)H production and matrix redox state modulation (97).

Mitochondrial Ca^{2+} regulates heart metabolism, where steady-state $[Ca^{2+}]_m$ is determined by the dynamic balance between MCU-based Ca^{2+} influx and NCLX-based Ca^{2+} efflux (98). It has been proposed that a novel role of NCLX is to regulate the automaticity of cardiomyocytes *via* modulating SR Ca^{2+} handling (99). NCLX has been proposed to be involved in several pathological conditions. In ischemia, NCLX acts as a key regulator of $[Ca^{2+}]_m$ accumulation (100), while in diabetic cardiac myocytes, NCLX is more susceptive to changes in the outside (cytosolic) Na^+ concentration, as compared with controls (101). Phosphorylation of NCLX has been reported to reverse Ca^{2+} mitochondrial overload and promote survival of PINK1-deficient dopaminergic neurons (102).

Other Proteins Proposed as Participating in or Mediating Ca^{2+} Efflux from Mitochondria

The transient opening of the mitochondrial permeability transition pore (MPTP or PTP) represents another mechanism for Ca^{2+} release from mitochondria. However, its function is probably related to non-physiological Ca^{2+} overload that depolarizes mitochondria by an irreversible opening of the PTP, leading to apoptotic and necrotic cell death associated with disease pathogenesis (103, 104). Multiple proteins have been proposed to constitute the PTP and thus to play a role in PTP opening by Ca^{2+} or ROS challenge, such as VDAC1 in the OMM, ANT in the IMM, and cyclophilin D in the matrix (105, 106). However, silencing approaches have only confirmed cyclophilin D as being essential for Ca^{2+}-sensitive PTP opening. One recent view considered parts of the F_oF_1 ATPase as components of the PTP, while other candidates has also emerged [for review, see Ref. (107)]. Recently, SPG7 at the IMM has been proposed as a key component of Ca^{2+}- and ROS-induced PTP opening, forming a complex with VDAC1 at the OMM and cyclophilin D in the matrix (108).

A potential candidate for the $[Ca^{2+}]_m/H^+$ anti-porter was suggested, the leucine zipper-EF-hand-containing transmembrane protein 1 (Letm1) (19, 92, 109–111). Letm1 has two Ca^{2+}-binding EF hand domains and catalyzes the electronic exchange of Ca^{2+} for H^+. Letm1 Ca^{2+} transport activity is pH-sensitive and is inhibited by RuR (111). Letm1 not only imports Ca^{2+} into the matrix through the IMM but can also extrude Ca^{2+} from the matrix when $[Ca^{2+}]_m$ concentration is high (19, 109, 110).

Channels function in Ca^{2+} transport in membranes other than in the mitochondria as ryanodine receptors (RyRs) and the transient receptor potential 3 (TRPC3) channel were reported to function in Ca^{2+} homeostasis [for review, see Ref. (112, 113)]; RyRs, the main Ca^{2+}-release channels in the SR/ER in excitable cells, were reported to be expressed at the IMM and mediate Ca^{2+} uptake in cardiomyocytes (114, 115). Recently, it was demonstrated that neuronal mitochondria express RyR at the IMM and accumulate Ca^{2+} in a manner that can be inhibited by dantrolene or ryanodine (116). Finally, canonical TRPC3 was shown to be located in the IMM and contributing to $[Ca^{2+}]_m$ uptake and thus functions in regulating $[Ca^{2+}]_m$ homeostasis (117).

