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REVIEW

Pore-Forming VDAC Proteins of the Outer Mitochondrial Membrane: Regulation and Pathophysiological Role

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Abstract—Voltage-dependent anion channels (VDAC1-3) of the outer mitochondrial membrane are a family of pore-forming β-barrel proteins that carry out controlled "filtration" of small molecules and ions between the cytoplasm and mitochondria. Due to the conformational transitions between the closed and open states and interaction with cytoplasmic and mitochondrial proteins, VDACs not only regulate the mitochondrial membrane permeability for major metabolites and ions, but also participate in the control of essential intracellular processes and pathological conditions. This review discusses novel data on the molecular structure, regulatory mechanisms, and pathophysiological role of VDAC proteins, as well as future directions in this area of research.

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Keywords: VDAC, mitochondria, membrane transport, cell death, cancer, diabetes mellitus, neurodegenerative diseases

INTRODUCTION

The functional activity of most eukaryotic cells is ensured by the constant production of energy by the mitochondria. Beside energy production in a form of ATP molecules, these organelles are involved in a variety of intracellular processes, including generation of reactive oxygen species (ROS), synthesis of metabolites, signal transduction, maintenance of ion homeostasis, and regulation of central metabolic pathways, that underly various physiological and pathological phenomena, such as thermogenesis, cell proliferation, cell death, inflammation, ischemic damage, neurodegeneration, and others. Mitochondrial dysfunction is an early sign of most cellular pathologies and in some cases is considered as their main pathogenetic factor [1-3].

The variety of functions performed by the mitochondria is due to the unique ultrastructure of these organelles, especially, the presence of two membranes limiting the intermembrane space [1]. A distinctive feature of the inner mitochondrial membrane (IMM) is its selective permeability to ions and metabolites. In contrast, the outer mitochondrial membrane (OMM) allows for a less selective but controlled movement of small hydrophilic molecules and ions between the cytoplasm and mitochondrial intermembrane space, thus modulating their overall accessibility to the mitochondrial transporters and enzymes. This primary controlled "filtration" at the OMM is provided by a family of voltage-dependent anion channels (VDACs). VDACs are the so-called β-barrel proteins composed of antiparallel amphipathic β-strands that form a hydrophilic pore [4-7]. VDACs were discovered

Abbreviations: ALS, amyotrophic lateral sclerosis; IMM, inner mitochondrial membrane; mPTP, mitochondrial permeability transition pore; OMM, outer mitochondrial membrane; ROS, reactive oxygen species; SOD1, superoxide dismutase 1; VDACs, voltage-dependent anion channels.

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in the mitochondria of *Paramecium aurelia* in 1976 [8]. These proteins are dominant in the OMM (up to 35%). The permeability of the pore formed by these proteins (porins) to various ions and intermediate metabolites is modulated by the transmembrane potential (an eponymous property of these channels) [9]. The capacity of VDAC proteins for temporary conformational transitions from the closed to the open state, as well as their interaction with a number of cytoplasmic and mitochondrial proteins, allows these channels not only to control the OMM permeability for key metabolites and ions, but also to participate in the regulation of vital cellular processes – from energy production to cell death. In this regard, it is not surprising that the VDAC family proteins are considered as the main pharmacological targets in the treatment of mitochondria-related diseases, including neurodegenerative and cardiovascular pathologies, various types of cancer, diabetes mellitus, autoimmune diseases, inflammation, and others [4, 7, 10-13].

In this review, we discussed the modern concepts on the molecular structure and mechanisms of regulation of VDAC proteins, as well as their role in physiological and pathological conditions in the cell.

ISOFORMES AND MOLECULAR ORGANIZATION OF VDAC PROTEINS

VDACs are a family of β-barrel proteins located in the OMM. They are encoded by three different genes sharing more than 70% nucleotide sequence similarity in eukaryotes. The genes encoding three human protein isoforms, hVDAC1, hVDAC2, and hVDAC3, are located on chromosomes 5, 10, and 8, respectively. Evolutionary analysis suggests that these isoforms have originated by the gene duplication in vertebrates, with *Vdac3* being the first to diverge from the primary *Vdac* and representing the oldest of the three paralog genes [14-16]. All three isoforms form virtually identical selective voltage-dependent ion channels for nucleotides (ATP, ADP, AMP, NADH), anionic metabolites (pyruvate, glutamate, succinate, malate), and lipids. Inorganic ions (K^+, Na^+, Ca^{2+}, Cl^-) and some organic ions can also pass through the porin channels [10, 17-21] (Fig. 1). A high content of VDACs in the OMM (10^3-10^4 protein molecules/μm^2), as well as the high rates of diffusion into the mitochondria for metabolites (10^2-10^3 molecules/ms) and ions (10^6 ions/ms), ensure constant exchange of substances

