



## ORAI channels in cellular remodeling of cardiorespiratory disease

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### ABSTRACT

Cardiorespiratory disease, which includes systemic arterial hypertension, restenosis, atherosclerosis, pulmonary arterial hypertension, asthma, and chronic obstructive pulmonary disease (COPD) are highly prevalent and devastating diseases with limited therapeutic modalities. A common pathophysiological theme to these diseases is cellular remodeling, which is contributed by changes in expression and activation of ion channels critical for either excitability or growth. Calcium ( $\text{Ca}^{2+}$ ) signaling and specifically ORAI  $\text{Ca}^{2+}$  channels have emerged as significant regulators of smooth muscle, endothelial, epithelial, platelet, and immune cell remodeling. This review details the dysregulation of ORAI in cardiorespiratory diseases, and how this dysregulation of ORAI contributes to cellular remodeling.

### 1. Introduction

Cardiovascular disease and chronic lower respiratory diseases are the first and fourth leading causes of death in the United States [1]. Over one in three Americans suffers from cardiovascular disease or chronic lower respiratory diseases (hereafter referred to as cardiorespiratory disease), and recent epidemiological evidence suggests that the prevalence of these diseases is on the rise [2–4]. These findings indicate that current therapeutic modalities are insufficient, and the need to identify novel molecular targets to treat these diseases.

A common theme in cardiorespiratory diseases is the molecular and cellular remodeling from a normal physiological phenotype to a dysfunctional diseased phenotype [5,6]. The ubiquitous intracellular second messenger, calcium ( $\text{Ca}^{2+}$ ), is necessary for many cellular functions such as secretion, exocytosis, contraction, metabolism, and activation of transcriptional programs that support proliferation and migration [7].  $\text{Ca}^{2+}$  signaling operates in highly specific spatio-temporal domains, and the molecular processes maintaining this specificity are exceedingly malleable and hence prone to dysfunction during cellular remodeling [8]. Dysfunction in  $\text{Ca}^{2+}$  signaling in cardiorespiratory disease has been previously described and is beyond the scope of this review (refer to reviews [9,10]). One significant modulator of  $\text{Ca}^{2+}$  signaling that has recently emerged to be dysregulated in cardiorespiratory diseases is the ORAI family of  $\text{Ca}^{2+}$  channels. ORAI

are highly  $\text{Ca}^{2+}$  selective channels located on the plasma membrane (PM) and are typically activated by the endoplasmic reticulum (ER) transmembrane proteins, Stromal Interaction Molecule (STIM), upon ER  $\text{Ca}^{2+}$  store depletion [11–13]. In addition, ORAI1/3 heteromeric channels have been described to be activated independently of ER  $\text{Ca}^{2+}$  store depletion by arachidonic acid and its metabolite, leukotriene  $\text{C}_4$  ( $\text{LTC}_4$ ) [14,15]. Activation of ORAI prompts large temporal  $\text{Ca}^{2+}$  signals that stimulate transcription of proliferative and migratory genes [16]. Herein, we describe how dysregulation in ORAI channels contribute to cardiorespiratory disease and the potential of these channels as attractive targets for future disease therapy. We briefly summarize the mechanisms of activation of ORAI channels and discuss their role in systemic arterial hypertension, restenosis, atherosclerosis, pulmonary hypertension, asthma, and chronic obstructive pulmonary disease (COPD).

### 2. Brief overview of ORAI channels

Before the discovery of the molecular identity of ORAI channels, the original idea that  $\text{Ca}^{2+}$  influx across the plasma membrane is stimulated by the fall of  $\text{Ca}^{2+}$  concentration within ER lumen was first introduced by Jim Putney in 1986 and originally termed capacitative  $\text{Ca}^{2+}$  entry [17]. This nomenclature was subsequently abandoned in favor of store-operated  $\text{Ca}^{2+}$  entry (SOCE), which implicitly indicate