VDAC1 AT THE NEXUS OF MITOCHONDRIA-MEDIATED APOPTOSIS

Mitochondria-mediated or intrinsic apoptotic pathway is activated *via* the release of mitochondrial pro-apoptotic proteins (e.g., Cyto *c*, AIF, Smac/DIABLO) from the IMS to the cytosol (32, 33, 52, 54, 118–126), leading to the activation of caspases. Some models for the release of apoptotic proteins suggest that release exclusively involves an increase in OMM permeability due to the formation of a channel large enough to allow the passage of apoptogenic proteins (32, 33, 124, 127–129), while others consider release to be due to disruption of OMM integrity (120, 130, 131). Recent studies demonstrated that upon apoptosis induction, VDAC1 is oligomerized to form a large pore, allowing the release of mitochondrial pro-apoptotic proteins (129, 132–139). VDAC1 oligomerization was found to be a general mechanism common to numerous apoptogens acting *via* different initiating cascades (135, 140, 141). Moreover, apoptosis inhibitors (135, 142) and recently identified VDAC1-interacting molecules [diphenylamine-2-carboxylate (DPC)] (40) and a molecule developed in our lab designated as VBIT-4 were found to prevent VDAC1 oligomerization and subsequent apoptosis (143). Furthermore, cyathin-R, a cyathane-type diterpenoid derived from a fungal secondary metabolite library from the medicinal fungus *Cyathus africanus*, was found to interact with purified VDAC1 and reduce its channel activity, as well as induce apoptosis *via* promoting VDAC1 oligomerization and the associated cytochrome *c* release in Bax/Bak-depleted cells but not when VDAC1 was depleted. Cyathin-R-induced apoptosis was inhibited by DPC (142).

VDAC1 also regulates apoptosis *via* the direct interaction with the anti-apoptotic protein HK (144–152), with apoptosis-regulating proteins, such as Bcl-2, Bcl-xL (33, 47, 48, 50, 144, 153–155), and with the pro-apoptotic proteins Bax and Bak (156).

Ca^{2+}-Induced Apoptosis through VDAC1 Overexpression

Apoptosis induction affects cell Ca^{2+} homeostasis and energy production (157). The intrinsic apoptotic pathway, initiated

in response to various stimuli, including high $[Ca^{2+}]_i$, oxygen radicals, activation of pro-apoptotic Bcl-2 family proteins, UV damage, and various anticancer drugs and cytotoxic agents, such as thapsigargin, staurosporine, As_2O_3, and selenite, disrupts cellular Ca^{2+} homeostasis and induces apoptosis (140). Indeed, the contribution of Ca^{2+} signals to cell death is well documented, and a few mechanisms that connect apoptotic stimuli, *via* a rise in $[Ca^{2+}]_i$, to cell death have been suggested (158–164).

Recently, it was demonstrated that a panel of apoptotic inducers, such as UV irradiation, H_2O_2, etoposide, cisplatin, or selenite, elevated $[Ca^{2+}]_i$ and upregulated VDAC1 expression levels in a Ca^{2+}-dependent manner (**Table 1**), resulting in VDAC1 oligomerization, Cyto *c* release, and subsequent apoptosis (140, 141) (**Figure 2**). Furthermore, direct elevation of $[Ca^{2+}]_i$ by the Ca^{2+}-mobilizing agents A23187, ionomycin, or thapsigargin led to VDAC1 overexpression, VDAC1 oligomerization, and apoptosis, while decreasing $[Ca^{2+}]_i$ using the cell-permeable Ca^{2+}-chelating reagent BAPTA-AM inhibited these events (141).

It has also been shown that the sensitivity of the CD45-positive ($CD45^+$) U266 myeloma cell line to various apoptotic stimuli is well correlated with the elevated levels of VDAC1 expression that follow Ca^{2+} signals in response to apoptosis stimulation (169, 175). This suggests that apoptosis-inducing agents act by increasing $[Ca^{2+}]_i$ and that this, in turn, leads to an upregulation of VDAC1 expression, which is connected to apoptosis induction (**Table 1**). The proposed sequence of events leading to VDAC1-mediated apoptosis can be schematically depicted as:

Support for this model comes with the findings that several VDAC-interacting molecules prevent its oligomerization, the elevation in $[Ca^{2+}]_i$ associated with apoptosis induction, Cyto *c* release, and apoptosis (40, 41, 141, 151, 165, 166, 170, 171, 173, 176–178). DIDS was shown to prevent the apoptosis stimuli-inducing increase in $[Ca^{2+}]_i$ levels (40) and Ca^{2+}-mediated oxidative stress and apoptosis, as induced by 5-aminolevulinic (41). The small molecules AKOS-022 and VBIT-4 that bind to VDAC1 prevent its oligomerization, the elevation $[Ca^{2+}]_i$ associated with apoptosis induction, Cyto *c* release, and apoptosis (166). Furthermore, mitochondria-mediated apoptosis was correlated with VDAC1 expression levels (141, 165–175). Thus, although different apoptosis inducers elicit cell death *via* different mechanisms, all induce VDAC1 overexpression in a Ca^{2+}-dependent manner, raising the possibility that elevating $[Ca^{2+}]_i$ represents a common mechanism for various apoptosis stimuli, subsequently leading to an elevation in VDAC1 expression. We, therefore, suggest that the upregulation of VDAC1 expression constitutes a major mechanism by which apoptosis inducers lead to apoptosis (**Figure 2**).