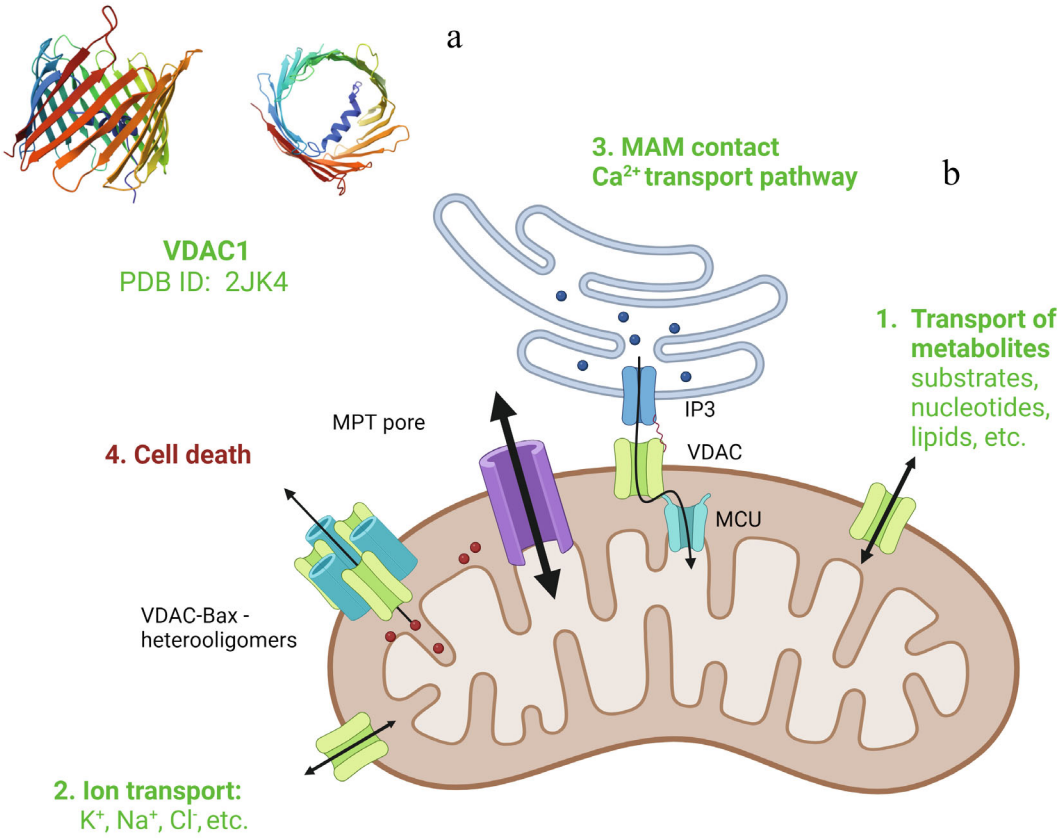

Fig. 1. VDAC1 structure (PDB ID: 2JK4) (a) and functional activity (b). In healthy cells, VDACs transport metabolites and ions through the OMM and participate in the regulation of cytoplasmic Ca^{2+} concentration through interactions with the endoplasmic reticulum. Under pathological conditions, VDACs become structural components of the mitochondrial permeability transition pore (mPTP) or participate in oligomerization with proapoptotic Bcl2 family proteins, leading to the mitochondrial dysfunction and cell death. The figure was created with the BioRender.com service.

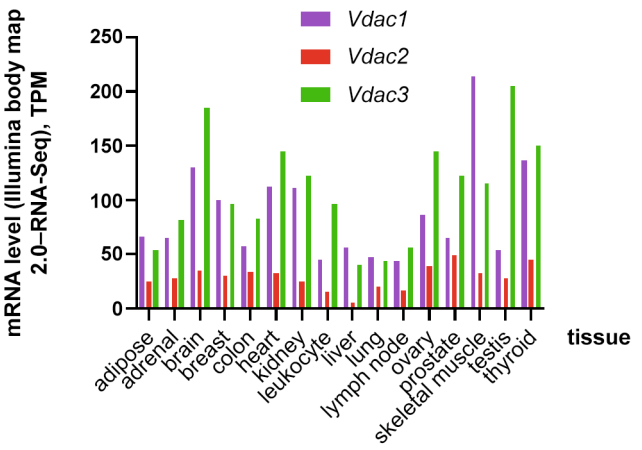

Fig. 2. Expression of *VDAC1-3* genes in human tissues (data obtained from the Illumina's Human BodyMap 2.0 project and downloaded from https://www.ebi.ac.uk/gxa/experiments/E-MTAB-513).

between the cytoplasm and these organelles [22]. The molecular weight of the three VDAC isoforms ranges from 30 to 35 kDa (VDAC1 and VDAC3 consist of 280 amino acid residues; VDAC2 consists of 291 residues), indicating a high degree of homology between these proteins [20]. Using the methods of X-ray diffraction, nuclear magnetic resonance, and cryoelectron microscopy, it has been shown that VDAC1 and VDAC2 have a β-barrel structure consisting of 19 mostly antiparallel β-strands (with the exception of β-strands 1 and 19). The N-terminus of these proteins is α-helical and is located inside the channel. A highly conserved glycine-rich sequence (Gly21-Tyr-Gly-Phe-Gly25) in VDAC1 connects the N-terminal α-helical fragment to the first β-strand, which allows this fragment to move into the pore and control the gate mechanism [23-26]. The three-dimensional structure of VDAC3 has not yet been determined. The internal space of a VDAC in the open state is an aqueous pore with a diameter of 3-3.8 nm, permeable to compounds weighing up to 5 kDa. When the N-terminal helix is located inside the pore, the pore diameter is reduced to 1.5 nm [22-25].

The three VDAC isoforms are widely represented in all mammalian tissues, with the highest expression levels observed in skeletal muscle [16, 27]. After termination of translation, mature proteins are delivered to the OMM, which is ensured by the presence of a specific signal sequence at the N-terminus. It should be noted that in a number of pathologies accompanied by VDAC overexpression, the directed transport of these proteins can be disrupted, leading to their integration into the plasma membrane [28]. Using Western blotting and real-time PCR, it was shown that under normal conditions, the expression levels of VDAC1 and VDAC2 are comparable, while the content of VDAC3 in most tissues and organs of mammals, excluding the testes, is significantly lower [27]. However, according to the expression atlases (for example, EMBL-EBI Expression Atlas), the *VDAC3* gene has a significantly higher transcription level in many tissues compared to the genes encoding two other isoforms (Fig. 2), presumably, due to a high activity of the *VDAC3* gene promoter. Also, the *VDAC3* promoter contains a polypyrimidine region, which is considered as a specific target of oxidative stress [29]. Therefore, the ratio between the three VDAC isoforms in the cell is regulated by different stability of their transcripts, and maintaining a high level of VDAC3 expression is necessary for a rapid cell response to changing external conditions [30]. This assumption was confirmed by the recently discovered features in the distribution of the three isoforms in the OMM. It was shown that hVDAC1 and hVDAC2 usually co-localize in the same relatively large domains (300-900 nm^2), while hVDAC3 is evenly distributed over entire OMM [31]. It is also important to note that the activity of transcription regulatory factors specific for the promoters of the three VDAC genes can differ depending on the conditions (see [5, 29, 32] for more details).

