Chapter

VRAC Channels and the Cellular Redox Balance

Alessia Remigante, Rossana Morabito, Sara Spinelli, Angela Marino, Silvia Dossena and Michael Pusch

Abstract

Volume-regulated anion channels (VRAC) are mainly involved in the regulated transport of osmolytes such as ions or small organic compounds across the plasma membrane during anisosmotic cell swelling. However, they also play additional roles in various pathophysiological processes, such as the transport of metabolites and drugs, extracellular signal transduction and anti-cancer drug resistance. These channels are formed by heteromers of LRRC8 proteins, of which LRRC8A is the essential subunit that combines with its paralogs LRRC8B–E to form hexameric complexes. Despite the extensive research devoted to the understanding of VRACs functions, different aspects of these channels are still to be characterized in depth. In this chapter, recent findings concerning the involvement of VRAC channels in the cellular redox balance will be summarized. Also, their relevance as potential targets of antioxidant therapies will be discussed.

Keywords: VRAC channels, oxidative stress, metabolite transport, drug resistance, cancer

1. Introduction

In physiological conditions, the production of reactive oxygen and nitrogen species (ROS/RNS) generated during cellular metabolism in biological systems is balanced by the ability of the latter to defend themselves through their antioxidant machinery [1, 2]. Nevertheless, when oxidants are produced in excess, or when the endogenous antioxidant defenses are inefficient, this balance could be perturbed, thus resulting in oxidative stress [3]. In these conditions, biomolecules can be altered via oxidation to an extent that exceeds repair capacity.

The term 'reactive species' (RS) is associated to a collection of highly reactive chemicals resulting from metabolic reactions that use oxygen and might represent a disturbance in the equilibrium of pro-oxidant/anti-oxidant reactions in living organisms. RS include the superoxide anion ($O_2^{\bullet -}$), which is generated at the level of the mitochondrial electron transport chain during energy transduction and can be converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) or by spontaneous dismutation. In the presence of transition metal ions, i.e. iron and copper ions, H_2O_2 can generate the highly reactive hydroxyl radical ($HO^{\bullet}$) via the Fenton reaction

[4]. Additionally, the free form of nitric oxide (NO) can react with $O_2^{\bullet -}$ to form the peroxynitrite anion ($ONOO^-$) and peroxynitrite radical ($ONOO^\bullet$) or, alternatively, the inducible nitric oxide synthase (iNOS) enzyme can form nitric oxide radicals ($NO^\bullet$) [5]. Defense systems include non-enzymatic molecules, such as glutathione, vitamins A, C, and E, and several anti-oxidants present in foods, as well as enzymatic scavengers of ROS, such as SOD, catalase (CAT), and glutathione peroxidase (GPx). RS not only lead to cell injury or death, but also have crucial physiological and signaling functions [6]. In fact, RS are well recognized for playing a dual role, since they can be either harmful or beneficial to living systems [7]. For example, ROS production is essential to maintain cellular functions and ensure cellular survival; this is achieved via the activation of transcription factors, such as NF-kappa-B (NF-κB) and hypoxia-inducible-factor-1α (HIF-1α). ROS have also been recognized to act as signaling intermediates for cytokines, including tumor necrosis factor (TNF-α) and interleukin-1β (IL-1β) [8].

As mentioned above, although mild concentrations of ROS/RNS play key roles in physiological processes, their over-production and failure of intracellular anti-oxidant defenses lead to oxidative injury [9]. Abnormal levels of RS can damage cellular proteins, lipids, or DNA by inhibiting their normal function. Because of this, oxidative stress has been involved in a number of human diseases as well as in the aging process [10–17]. The sensitive balance between beneficial and harmful effects of free radicals is a very important aspect of living organisms and is achieved by mechanisms called redox regulation [18]. In fact, the redox equilibrium is essential in preserving the correct functionality of vital cellular functions.