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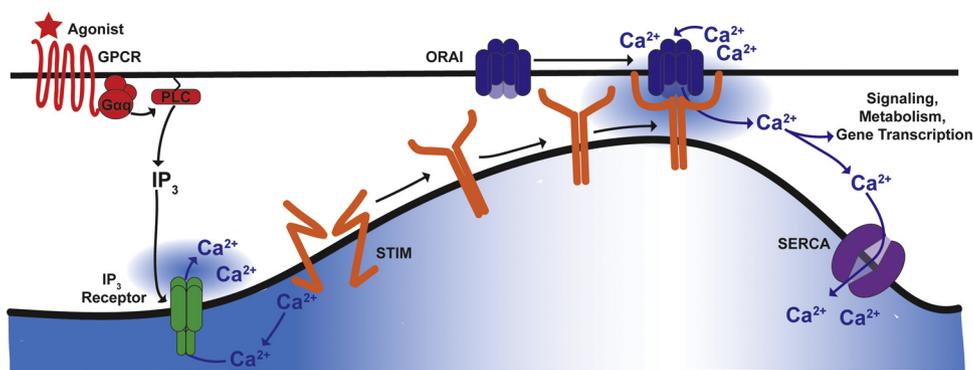
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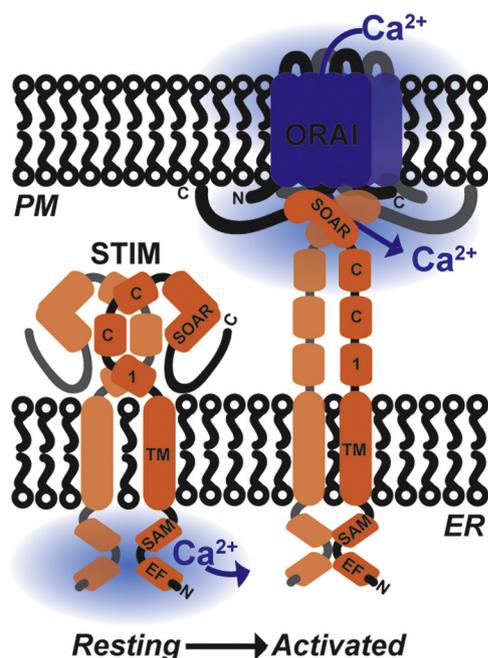
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**Fig. 1. SOCE mediated by ORAI channels.** Binding of agonists to PLC-coupled receptors generates the secondary messenger IP<sub>3</sub>. IP<sub>3</sub> induces ER Ca<sup>2+</sup> release through the IP<sub>3</sub> receptor. Following ER Ca<sup>2+</sup> release, Ca<sup>2+</sup> dissociates from the EF hand of STIM and triggers STIM to oligomerize, migrate towards the ER-PM junction, trap, and activate ORAI channels. This activation causes a large Ca<sup>2+</sup> influx from the extracellular milieu into the cytosol, which refills ER Ca<sup>2+</sup> stores through SERCA and create cytosolic Ca<sup>2+</sup> microdomains that are sensed by downstream cell signaling effectors and transcription factors, activating gene programs to support metabolism, proliferation and migration.



**Fig. 2. Mechanism of SOCE activation.** Under resting conditions, STIM proteins exist as inactive dimers in the ER membrane. STIM low-affinity luminal EF hand domains are bound to Ca<sup>2+</sup>, and the inhibitory coiled-coil-1 (CC1) domain occludes the STIM-ORAI activating region (SOAR). Upon ER Ca<sup>2+</sup> store depletion, Ca<sup>2+</sup> dissociates from EF hand domains, which causes STIM to undergo a conformational change and gain an extended conformation, which exposes its cytosolic SOAR domain. SOAR dimers are then able to physically trap and gate PM-located ORAI Ca<sup>2+</sup> channels leading to activation of SOCE.

that activation of Ca<sup>2+</sup> influx from the outside is controlled by the state of filling of ER Ca<sup>2+</sup> stores. Physiologically, binding of agonists to receptors coupled to phospholipase C (PLC) isoforms causes the cleavage of the acidic lipid phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) into the soluble head group inositol-1,4,5-trisphosphate (IP<sub>3</sub>) and the membrane-bound diacylglycerol (DAG). Diffusible IP<sub>3</sub> stimulates ER Ca<sup>2+</sup> release by activating the IP<sub>3</sub> receptor [18]. The subsequent depletion of ER Ca<sup>2+</sup> causes Ca<sup>2+</sup> to dissociate from the low-affinity luminal EF-hand domains of STIM proteins (mammals have two STIM homologs, STIM1 and STIM2) [19–22] (Fig. 1 and 2). This dissociation causes STIM to undergo a conformational switch, oligomerize and migrate towards the ER-PM junctions [23,24]. At the ER-PM junctions, STIM exposes its C-terminal STIM-ORAI activating region (SOAR), which physically traps and activates ORAI channels [22,25–27] (Fig. 2). Both STIM1 and STIM2 are capable of activating ORAI1; however, STIM2 is a weaker activator likely due to differences in the SOAR [28,29] and sterile α-motif (SAM) domains [30]. Yet, the EF hand of STIM2 has a