TABLE 1 | Anticancer, pro-apoptotic drugs, and chemical agents that increase voltage-dependent anion channel 1 expression level in cancer cells.

Drugs or chemical agent	Cancer cell type	Reference
Prednisolone—synthetic glucocorticoid, a derivative of cortisol, used to treat a variety of inflammatory and autoimmune conditions and some cancers	Acute lymphoblastic leukemia cell lines, REH, 697, Sup-B15, and RS4;11	(165)
Cisplatin—a chemotherapy drug, the first member of a class of platinum-containing anticancer drugs	Cervix squamous cell carcinoma line (A431), human cervical adenocarcinoma (HeLa), non-small human lung carcinoma (A549), and human ovarian carcinoma (SKOV3)	(141, 166)
Mechlorethamine and its derivative, melphalan—DNA cross-linking agents, a group of anticancer chemotherapeutic drugs	Human cervical adenocarcinoma (HeLa)	(167)
ROS—reactive oxygen species (H_2O_2 and sodium nitroprusside)	Human cervical adenocarcinoma (HeLa), non-small human lung carcinoma (A549), human ovarian carcinoma (SKOV3), and rat PC12 cells	(141, 168)
UV irradiation	B cell mouse lymphoma (LYas)	(169)
Arbutin—(hydroquinone-*O*-β-D-glucopyranoside), tyrosinase inhibitor, and potential anticancer agent, extracted from the bearberry plant	Human malignant melanoma cells (A375)	(170, 171)
Orf3—hepatitis E virus protein	Hepatoma cells	(172)
Somatostatin—a peptide hormone	Human prostate cancer cell line (LNCaP)	(173)
Endostatin—20-kDa C-terminal fragment derived from type XVIII collage	Human microvascular endothelial cells	(126)
Selenite—inorganic compound	Human cervix carcinoma (HeLa) cells	(141, 174)
Thapsigargin—non-competitive inhibitor of the sarco/endoplasmic reticulum Ca^{2+}-ATPase, extracted from the plant *Thapsia garganica*	U266 myeloma cells and human cervical adenocarcinoma (HeLa) cells	(141, 175)
Etoposide—topoisomerase inhibitor, cytotoxic anticancer drug	Human cervical adenocarcinoma (HeLa), non-small human lung carcinoma (A549), and human ovarian carcinoma (SKOV3)	(141)
Arsenic trioxide $\left(As_2O_3^-\right)$—inorganic compound	Human cervical adenocarcinoma (HeLa), non-small human lung carcinoma (A549), and human ovarian carcinoma (SKOV3)	(141)