By representing the boundary between the cell interior and the extracellular environment, cell membrane is most vulnerable to free radical attack. The plasma membrane is characterized by a wide range of proteins and lipids that elicit distinct cellular reactions in response to extracellular stimuli and stressors and each of these can be target of oxidative stress. Among plasma membrane proteins, ion channels are multimeric proteins forming ion-selective pores that open or close in response to specific stimuli such as membrane potential, ligand binding, temperature, and mechanical stimuli [19]. Reactive oxygen and nitrogen species can directly induce post-translational modification of ion channels leading to oxidation, nitrosylation, and/or nitration of specific amino acid residues—sulfhydryl groups or disulfide linkages involving cysteine residues—or indirectly modulate channel activity by affecting the signaling pathways that control gene transcription as well as protein trafficking and turnover [20]. Multiple studies have reported the involvement of ion channels in the development of pathologies where oxidative stress plays a major role [16, 21–24]. Ion channel activity can be stimulated or blocked during oxidative stress and, in turn, these molecular entities can even be involved in determining or attenuating oxidative stress levels. Indeed, the relationship between ion transport and cellular redox balance is very complex. In this context, the ubiquitously expressed volume-regulated anion channels (VRACs) play an important role because they can be either activated or inhibited by oxidative stress depending on their subunit composition and because VRAC activity in turn has an influence on cell oxidative stress [25, 26].

This chapter will provide a broad overview of the impact of oxidative stress on VRAC channels, which are essential for maintaining crucial homoeostatic functions, such as the regulation of cell volume. However, VRAC channels have other roles beyond cell volume regulation. These additional roles, including the transport of organic osmolytes and metabolites that are involved in cellular signaling and even

clinically important drugs, will be discussed in the context of oxidative stress-related conditions. Finally, their relevance as potential targets of antioxidant therapies will be emphasized.

2. Structure-function relationships of VRACs

Volume-regulated anion channels (VRACs) are important constituents of the cellular response to osmotic swelling. This process occurs as a consequence of aquaporin-mediated water influx into cells under hypotonic conditions. Cell swelling is a threat to normal cell function and may lead to cell bursting. To prevent the volume increase and avoid osmotic lysis, different ion transport proteins are activated to enable a controlled efflux of ions, organic osmolytes, and consequently water, and allow cells to restore their original state [27]. The proteins involved in this phenomenon, which is called regulatory volume decrease (RVD), include several cation-selective ion channels, secondary-active transporters, and VRACs. In particular, RVD involves the exit of potassium (K^+) and chloride (Cl^-) through electroneutral K^+/Cl^- cotransporters or through the parallel activation of K^+ and Cl^- channels, including VRACs, as well as an efflux of organic osmolytes through VRACs [28]. The ion currents of VRACs have been identified more than 30 years ago in lymphocytes and cultured epithelial cells [29, 30] and since then have been found to be ubiquitously present in practically every vertebrate cell type studied [31–33]. These currents show moderate outward rectification, a variable degree of inactivation at positive voltages and a $I^- > Cl^- > Br^-$ selectivity. VRACs are reported to require intracellular ATP and basal levels of intracellular calcium and can be inhibited by various drugs, none of which is, however, specific [34]. In addition to their participation in RVD mechanisms, VRACs have also been proposed to play a role in apoptosis, cell proliferation and migration, cancer drug resistance, membrane potential modulation, cell–cell communication, secretion and epithelial transport [28, 35–38]. The identification of the genes that encode the protein(s) underlying VRACs has been challenging. Although investigated by electrophysiology for decades, the molecular identity of VRACs was unknown until 2014, when two groups independently identified LRRC8A as an essential component of the channel [39, 40]. LRRC8A is a member of the LRRC8 protein family, which is found exclusively in vertebrates. In mammals, the family encompasses five paralogues (LRRC8A–E), all of which share a high degree of sequence similarity [41]. Voss et al. demonstrated that VRACs are hetero-multimers composed of the essential LRRC8A subunit and one or more among the LRRC8B-E subunits [40]. Each LRRC8 subunit consists of ~800 amino acids with a molecular mass of ~95 kD. These are characterized by a transmembrane pore domain that is remotely related to pannexin proteins, followed by a C-terminal domain containing 15–17 predicted leucine-rich repeat.