Beside regulating exchange of ions and metabolites between the mitochondria and the rest of the cell, VDACs can affect a variety of other intracellular processes. VDAC1 is considered to be a key participant in the mitochondria-mediated apoptosis, while VDAC2, on the contrary, prevents the programmed cell death [5, 27, 32, 33]. It is likely that by colocalizing in the same subcompartments of the OMM, these two proteins regulate the balance between pro- and antiapoptotic signals in the cell. Although there are no data on the role of VDAC3 in the initiation of apoptosis, the participation of this isoform in the regulation of ROS production and functioning of the mitochondrial quality control system has been proven [5, 16].

REGULATION OF FUNCTIONAL ACTIVITY OF VDAC PROTEINS

Regulation of VDAC activity by the transmembrane potential. VDACs form a large hydrophilic pore, so that the regulation of its functional activity might be problematic. However, the possibility of such regulation has been demonstrated in a number of electrophysiological studies in artificial membranes. It has been shown that the throughput performance of this channel depends on the presence of a variety of natural and synthetic ligands, lipid environment, and transmembrane potential (it is the change in the VDAC activity in response to a shift in the transmembrane potential that allowed these channels to be called voltage-dependent). Thus, when VDAC1 was incorporated into a bilayer lipid membrane (BLM) at a low membrane potential (±20 mV) or in its absence, the formed

channel had a high conductance (was in the open state) and allowed free transport of predominantly anionic metabolites and monovalent ions. An increase or decrease in the transmembrane potential on the BLM within a range of ±20 to ±40 mV led to the suppression of the VDAC permeability for anionic metabolites, nucleotides, and monovalent ions [16, 34], while the rate of Ca^{2+} transport through the membrane increased significantly (4 to 10 times) [35]. It should be noted that in *in vitro* experiments on artificial membranes, only VDAC1 and VDAC2 demonstrated high conductance [36], whereas hVDAC3 was characterized by a very low conductance (100 pS versus 3.5-4 nS for VDAC1 and VDAC2 in 1 M KCl) and its activity did not depend on the membrane potential [37, 38].

The mechanism of the VDAC permeability regulation is associated with the conformational changes in the protein, in particular, with the position of the N-terminal fragment that acts as a voltage sensor. These conformational changes lead to a decrease in the pore diameter and changes in the channel selectivity [38, 39].

The dependence of the VDAC activity on the transmembrane potential in *in vitro* experiments suggests that the same regulatory mechanism exists in living cells. Indeed, the transition from the open (permeability to metabolites) to the closed (permeability predominantly to Ca^{2+}) state can lead to the switch from the normal functioning of mitochondria and cells to the damage to organelles and cell death due to the excessive entry of Ca^{2+} into the mitochondrial matrix and opening of the calcium-dependent non-selective mitochondrial transition permeability pore (mPTP). Lemeshko [6, 40] has proposed a theoretical model suggesting that the potential on the OMM can reach –50 mV (with negative charge on the cytosolic side of the membrane). However, it is important to note that the VDAC conformational transition from the open to the closed state in a living cell may occur due not so much to the shift in the membrane potential, but rather to changes in the VDAC interaction with intracellular proteins (tubulin, α-synuclein, hexokinase, etc.) and small endogenous regulatory molecules [10, 33, 34].

Protein–protein interactions mediating the opening/closing of VDACs. VDACs have a simultaneous access to otherwise strictly separated cytoplasmic and mitochondrial proteins and cell regulators, including glycolytic enzymes, neuronal proteins, and cytoskeletal components, which allows for a fine regulation of their function in health and disease.

The binding of tubulin and α-synuclein to VDACs leading to the VDAC transition to the closed (blocked) state at a low membrane potential has been studied in most detail. Addition of nanomolar concentrations of α-synuclein or the α/β-tubulin heterodimer to the BLM containing incorporated VDACs resulted in a partial decrease in the channel conductance for anionic metabolites (~60% of the open state conductance) and a significant increase in the channel conductance for Ca^{2+} [34]. This effect was due to the presence of the polyanionic C-terminus in the interacting proteins [34, 41, 42], which penetrated into the VDAC pore and screened the inner part of the channel. Since the polyanionic C-terminus of α-synuclein is longer than that of tubulin, regulation of VDAC conductance by this protein depends on which part of α-synuclein molecule is located inside the pore at a particular time. The blockade of the VDAC pore was found to cause disruption of metabolite and Ca^{2+} exchange between the cytoplasm and mitochondria, decrease in the mitochondrial membrane potential, and suppression of oxidative phosphorylation [34].

The blockade of VDACs by tubulin and α-synuclein also depends on the lipid environment. Thus, phosphatidylethanolamine stabilized the amphipathic protein domains on the membrane surface due to electrostatic and hydrophobic interactions and prevented their complete immersion into the VDAC pore [43, 44]. It is also important to note that tubulin and α-synuclein inhibited the conductance of VDACs incorporated into artificial membranes only when added to the negatively charged side of the membrane [45, 46]. The effects of these proteins differed from the effects caused by the 20 to 40-mV shift (either increase or decrease) in the membrane potential [6, 34].

A number of studies have indicated that the intermediate filament proteins desmin and vimentin can also interact with VDACs and modulate the functional activity of the mitochondria [47, 48]. This interaction occurs through the N-terminal fragments of these proteins, which, however, lack the polyanionic region, so that the exact mechanism of the VDAC activity regulation by the intermediate filament proteins remains unclear [49]. The data on the effect of these proteins on the functioning of mitochondria are conflicting – from the stimulation of mitochondrial bioenergetics to the suppression of cellular respiration and activation of ROS generation [47-50].

The interactions of tubulin and α-synuclein with the mitochondria and VDAC proteins play an important physiological role. In particular, regulation of VDAC permeability by β-tubulin is considered as one of the key mechanisms in the switch of cellular metabolism from oxidative phosphorylation to glycolysis. This metabolic flexibility underlies the Warburg effect and is of great importance for the growth and proliferation of cancer cells [11, 20, 34, 51]. Indeed, β3-tubulin, which is the most active VDAC blocker, is widely expressed in tumor cells [52]. The aggregation of α-synuclein is believed to be one of the main causes of the dopaminergic neuron degeneration in the Parkinson's disease [53]. It is possible that the initiation and progression of mitochondrial dysfunction in this disease are caused not only by the disruption of selective elimination

of these organelles (mitophagy), but also by suppression of oxidative phosphorylation and induction of mPTP through direct α-synuclein interaction with the mitochondria and VDACs [54, 55].