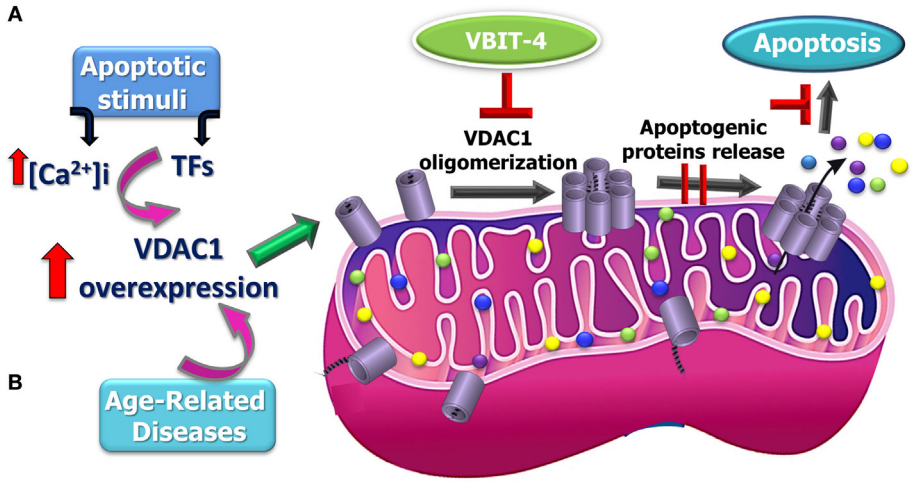

FIGURE 2 | Proposed model for apoptosis stimuli-induced increase in voltage-dependent anion channel 1 (VDAC1) expression levels leading to VDAC1 oligomerization, Cyto *c* release, and apoptosis and possible inhibition steps. A schematic model describing the novel pathway proposed for apoptosis induction involving elevation of $[Ca^{2+}]_i$ leading to VDAC1 overexpression. **(A)** This facilitates VDAC1 oligomerization to form a large channel mediating cytochrome *c* release from the mitochondrion into the cytosol, resulting in apoptosis activation. It is proposed that the overexpression of VDAC1 in diseases such as Alzheimer's disease, cardiovascular diseases, and diabetes is associated mitochondrial dysfunction, including apoptosis induction **(B)**.

Although many studies in various experimental systems have demonstrated increased VDAC1 expression levels following apoptosis stimulation (**Table 1**), only a few have linked VDAC1 overexpression to the rise in $[Ca^{2+}]_i$ following apoptosis induction. Indeed, the expression level of VDAC1 has been shown to be a crucial factor in the process of mitochondria-mediated apoptosis (141, 165–175). Moreover, exogenous VDAC1 expression leads to apoptosis in the absence of any apoptotic stimulus (32, 34, 137, 141, 144, 151, 165, 179). There are several potential Ca^{2+}-dependent steps that could contribute to the process of gene expression and a few, such as mRNA transcription, elongation, splicing, stability, and translation, have been suggested as being regulated by Ca^{2+} (180, 181).

This new mode of action for apoptosis stimulus involving increased expression of VDAC1 leading to dynamic VDAC1 oligomerization, release of Cyto *c*, and apoptosis provides a platform for developing a new class of anticancer drugs modulating VDAC1 expression *via* its promoter.

VDAC1 AND Ca^{2+} IN CANCER AND OTHER DISEASES

Various cancer hallmarks, such as proliferation, migration, angiogenesis, invasion abilities, and resistance to cell death, are associated with alterations of Ca^{2+} homeostasis (2). As a transporter of metabolites and Ca^{2+}, VDAC1 contributes to the metabolic phenotype of cancer cells, possibly as reflected in its overexpression in many cancer types (182, 183). Moreover, its downregulation resulted in reduced metabolite exchanges between mitochondria and cytosol and inhibited cell and tumor growth (122, 176, 182, 184, 185).

Tumor cells exhibit a well-developed capacity for modulating $[Ca^{2+}]_i$ levels by remodeling the cellular machinery that participates in processes that determine Ca^{2+} dynamics and homeostasis, as well as changes in sensitivity to cell death induction (186). It was recently demonstrated that the basal $[Ca^{2+}]_m$ uptake *via* the ER–mitochondria junction is essential for tumorigenic cell viability, and that inhibition of this pathway in cancer cells might be used as a therapeutic approach (187). Moreover, some cancer cells are addicted to such constitutive Ca^{2+} transfer to sustain their mitochondrial metabolism, particularly nucleoside production (188). Thus, the increase in VDAC1 levels in cancer (182, 183) also contributes to this enhanced transport of Ca^{2+}.

In diabetic mouse coronary vascular endothelial cells (MCECs), VDAC levels were increased, as were $[Ca^{2+}]_m$, mitochondrial O_2 production, and PTP opening activity (189). Downregulation of VDAC1 in diabetic MCECs decreased $[Ca^{2+}]_m$ and subsequently normalized the levels of PTP activity and mitochondrial ROS production (190). VDAC1 has proposed to mediate the protective effects of hesperidin, a bioactive flavonoid compound, against amyloid β-induced mitochondrial dysfunction, mitochondrial PTP opening, $[Ca^{2+}]_i$ increase, and ROS production (191). It has also shown that blocking of VDAC1-mediated $[Ca^{2+}]_m$ release in Schwann cells prevented demyelinating neuropathies (192). Thus, VDAC function in Ca^{2+} homeostasis is connected to several diseases.

AUTHOR CONTRIBUTIONS

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