The general structure of VRACs was revealed from cryogenic electron microscopy (cryo-EM) of homomeric LRRC8A in 2018 [42]. Structure–function studies of VRAC heteromeric channels are complicated by their likely variable and experimentally uncontrollable stoichiometry. Indeed, the organization of heteromeric channels has remained elusive. Recently, two cryoEM structures of heteromeric LRRC8A/LRRC8C complexes confirmed a hexametric architecture, albeit with different stoichiometries [43, 44]. In contrast, unphysiological LRRC8C homomers, and functional homomeric chimeric LRRC8C channels harboring an intracellular loop from LRRC8A, displayed a presumably non-physiological heptameric stoichiometry [43, 44]. VRACs are permeable to Cl^- ions but, depending on their subunit composition, exhibit several

biophysical properties and significant permeability to various substrates that vary strongly in charge and size. To name just a few examples, LRRC8A/D heteromers have been shown to be involved in the transport of large molecules such as neurotransmitters (Gamma-aminobutyric acid—GABA—and glutamate) from astrocyte as well as different amino acids (taurine, lysine and aspartate) [45] or nucleotides such as ATP [46], but are also involved in the uptake of antibiotics and some anticancer drugs (cisplatin and carboplatin) [28]. Conversely, the combination of LRRC8A/C shows a significant conductance to Cl^- but is less permeable to larger anions, while LRRC8A/E heteromers are more permeable to aspartate and negatively charged osmolytes. VRACs currents begin to activate within seconds after cell swelling is induced and can take up to different minutes to reach their maximum activity. A distinctive characteristic of VRACs composed of different subunits is the degree and kinetics of current inactivation at positive voltage [47]. Complexes formed by LRRC8A/E subunits show profound and rapid inactivation at positive voltages, similar to what is seen, for example, for endogenous VRAC in glioblastoma cells [48]. Heteromeric LRRC8A/D channels exhibit similar but slower and less pronounced inactivation, while LRRC8A/C heteromers show much less and slower inactivation, similar to VRAC channels in lymphocytes or pancreatic cancer cells (Panc-1) derived from a pancreatic duct carcinoma [49].

Signaling pathways other than an osmotic cell swelling were reported to facilitate VRAC activation, often under isotonic conditions. These include G-protein-coupled receptors, purinergic signaling, ROS, calcium signaling, and phosphorylation cascades [50]. Despite the plethora of findings on VRACs, many aspects of these anion channels are still to be discovered. One of the biggest challenges is to unravel the complex relationship between VRACs and the cellular redox balance. In general, VRAC activation is associated with an increase in ROS levels [26, 51, 52]. However, the main problem regarding the role of ROS in the VRAC channel activation is whether oxidation directly acts on the channel-forming proteins (i.e. LRRC8 proteins) or other intracellular cellular components involved in the activation machineries.

3. VRAC channels and the redox balance: a complex interplay

3.1 VRAC-generated currents during ROS production

As the molecular entity of VRACs has remained unknown until recently although their biophysical characteristics, as well as diverse physio-pathological roles have been extensively studied, for decades the only way of characterizing their functions has been the use of inhibitors. Unfortunately, none of these chemicals was specific enough to unequivocally discriminate the VRAC channel component in the various physio-pathological mechanisms. Thus, studies published before the definition of the molecular identity of VRACs that relied on unspecific inhibitors will not be considered here, as these inhibitors could have targeted other channels and/or other cellular targets and could have led to incorrect interpretations. Nevertheless, several important discoveries on VRAC regulation by ROS have been made in these years by studying the VRAC-mediated currents.

In 2004, it has been demonstrated that H_2O_2 triggers a Cl^- conductance with VRAC properties (outward rectification, deactivation at positive potential, and inhibition by DCPIB). Exogenous H_2O_2-activated VRAC currents were inhibited by both CAT and the reducing agent dithiothreitol (DTT) in Hela and HTC cell lines,

respectively [53, 54]. Instead, other studies have shown that H_2O_2-activated VRAC currents were inhibited by the ROS scavenger N-acetyl-L-cysteine (NAC) [55]. More recently, this phenomenon has been further confirmed by demonstrating that treatment of neurons with H_2O_2 was able to induce VRAC activation [56]. Many studies have also reported a direct involvement of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase, NOX) family members in the VRAC activation during hypotonic stimulation. NOXs are key producers of ROS in many cells and their activation leads to the reduction of oxygen into $O_2^{\bullet-}$, which is spontaneously or enzymatically converted into H_2O_2. Specifically, Crutzen and collaborators [52] have shown the complex co-regulation of VRACs and ROS in pancreatic β-cells. H_2O_2 was able to trigger VRAC currents and insulin release, a process inhibited by both NOX4 inhibitors and ROS scavengers. Inhibition of VRAC channels reduced oxidative stress levels as well as insulin release. Additionally, Deng and co-authors demonstrated that VRAC activation was dependent on ROS produced by NOX2 in cardiac myocytes [57].