Another cytoplasmic protein that causes the closure of VDACs is hexokinase [4, 20, 41, 56-61]. Hexokinase isozymes I and II are key components in the first rate-limiting step of the glycolytic pathway, and their interaction with VDACs can lead to a shift in energy metabolism towards glycolysis. In addition, the binding of hexokinase to VDAC suppresses induction of cell death via necrosis (by inhibiting emergence of the calcium-dependent mPTP) or apoptosis (by blocking VDAC oligomerization with the Bcl2 family proteins) [56, 62, 63], which promotes tumor growth. Importantly, cancer cells were found to overexpress mitochondrial-associated hexokinases I and II [64].

It is believed that the key amino acid residue mediating interactions with the hydrophobic N-terminus of hexokinase is glutamic acid at position 73 of the VDAC polypeptide chain [65, 66]. This residue is also involved in the VDAC oligomerization and binding with various molecules, including those regulating VDAC interaction with hexokinase [66, 67].

Recent studies have shown that VDAC opening can be modulated by a number of other proteins, including mitochondrial creatine kinase, TSPO (translocator protein, 18 kDa; also known as peripheral benzodiazepine receptor), p53, actin, and others [4, 68-71]. The activity of VDACs is also regulated through the post-translational protein modification. In particular, phosphorylation of VDAC at serine or threonine residues by protein kinase A (PKA), protein kinase C (PKCε), and glycogen synthase kinase-3β (GSK3β) modulated the activity of VDACs and their interaction with other proteins, in particular, β-tubulin [72]. Mass spectrometry data showed that VDACs contain cysteine residues accessible to soluble oxidants and, therefore, sensitive to the dithiol-disulfide exchange [73]. In particular, it was proposed the cysteine residues of VDAC3 play a key role in the modulation of mitochondrial ROS production [74]. These data suggest that the VDAC proteins can be used as biomarkers of the redox state of the mitochondrial intermembrane space.

Protein–protein interactions mediating VDAC oligomerization. Oligomerization of VDACs in the OMM with the formation of complexes composed of the channel molecules only or heterooligomers containing other proteins is a key pathophysiological process.

Large dynamic VDAC oligomeric channels (pores) can form in the OMM in response to various proapoptotic signals, leading to the release of proapoptotic molecules, including cytochrome *c*, apoptosis-inducing factor (AIF), Smac/Diablo, and others, from the mitochondrial intermembrane space or mitochondrial matrix (in the case of IMM damage) [10]. After exiting to the cytoplasm, these molecules trigger caspase-dependent (cytochrome *c* and Smac/Diablo) or caspase-independent (AIF) cascade of degradation processes resulting in cell death.

VDAC1 can form dimeric, trimeric, and multimeric complexes in the membrane [4]. It was assumed that Glu73 and Ser43 residues play a key role in the molecule dimerization [75]. VDAC1 dimerization occurs at low pH and is abolished by the replacement of Glu73 with alanine or glutamine. The process of oligomerization also depends on the lipid environment (in particular, the presence of cholesterol in the animal membranes and ergosterol in the membranes of plants and fungi) and interaction with the apoptosis-related p53 protein [4, 66, 70]. VDAC1 oligomerization is triggered by a wide range of apoptotic death inducers [staurosporine, curcumin, As_2O_3, cisplatin, H_2O_2, tumor necrosis factor alpha (TNF-α), etc.] in various cell lines. It is accompanied by the increase in the expression of VDAC1 [4, 76], which is believed to be associated with the post-translational phosphorylation of the channel by GSK3β, increased cytoplasmic Ca^{2+} concentration, and activation of transcription factors [4, 10, 72, 76, 77].

Proapoptotic proteins of the Bcl2 family (e.g., Bax, Bak, tBid) also contribute to the induction of the rapid increase in the OMM permeability. Bcl2 family proteins are characterized by the presence of the Bcl2 homology (BH) domain. In addition to the proapoptotic proteins (Bax, Bak, Bim, Bid, BAD), this family also includes the antiapoptotic proteins (Bcl2, Bcl-XL). Bcl2 proteins localize predominantly to the cytosol, but can move to the mitochondria under the action of certain stimuli and participate in the OMM permeabilization [78] through the formation of the Bax/Bak homo- and heterooligomeric complexes or VDAC1/Bax heterooligomeric channels.

As mentioned above, VDAC1 protein, whose expression increases upon exposure to apoptotic agents, is capable of forming oligomeric or heterooligomeric (with Bax or Bak) megachannels [4, 79, 80] that facilitate the release of proapoptotic proteins and mitochondrial DNA from the mitochondria. Therefore, the regulation of the VDAC1 functional activity is currently considered as a promising therapeutic strategy for the initiation or suppression of programmed cell death in various pathological conditions.

Elucidation of the role of VDAC2 in the induction of cell death has turned out to be a much more difficult task. On one hand, the knockout of VDAC2 in mouse embryos was either lethal or led to serious developmental impairments in newborn animals [81]. Experiments in cell cultures have yielded conflicting results. In particular, proapoptotic stimuli promoted death of VDAC2-deficient mouse embryonic fibroblast (MEFs), presumably, due to the specific VDAC2 binding to Bak monomers and prevention of megachannel formation [81].

On the other hand, VDAC2-deficient cells were insensitive to the tBid-induced OMM permeabilization and apoptosis. The authors explained this by the VDAC2 involvement in the incorporation of the proapoptotic Bak and Bax proteins into the OMM. Disruption of this process prevented anchoring of these proteins in the membrane and subsequent OMM permeabilization. It is believed that residues 123-179 in the VDAC molecule (mainly Thr168 and Asp170) are responsible for the Bak incorporation into the membrane [82]. Interestingly, suppression of the VDAC2 expression disrupted the interaction of Bax and Bak with the VDAC-containing mitochondrial complexes and suppressed apoptosis induced by Bax, but not Bak [83]. Hence, VDAC2 can also act as a proapoptotic protein by participating in the Bak (Bax) incorporation into the OMM; however, in some cases, it can inhibit apoptosis by suppressing the formation of large pores by the Bak (Bax)/VDAC1 heterooligomers. It is possible that the pro- and antiapoptotic effects of VDAC2 are determined by its environment. A similar hypothesis has been suggested for VDAC1. Thus, it was shown that VDAC1 formed large (120 to 500 kDa) heterooligomers with Bax. However, these structures were stable only in healthy cells, while apoptotic cells demonstrated protein rearrangement with the formation of 170-kDa oligomeric channels that contained no VDAC1 protein [84]. Based on these data, the authors suggested that in healthy neurons, VDAC1 inhibited premature assembly of Bax oligomeric channels (similar to the mechanism taking place in the case of interactions between VDAC2 and Bak).