Other stimuli have been reported to activate VRACs. To name just a few examples, acute exposure to the epidermal growth factor (EGF), known to increase intracellular ROS levels, has been shown to activate VRACs in a process inhibited by diphenyleneiodonium (DPI) [54]. Staurosporine (STS), known to induce apoptosis, activated a normotonic cell shrinkage called Apoptotic Volume Decrease (AVD) via ROS increase, which in turn activated VRAC channels. STS-triggered VRACs currents, AVD as well as caspase-3 activation, were inhibited by DPI and NAC [55, 58]. Finally, in a follow up manuscript, Deng et al. also demonstrated that the anti-retroviral drugs, namely ritonavir and lopinavir, could induce VRAC activation through mitochondrial ROS production [59].

3.2 Regulation of LRRC8/VRAC activity by oxidation and reduction

After the identification of LRRC8 proteins underlying VRACs, numerous investigations have been carried out to establish the impact of oxidative stress on VRAC activity. However, the results are very divergent, most likely due to differences in the LRRC8 subunit composition and the cellular context. Different subunit combinations trigger currents with specific biophysical properties in terms of open probability, ion selectivity and single channel conductance [60]. In 2017, it was found that C-terminally tagging LRRC8 proteins with fluorescent proteins gives rise to constitutive currents in *Xenopus* oocytes [61]. This allowed to clarify direct effects of oxidizing and reducing agents on LRRC8-mediated VRAC channel function. Gradogna and collaborators shed light on the modulation of VRAC currents by oxidation [26, 62]. Heterologous co-expression of LRRC8A/C, A/D and A/E isoforms in *Xenopus* oocytes resulted in currents with differential sensitivity to the oxidant Chloramine-T (Chlor-T) as well as tert-butyl hydroperoxide (TBHP). In particular, Chlor-T activated A/E channel currents, but inhibited A/C and A/D channel currents. Conversely, TBHP was able to induce activation of A/E channel currents, but no effect on LRRC8A/C and LRRC8A/D channel currents was shown, respectively (**Figure 1**) [26]. Such a differential effect of Chlor-T on the different heteromers strongly suggested that the relevant oxidation reactions directly affect the channel protein. In this regard, Bertelli and co-workers later identified two cysteines, C424 and C448, in LRRC8E as the targets of oxidation. Probably, oxidation results in the formation of a disulfide bond between the two cysteines, which, in turn, induces a conformational change leading to channel activation (**Figure 1**) [63]. Instead, the lack of effects of cysteine modifiers on the Chlor-T response of LRRC8A/C heteromers suggested that their inhibition is unlikely mediated

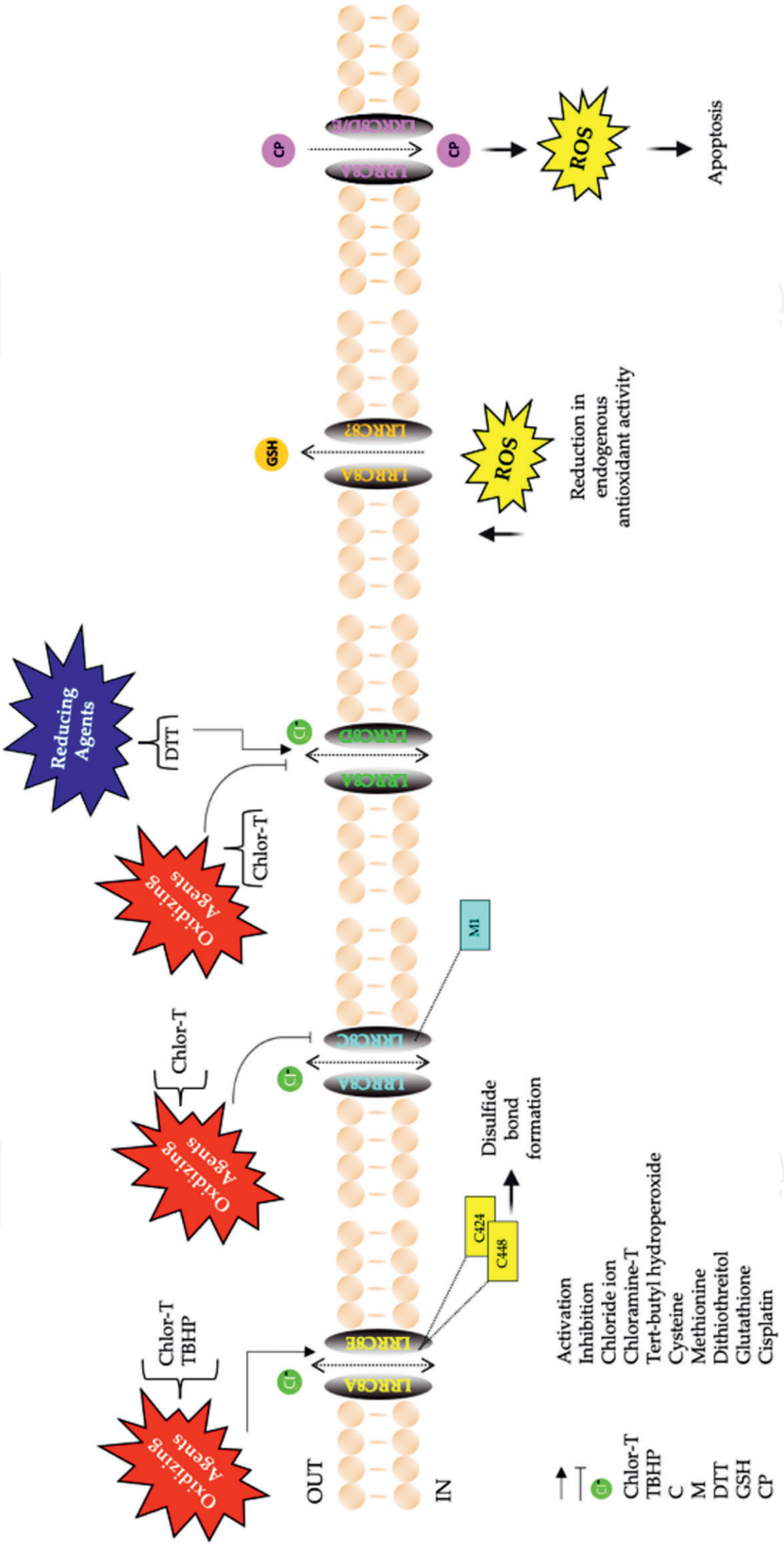

Figure 1.
Regulation of the volume-regulated anion channels (VRACs) by oxidation depending on the LRRC8 subunit composition. Amino acids targeted by oxidation are boxed. The role of VRACs in the permeation of glutathione and cisplatin is also shown.

by oxidation of cysteine residues. In fact, LRRC8C inhibition was caused by oxidation of the first methionine (**Figure 1**) [63]. In addition, the same group has also shown that Chlor-T inhibits the hypotonicity-triggered current of endogenous LRRC8/VRACs in Jurkat cells expressing mainly LRRC8A/C [26]. On the contrary, DTT exposure induced a significant increase of the hypotonicity-induced VRAC current in HEK293 cells, which mainly express the LRRC8A/D subunit combination (**Figure 1**) [64]. Therefore, differential VRAC sensitivity to oxidative stress could be the result of a modification in their subunit stoichiometry.

3.3 Modulation of redox balance by LRRC8/VRAC

As illustrated above, VRAC activity appears to be directly modulated by oxidizing and reducing agents, with an effect depending on the cellular context and composition in LRRC8 subunits. However, there are also different pieces of evidence showing that VRAC activity can, in turn, modulate the cellular redox balance.