Intermembrane interactions involving VDACs and affecting intracellular Ca^{2+} homeostasis. As stated above, VDAC isoforms are key regulators of Ca^{2+} transport in the mitochondria: when VDACs are closed, the rate of Ca^{2+} transport into the mitochondrial matrix increases significantly. Moreover, VDAC proteins have a Ca^{2+}-binding site that regulates their activity; the VDAC-dependent Ca^{2+} transport is suppressed by La^{3+} and ruthenium red (RuR) [38, 85]. Therefore, many important mitochondrial processes, such as the functioning of the Ca^{2+}-dependent enzymes of the Krebs cycle (pyruvate dehydrogenase, NAD-isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase), maintenance of Ca^{2+} homeostasis and opening of the calcium-dependent nonselective pore, directly depend not only on the Ca^{2+} transport systems of the IMM, but also on VDACs that act as the "primary filters" in the OMM [86].

Rapid uptake of Ca^{2+} by the mitochondria is closely associated with the release of this ion from the endoplasmic reticulum and occurs under control of specialized structures named mitochondria-associated membranes (MAMs) formed as a result of reversible interaction between the membrane proteins of the endoplasmic reticulum and the OMM. MAMs play an important role in the bidirectional regulation of organelle functioning, signaling, and maintenance of intracellular homeostasis under physiological conditions and during the development of neurodegenerative diseases (see reviews [87, 88] for more details). MAMs (10-30 nm in size) are highly dynamic structures prone to rearrangement. Proteomic analysis revealed approximately 1000-2000 different proteins that can be involved in the formation and regulation of MAMs (70 of them are considered essential), which suggests the role of MAMs as multifunctional molecular platforms for signaling. Among the variety of MAM proteins, inositol 1,4,5-triphosphate receptor (IP_3R) and ryanodine receptors (RyRs) are responsible for the Ca^{2+} release from the endoplasmic reticulum, while VDAC1 provides the transport of this ion across the OMM. These proteins are the most abundant at MAMs, and their structural and functional coupling is ensured by the chaperone GRP75 (glucose-regulated protein 75) [86-90]. Changes in the density or distribution of MAMs can lead to the disruptions in Ca^{2+} homeostasis in the mitochondria. In particular, we showed that skeletal muscles of the dystrophin-deficient C57BL/10ScSn-*mdx* mice (a model of Duchenne muscular dystrophy) had an increased number of MAMs, which was accompanied by a higher Ca^{2+} content in the mitochondria and decrease in the mitochondrial Ca^{2+} retention capacity [91].

VDAC1 is considered as the main protein involved with IP_3R in the formation of active Ca^{2+} transport complex at MAMs in most cell types and tissues [38, 92]. However, VDAC2 also participates in the formation of this complex [93]. Moreover, efficient Ca^{2+} transport in cardiomyocytes occurs as a result of interaction between VDAC2 and RyR [94]. The role of VDAC3 in the regulation of intracellular Ca^{2+} signaling has not yet been fully established. As mentioned above, this isoform is weakly regulated by the conformational transitions between the open and closed states and, therefore, does not affect the rate of Ca^{2+} flux across the mitochondrial membranes, which might be due to the loss of Glu73 in its structure [38].

After Ca^{2+} enters the mitochondrial intermembrane space through VDACs, it is transported to the matrix via the Ca^{2+} uniporter, a multicomponent protein complex that includes the mitochondrial calcium uniporter (MCU, the channel-forming subunit), its dominant negative form MCUb, regulatory subunits MICU1, MICU2, EMRE, and MCUR1, and other proteins [86]. It has been shown that MCU and VDAC1 form a complex involved in the transport of Ca^{2+} to the mitochondrial matrix [95]. The authors suggested that the inhibition of the MCU activity or disruption of its complex with VDAC1 could be a strategy in the treatment of nervous system diseases. However, the mechanism of interaction between the VDAC isoforms and the MCU subunit has not been fully understood.

Compounds regulating VDAC activity

Name	Effect on VDAC	Effect on cells and organism	References
Erastin	VDAC “opener,” disrupts VDAC interaction with tubulin	ferroptosis inducer, anticancer effect	[98, 99, 102]
VBIT-4	inhibitor of VDAC oligomerization and conductance	apoptosis inhibitor, protective effect in diabetes mellitus, Alzheimer’s disease, ALS, hyperaldosteronism	[105-107]
Olesoxime	inhibitor of VDAC activity and oligomerization	cell death inhibitor, protective effect in ALS and acute kidney damage	[108,109]
DIDS	VDAC oligomerization inhibitor	apoptosis inhibitor, apoptosis inducer, neuroprotective and neurotoxic effect	[110]
Konig’s polyanion	VDAC inhibitor	apoptosis inducer, anticancer effect	[10, 119]
Ethanol	VDAC inhibitor	apoptosis inducer, anticancer effect	[114-116]
Aspirin	interrupts VDAC interaction with hexokinase	apoptosis inducer, anticancer effect	[111]
3-bromopyruvate	interrupts VDAC interaction with hexokinase	apoptosis inducer, anticancer effect	[112]
Clotrimazole	interrupts VDAC interaction with hexokinase	apoptosis inducer, anticancer effect	[113]
G3139	VDAC inhibitor, disrupts interaction with Bcl2 proteins	apoptosis inducer, anticancer effect	[117,118]
Ruthenium red	VDAC inhibitor	apoptosis inhibitor	[103, 104]
SC18	VDAC inhibitor	cell death inducer, anticancer effect	[120]