Tumor necrosis factor-α (TNFα) stimulates the production of superoxide $O_2^{\bullet-}$ by activating Nox1 at the plasma membrane. Superoxide $O_2^{\bullet-}$ production continues within signaling endosomes formed by TNFα receptor internalization. Inhibition of Nox1 activity blocks TNFα inflammatory signaling in vascular smooth muscle cells (VSMCs). In this context, Choi and co-authors demonstrated that LRRC8A/VRAC is part of the multi-protein Nox1 signaling complex in VSMCs [65]. Specifically, LRRC8A associates with NOX1, and VRAC activity is required for Nox1 activation in response to TNFα. In VSMCs, LRRC8A/C knockdown inhibited TNFα-induced $O_2^{\bullet-}$ production and NF-κB activation, while LRRC8A/D knockdown enhanced NF-κB activation, thus increasing the inflammation state. Thus, changing patterns of LRRC8 isoform expression and differential regulation by oxidation may represent important mechanisms for modulation of the inflammatory response. These findings suggest the presence of different distinct LRRC8 heteromers possessing their own regulation mechanisms and functions during redox balance variations rather than a unique VRAC. Additionally, endothelial VRAC has been proposed to be mechanosensitive in the regulation of vascular functions including vascular tone, blood pressure, and blood flow. In particular, endothelial LRRC8A regulates AKT-endothelial eNOS signaling, thus forming a GRB2-Cav1-eNOS signaling complex, which is required for endothelial cell alignment to laminar shear flow [66]. Endothelium-restricted LRRC8A KO mice develop hypertension in response to chronic angiotensin-II infusion and exhibit impaired retinal blood flow with both diffuse and focal blood vessel narrowing in the setting of Type 2 Diabetes mellitus (T2D). These data demonstrate that LRRC8A regulates AKT-eNOS in endothelium and is required for maintaining vascular function, particularly in T2D [66].

Wang et al. showed a Cl^- current mediated by VRAC channels in 77% of isolated nodose neurons after a brief exposure to extracellular acid pH. This activation involved proton efflux, intracellular alkalinity, and an increase in NOX-derived H_2O_2 [56]. The molecular identity of VRAC was confirmed by cre-flox-mediated KO, shRNA-mediated knockdown, and CRISPR/Cas9-mediated LRRC8A deletion in HEK cells and in primary nodose neuronal cultures, respectively. Activation of VRAC channel by low pH reduced neuronal damage during simulated ischemia and N-methyl-D-aspartate-induced apoptosis. These findings identify VRAC as a dual sensor of hypo-osmolarity and low pH in vagal afferent neurons and determine the mechanisms of its activation and its neuroprotective potential.

Glutathione (GSH) contributes to the regulation of the redox state within the cell. This tripeptide reacts with peroxide residues and subsequently scavenges ROS while being oxidized to glutathione disulfide (GSSG). The intracellular concentration of GSH is in the mM range, which is 10 to 1000-fold higher than its extracellular levels. Thus, GSH transporters and channels usually support an efflux of GSH and consequently lower the cellular antioxidant potential [12, 67] or increase the antioxidant defense of the mucosal surface, as in the case of CFTR [68]. The first evidence of GSH efflux during RVD was shown in 1990 by Hussinger and co-authors [69] in hepatocytes. Afterwards, Sabirov et al. showed that the osmosensitive release of GSH in rat thymocytes was inhibited by VRAC inhibitors, including DCPIB [70]. Recently, a significant permeability to GSH of VRAC channels has been also demonstrated in epithelial-to-mesenchymal transition (EMT), a cellular process in which an increase in the cellular oxidative status is a crucial step [71]. First, the authors showed that a hypotonic condition induced a LRRC8/VRAC-dependent GSH conductance and a decrease in intracellular GSH levels in HEK293-WT cells (**Figure 1**). GSH currents and GSH intracellular decrease were both inhibited by DCPIB and were not observed in HEK293-LRRC8A KO cells. Successively, renal proximal tubule epithelial (HK2) cells were exposed to the pleiotropic growth factor TGFβ1 (a key inducer of EMT), and the contribution of LRRC8/VRAC in this process by measuring EMT marker expression, cell morphology and increase in migration ability were measured. Interestingly, pharmacologic targeting of LRRC8/VRAC with DCPIB or RNA interference-mediated inhibition of LRRC8A attenuated the TGFβ1-induced EMT response both at the gene and protein levels by controlling GSH and ROS levels in HEK293 and HK2 cell lines. These findings suggest that LRRC8/VRAC plays a critical role in EMT owing to its native permeability to GSH and thus its ability to modulate ROS levels and might contribute to other physiological and pathophysiological processes associated with oxidative stress.

Cisplatin is a widely used and highly effective chemotherapy medication with the ability to crosslink with the purine bases on the DNA, which interferes with DNA repair mechanisms, causes DNA damage, and subsequently induces apoptosis in cancer cells [72]. Oxidative stress plays a major role in cisplatin mechanism of action and, at the same time, is one of most important processes involved in cisplatin toxicity. The first evidence of a permeation of cisplatin via LRRC8/VRAC was provided by Planells-Cases et al. in 2015 [73]. In their seminal work, these authors showed that about 50% of cisplatin uptake in isotonic conditions depended on LRRC8A and LRRC8D, but not LRRC8C or LRRC8E, and was strongly enhanced by cell swelling. These findings imply that downregulation of VRAC activity is causatively involved in acquisition of cisplatin resistance in cancer cells and were later confirmed by other studies [74–77]. In addition, exposure to cisplatin dramatically activated constitutively active forms of LRRC8A/E and LRRC8A/D expressed in *Xenopus* oocytes. These results strongly suggest that cisplatin entered the oocytes through the constitutively active LRCC8 channels and further stimulated the channel activity [78], possibly via oxidative stress. Overall, these studies support the concept that modulation of LRRC8A/VRAC activity and subunit composition has an impact on cisplatin sensitivity of cancer cells through two distinct effects, i.e. limitation of drug uptake as well as impairment of the initiation of AVD and intracellular apoptotic cell signaling (**Figure 1**).

Bach and collaborators have demonstrated that both ROS and the cell growth-associated kinases Akt/mTOR play a role in the regulation of VRAC in human alveolar cancer cells [79]. Long-term exposure to hypotonicity, cisplatin, and ROS impaired VRAC activity through oxidation of phosphatases and/or kinases in the PI3K/Akt/

mTORC2 pathway rather than reduction in LRRC8A expression or availability at the plasma membrane. Instead, an inhibition of LRRC8/VRAC current was paralleled by an increase of LRRC8A expression, which correlated with an increased activation of the pro-apoptotic transcription factor p53. This evidence suggests that LRRC8A protein expression increase can be associated with reduced VRAC activity and can be identified as an indicator of cellular injury.

4. Conclusions and remarks

Molecular and electrophysiological approaches have explored the functional role of VRAC in the redox balance and have offered insights into the underlying mechanisms to some degree. VRAC activity could be directly modulated by oxidation or reduction depending on the different combinations of LRRC8 subunits and, alternatively, VRAC function could affect the redox balance via the involvement of intracellular specific pathways. However, further investigation of the specific molecular mechanisms that modulate VRAC function in oxidative stress-related processes, including cancer, will be needed. The development of specific inhibitors and/or agonists of LRRC8/VRAC would be of great significance to promote research in this field.

Conflict of interest

The authors declare no conflict of interest.

Nomenclature and abbreviation

ATP	adenosine triphosphate
AVD	apoptotic volume decrease
C424	cysteine
C448	cysteine
CAT	catalase
Cl^-	chloride
Chlor-T	chloramine
Crio-EM	cryogenic electron microscopy
DPI	diphenyleneiodonium
DTT	dithiothreitol
EGF	epidermal growth factor
EMT	epithelial-to-mesenchymal transition
GABA	gamma-aminobutyric acid
CFTR	cystic fibrosis transmembrane conductance regulator
GPx	glutathione peroxidase
GSH	gluthatione
K^+	potassium
GSSG	glutathione disulfide
H_2O_2	hydrogen peroxide
HIF-1α	hypoxia-inducible-factor-1α
HK2	renal proximal tubule epithelial cell
$HO^•$	hydroxyl radical

IL-1β	interleukin-1β
iNOS	oxide synthase
LRRC8	leucine-rich repeat 8
NAC	N-acetyl-L-cysteine
NADPH	nicotinamide adenine dinucleotide phosphate
NF-κB	nuclear factor κB
NO	nitric oxide
$NO^{\bullet}$	oxide radical
NOX	NADPH-oxidase
$ONOO^{-}$	peroxynitrite anion
$ONOO^{\bullet}$	peroxynitrite radical
$O_2^{\bullet -}$	superoxide anion
ROS	reactive oxygen species
RS	reactive species
RNS	reactive nitrogen species
RVD	regulatory volume decrease
SOD	superoxide dismutase
STS	staurosporine
T2D	type 2 diabetes
TBHP	tert-butyl hydroperoxide
TGF-β1	transforming growth factor
TNF-α	tumor necrosis factor
VRAC	volume-regulated anion channel
VSMC	vascular smooth muscle cell

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