Another important role of VDAC is associated with a pathological phenomenon known as the mPTP opening caused by the excessive accumulation of Ca^{2+} in the mitochondria. It has been found that accumulation of Ca^{2+} in the mitochondrial matrix can lead to the formation of a large non-selective channel (pore) in the IMM (see reviews [63, 86, 96, 97] for more details). This results in the disruption of mitochondrial respiration and oxidative phosphorylation, collapse of the membrane potential, dissipation of ion gradients across the IMM, and, ultimately, swelling of the matrix and destruction of the mitochondria. Currently, it is believed that the main components forming the channel in the IMM are ATP synthase (in different configurations) and adenine nucleotide translocator (ADP/ATP translocase). The regulatory protein that ensures the mPTP opening is peptidyl-prolyl *cis-trans* isomerase cyclophilin D [63, 86]. In isolated mitochondria, mPTP formation does not require VDACs [63]. At the same time, VDAC is important for the generation of the Ca^{2+}-dependent mPTP in living cells, since it provides the main route for Ca^{2+} entry to the mitochondria. Under physiological conditions, GSK3β, PKA, and other protein kinases phosphorylating VDACs reduce the threshold for mPTP opening for various inducers, while phosphorylation of these kinases leads to the suppression of mPTP opening and reduces the risk of severe pathologies associated with the hypoxia-induced tissue damage.

Small molecules regulating VDAC activity. Studying electrophysiological characteristics of VDACs and their role in the mitochondria and cells mostly involves elucidation of the effects of small molecules on these channels. When interacting with VDAC proteins, most known ligands reduce the channel activity, but their effect on the induction of apoptosis can be different.

The only known “opener” of VDACs is the low-molecular-weight compound erastin that prevents VDAC interaction with tubulin [98]. Erastin preferentially binds with VDAC2 and VDAC3 isoforms [99]. It is believed that

such activation leads to the hyperpolarization of mitochondria and increased ROS production in these organelles [10, 100, 101]. This is one of the reasons why erastin initiates oxidative programmed necrotic cell death associated with the iron-dependent lipid peroxidation (ferroptosis) [102].

One of the key targets for various inhibitors in the VDAC molecule is Glu73(72) residue [66]. It is believed that this residue binds, for example, with RuR, which not only blocks VDAC1, but also competes with hexokinase for the binding site [10, 66]. RuR is commonly considered as a blocker of apoptosis triggered by various proapoptotic stimuli. The substitution of Gln73 for Glu73 suppresses the RuR-mediated inhibition of VDAC1 conductance and apoptotic cell death [65, 66, 103, 104]. Therefore, RuR, on the one hand, blocks VDACs, thus increasing their conductance for Ca^{2+}; on the other hand, it prevents the influx of Ca^{2+} into the matrix by inhibiting the MCU [66, 86].

Some agents capable of triggering apoptosis (e.g., myostatin and cisplatin) upregulate VDAC1 expression. It is believed that the increased expression of VDAC1 promotes oligomerization of this protein, which can cause apoptotic cell death (see review [10] for more details).

On the other hand, a number of compounds suppress VDAC oligomerization and, hence, display the antiapoptotic effect. These compounds include VBIT-4 (voltage-dependent anion channel 1 oligomerization inhibitor), DIDS (4,4′-diisothiocyano-2,2′-stilbene-disulfonic acid), and olesoxime (TRO19622) [10, 105-110].

According to the published data, there is a wide range of compounds that influence the activity of isolated VDACs incorporated in BLMs, as well as mitochondria and cells. These agents include the Konig's polyanion, dicyclohexylcarbodiimide (DCCD), ethanol, phosphorothioate antisense oligonucleotide G3139, 3-bromopalmitate, and others (table) [111-120]. However, these compounds target many molecules, interaction with which can affect the functioning of mitochondria and lead to the cell death. Therefore, the use of these small compounds as intracellular modulators of VDACs channels should be considered only with a great degree of assumption.

ROLE OF VDACs IN PATHOLOGIES

VDACs and carcinogenesis. The content and conformational state of VDAC proteins determine the cytosolic ATP/ADP ratio through the regulation of the substrate flux and, therefore, play a critical role in the switch to the Warburg proliferative phenotype characteristic of cancer cells. Specifically, the open state

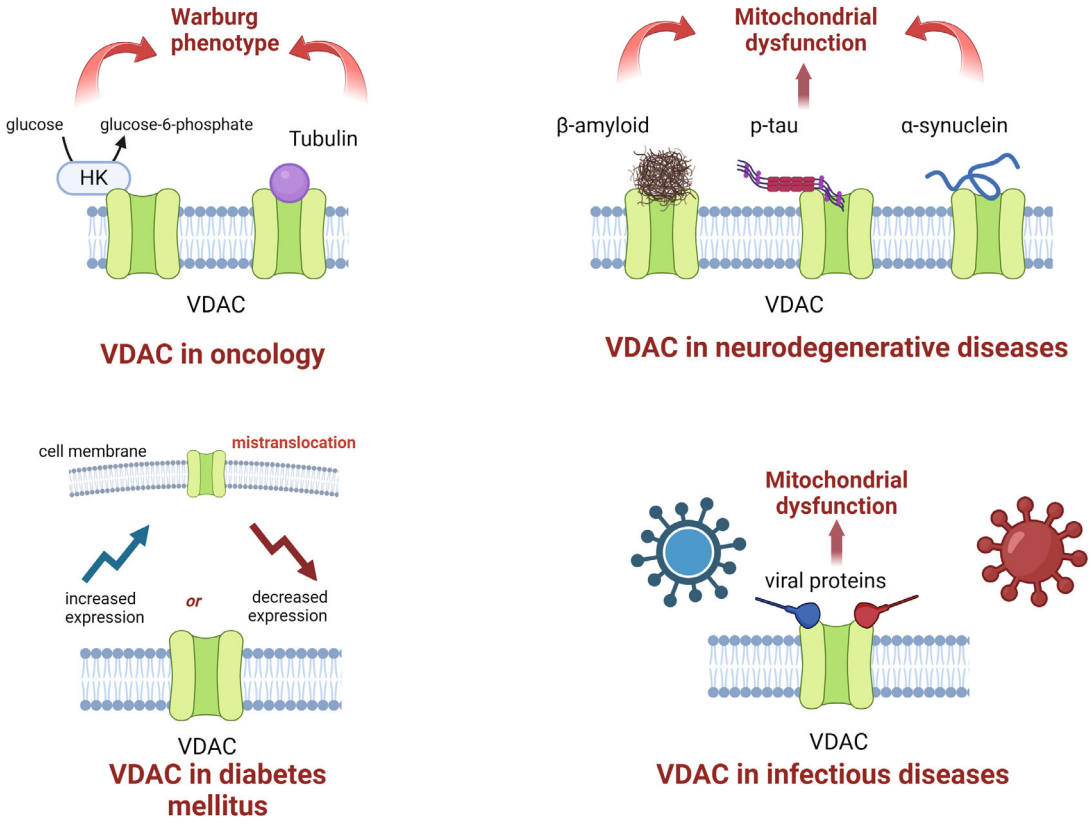

Fig. 3. Participation of VDACs in pathological processes in the development of oncological diseases, neurodegenerative disorders, diabetes mellitus, and infectious diseases (see the text for explanation). The figure was created with the BioRender.com service.

of VDACs promotes the maximal metabolite flux for the optimal mitochondrial function, whereas the closed state minimizes the mitochondrial metabolic rates and promotes the Warburg phenotype upon accumulation of precursors for macromolecular biosynthesis required for the proliferation of tumor cells [4, 10, 11, 20]. It is believed that stimulation of mitochondrial metabolism, in part through the modulation of the VDAC open conformation, leads to the activation of oxidation of physiological substrates and ATP generation, but also promotes ROS generation followed by the development of oxidative stress and elimination of cell with the Warburg phenotype (Fig. 3).

VDAC isoforms can be used as prognostic biomarkers in different types of human cancer [4, 10, 121, 122]. In particular, development of lung, breast, and liver cancers is accompanied by the increased levels of VDAC1; high VDAC1 expression is closely associated with lower survival rates in cancer patients [121, 123]. It has been suggested that VDAC1 acts as an oncogene in tumorigenesis and tumor progression. VDAC1 overexpression in cancer cells can result in its interaction with hexokinase, leading to glucose phosphorylation by the mitochondrial ATP and development of the Warburg effect. VDAC interaction with hexokinase may prevent its binding to the proapoptotic factors, in particular, Bax [124]. Thus, VDAC1 interacts with hexokinase and antiapoptotic proteins overexpressed in cancer (Bcl2, Bcl-XL) and blocks the release of cytochrome *c* from the mitochondria, thus preventing the apoptosis of cancer cells [4, 10, 11, 20]. It was found that the effect of some anticancer agents (clotrimazole, 3-bromopyruvate) was due to their ability to cause the dissociation of the VDAC1 complex with hexokinase [112, 113].

VDAC1 overexpression can contribute to tumorigenesis by suppressing activation of NK cell that eliminate neighboring cells carrying surface markers associated with the oncogenic cell transformation [125]. It has been shown that the mitochondrial fission factor (MFF), which binds to VDAC1 and regulates its conformational state, can be overexpressed in non-small cell lung cancer, while destruction of the MFF–VDAC1 complex with MFF mimetics leads to mitochondrial depolarization and triggers cell death in various types of cancer cells, including melanoma [126]. miR-7 reduced VDAC1 expression, resulting in the inhibition of cell proliferation and metastasis in hepatocellular carcinoma [127]. In contrast, activation of VDAC1 through the downregulation of miR-320a expression induced proliferation and invasion of non-small cell lung cancer cells [128]. As stated above, blocking the inhibitory effect of tubulin on VDACs with erastin or erastin-like compounds activated mitochondrial metabolism, promoted oxidative stress, and was accompanied by the inhibition of glycolysis [100, 129]. This approach can be used to induce the damage and death of cancer cells by both blocking the supply of building materials necessary for cell proliferation and inducing the oxidative burst (ROS burst) in the mitochondria. Thus, a combination of the oxidative stress modulation and the Warburg effect induced by erastin-like compounds caused cell death in various human hepatocarcinoma cell lines and slowed tumor growth in a xenograft model of Huh7 hepatocarcinoma cells [129, 130]. Recently, it was shown that NADH bound to the NADH-binding pocket in various VDAC isoforms and closed the channel [131]. In this case, the small molecule SC18 capable of interaction with the NADH-binding pocket, maintained VDACs in the open configuration, which caused mitochondrial dysfunction and reduced the proliferation of human hepatocarcinoma cells [120]. Importantly, the effect of SC18 was independent of the VDAC isoform. On the other hand, it should be taken into account that due to the heterogeneity of cancer cells, the effects of the VDAC-mediated switch in the mitochondrial metabolism can be unpredictable, and therefore, this issue requires detailed studying.

VDACs and neurodegenerative diseases. VDACs are involved in the development of some neurodegenerative diseases due to their interactions with misfolded and aggregated proteins that lead to changes in the channel conductance and contributes to the progression of mitochondrial dysfunction [4, 10, 12]. In particular, this is observed in the case of Alzheimer's disease, when accumulation of β-amyloid peptide and hyperphosphorylated tau promotes their interaction with the mitochondrial VDAC1, whose level is significantly increased in this disease [132]. This process is accompanied by a decrease in the VDAC1 conductance and inhibition of mitochondrial function. The modulating effect of β-amyloid on VDAC1 conductance was also shown in *in vitro* experiments using BLMs [133]. The interaction of β-amyloid with VDAC1 was demonstrated in SH-SY5Y neuroblastoma cells, in which VDAC1 phosphorylation promoted release of proapoptotic molecules from the mitochondria and exacerbated the neurotoxic effect of β-amyloid [134]. Reducing VDAC1 levels in VDAC1$^{+/-}$ mice protected brain cells from degenerative changes by preserving their mitochondrial function [135].

The binding of VDAC1 to misfolded proteins and accompanying disturbances in the mitochondrial homeostasis were also found in amyotrophic lateral sclerosis (ALS), a progressive disease characterized by muscle paralysis caused by degeneration of motor neurons [136, 137]. The main cause in the development of hereditary ALS forms is aggregation of mutant superoxide dismutase 1 (SOD1) [134]. It is assumed that mutant SOD1, alone or together with other intracellular components, forms oligomers, which is followed by the generation of high-molecular-weight aggregates. In the SOD1*G93A transgenic rat model of ALS, mutant SOD1 protein was found to bind specifically to VDAC1 and

to suppress ADP transport to the mitochondria isolated from the spinal cord [138]. The binding of misfolded SOD1 to the N-terminal domain of VDAC1 was recently characterized in [136]. The authors of [72] identified post-translational modifications of VDAC1 residues in NSC-34 cells expressing mutant SOD1 protein, which suggested changes in the VDAC1 structure and, consequently, in the energy metabolism of motor neurons in ALS [72]. The inhibitor of VDAC oligomerization olesoxime delayed the death of motor neurons in the mouse model of ALS but was ineffective in clinical trials in humans with advanced ALS [139, 140].

The interaction of VDACs and abnormally aggregated α-synuclein may contribute to the onset and progression of the Parkinson's disease. α-Synuclein localizes primarily to the presynaptic terminals in various parts of the brain and is the main component of Lewy bodies in the Parkinson's disease. Accumulation and aggregation of α-synuclein have been shown to contribute significantly to the neurotoxicity and to be a leading cause of the degradation of brain dopaminergic neurons [53, 141]. Monomeric α-synuclein can be transported through the channels formed by all three VDAC isoforms and reach the IMM. Accumulation of α-synuclein in the IMM led to the mitochondrial dysfunction, activation of ROS production by these organelles, and mitophagy [46, 142]. It was also found that overexpression of α-synuclein in the substantia nigra of the rat brain promoted its interaction with VDAC1 and mPTP opening, thus causing degeneration and death of dopaminergic neurons [54, 55]. Accumulation of α-synuclein in the neurons of substantia nigra in Parkinson's disease patients has been shown to downregulate VDAC1 expression, which could lead to the disruption of calcium homeostasis and promote mitochondrial dysfunction and cell death [143].

VDACs and diabetes mellitus. The development of diabetes mellitus is accompanied by changes in the content of VDAC1 in the mitochondria, which may be one of the pathogenetic factors in this pathology [4, 12, 28, 144]. However, changes in the expression level of mitochondrial VDAC1 are tissue-specific [28, 144-148]. It has been established that during hyperglycemia, VDAC1 in pancreatic beta cells is erroneously translocated to the cytoplasmic membrane, which contributes to a significant decrease in the ATP pool and impairs insulin secretion [28]. It was suggested that VDAC1 overexpression can lead to the apoptotic death of coronary endothelial cells in a mouse model of diabetes [144]. Recently, it was found [149] that the skeletal muscle mitochondria of the offspring of female Japanese macaques fed a Western-style diet (high in carbohydrates and saturated fats) underwent metabolic reprogramming characterized, among other things, by a decreased content of VDAC proteins and respiratory complex I, and this correlated with the reduction in the biomarkers of oxidative stress. The authors suggested that the decrease in the content of these proteins may be an adaptive response to the diabetes development at the early stage of animal's life, aimed at reducing excessively generated ROS. It is important to note that suppression of VDAC oligomerization with the new inhibitor VBIT-4 helped to eliminate mitochondrial dysfunction in endothelial cells upon hyperglycemia induction and also attenuated the development of experimental diabetes in mice [28, 148]. The knockdown of the *VDAC1* gene in human skin fibroblasts facilitated suppression of negative effects of hyperglycemic stress [148]. Therefore, genetic or pharmacological modulation of VDAC1 may suppress the negative effects of chronic high glucose on the mitochondrial function in different types of cells.

VDACs and infectious diseases. VDAC is a molecular target of viruses and, therefore, plays an important role in the development of mitochondrial dysfunction accompanying infectious diseases [4, 12]. Viruses can influence expression of VDACs and directly interact with these proteins. In particular, the hepatitis B virus X protein (HBx) binds to VDAC3, leading to mitochondrial depolarization [150] VDAC1 has been shown to interact with the ORF3 protein of the hepatitis E virus [151]. Human immunodeficiency virus (HIV-1) R protein is also known to interact with VDAC1, which triggers the apoptosis of infected T lymphocytes [152]. The interaction of the influenza A virus PB1-F2 protein with ANT3 and VDAC1 leads to the mPTP induction, mitochondrial depolarization, and release of cytochrome *c*. It is assumed that the death of cells (primarily, immune cells) caused by the PB1-F2 protein through the mitochondrial dysfunction contributes to the influenza virus pathogenicity [153]. A number of studies have also revealed interactions between VDAC1 and the E protein of the Dengue virus [154], VP5 protein of the infectious bursal disease virus [155], and some other viral proteins, which also upregulated VDAC1 expression and caused the development of infectious process and cell death. The SARS-CoV-2 virus responsible for the COVID-19 pandemic also stimulated VDAC1 expression and caused the development of mitochondrial dysfunction in T lymphocytes [12, 156]. In this case, the VBIT-4 inhibitor was able to block the apoptosis of T lymphocytes [156].

Today, the role of mitochondrial VDAC1-3 proteins as critical coordinating centers ensuring bilateral regulation of processes at the border between the mitochondria and cytosol, as well as integration of mitochondria into metabolic pathways under normal conditions and in pathologies, has become increasingly obvious. Numerous data suggest that the pathophysiological function of VDAC proteins depends on their structural flexibility, which allows them to respond to various stimuli through the conformational switches,

and is also due to the interaction with multiple, strictly compartmentalized proteins of the mitochondrial intermembrane space and cytosol. The determination of the three-dimensional structure, as well as the recognition of different functional properties of the three VDAC isoforms, were important discoveries that have stimulated the search for regulatory molecules of endogenous and exogenous origin. At the same time, the use of existing natural and synthetic VDAC regulators encounters a number of difficulties associated with their low specificity and lack of known mechanisms of action. Addressing these issues may help researchers to pave the way to resolving the long-standing issue of pharmacological regulation of VDAC proteins as promising molecular targets in the treatment of pathological conditions associated with the mitochondrial dysfunctions and metabolic reprogramming.

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