lower affinity for Ca<sup>2+</sup>, which allows STIM2 to activate ORAI1 at lower levels of agonist-induced stimulation and subsequent ER store depletion [31,32]. In whole-cell-patch-clamp electrophysiological recordings, SOCE manifests as a highly Ca<sup>2+</sup> selective inwardly-rectifying current termed Ca<sup>2+</sup> release-activated current (I<sub>CRAC</sub>) [12]. Although very small in size (less than 1 pA/pF), native I<sub>CRAC</sub> has been measured in a variety of cell types [33–37]. Numerous SOCE inhibitors have been used including lanthanides such as Lanthanum (La<sup>3+</sup>) and Gadolinium (Gd<sup>3+</sup>) when used at low concentrations (1–5 μM) [38], 2-Aminoethoxydiphenyl borate (2-APB), 3,5-bistrifluoromethyl pyrazole (BTP<sub>2</sub>), Synta66, and GSK-7975A [13]. However, these SOCE inhibitors have never reached the clinic mostly as a result of either their toxicity or poor specificity. They have been reported to block other Ca<sup>2+</sup> channels including transient receptor potential (TRP) channels and IP<sub>3</sub> receptors [39]. This highlights the need for more detailed studies focusing on ORAI channel structure and regulation, which would help in identifying specific inhibitors.

ORAI1, the canonical ORAI isoform, was identified in 2006 [40–42]. ORAI1 is a 33 kDa protein with four transmembrane domains where both the N- and C-termini are located in the cytosol. The functional channel is a hexamer of six ORAI subunits with the transmembrane domains arranged in concentric layers [43–45]. Mammals have three ORAI homologs (ORAI1–3), and each individual homolog is capable of mediating I<sub>CRAC</sub> when overexpressed with STIM1; however, ORAI2 and ORAI3 mediate smaller currents compared to ORAI1 [46], and with the exception of few specific instances [47–49], their role in mediating native Ca<sup>2+</sup> entry pathways remain largely unknown [50]. ORAI1 also has two variants generated through two alternative translation-initiation sites at Methionine 1 and Methionine 64 from the same messenger RNA. While both the long (ORAI1α) and the short (ORAI1β) mediate I<sub>CRAC</sub> [51,52], ORAI1β has a significantly smaller Ca<sup>2+</sup> dependent inactivation (CDI) [52]. Physiologically, different ORAI homologs and translational variants appear to form heteromeric channels that are differentially expressed depending on cell types [53]. One such heteromeric channel is formed by ORAI1α and ORAI3, is activated by STIM1 independently of depletion of ER Ca<sup>2+</sup> stores and requires instead arachidonic acid or its metabolite LTC<sub>4</sub> [14]. These channels which are also highly Ca<sup>2+</sup> selective are termed arachidonate-regulated Ca<sup>2+</sup> (ARC) channels. Unlike I<sub>CRAC</sub>, I<sub>ARC</sub> is not inhibited by the SOCE inhibitor 2-Aminoethoxydiphenyl borate (2-APB), and their monovalent currents measured under divalent free bath solutions do not rapidly depotentiate [54,55].

At low physiological concentrations of agonist stimulation, the Ca<sup>2+</sup> signal emanating from ORAI1 most notably takes the form of regenerative oscillations. These Ca<sup>2+</sup> oscillations, which are believed to be mediated by the oscillatory activity of IP<sub>3</sub> receptors, quickly run-down in the absence of SOCE that is needed to refill ER Ca<sup>2+</sup> stores [56]. Changes in the frequency and amplitude of Ca<sup>2+</sup> oscillations produce precise spatiotemporal Ca<sup>2+</sup> microdomains with specific signatures that activate distinct Ca<sup>2+</sup>-dependent transcription factors

[57]. These include isoforms of the transcription factor, nuclear factor of activated T cells (NFAT) [58]. The  $\text{Ca}^{2+}$ -dependent phosphatase calcineurin, which localizes to the proximity of ORAI1 channels [59], dephosphorylates NFAT isoforms, and causes their nuclear translocation and subsequent transcriptional regulation [58,60]. Another transcriptional regulator that is specifically activated by the local  $\text{Ca}^{2+}$  signal generated by SOCE is c-Fos, which upon activation forms a transcriptional complex with c-Jun known as AP-1 that regulates the expression of inflammatory genes [61]. SOCE can also lead to phosphorylation of the transcription factor, cAMP response element-binding protein (CREB), which transcribes metabolic genes and indirectly induces cellular proliferation in T lymphocytes [62]. Recently, SOCE has also been shown to suppress the transcriptional coactivators of the Hippo pathway Yes-associated Protein (YAP) and PDZ-binding motif (TAZ) [63].

The physiological significance of ORAI1 is underscored with patients that have loss of function mutations in ORAI1. The most characterized mutation is R91W, which is located on the cytoplasmic side of the first transmembrane domain. These patients have drastically reduced SOCE and  $I_{\text{CRAC}}$  and show severe combined immunodeficiency (SCID), autoimmunity, muscular myotonia, and ectodermal dysplasia [64]. Global ORAI1 knockout mice are small in size, and most die perinatally and fail to thrive [65], suggesting a crucial role for ORAI1 channels beyond their established role in specific immunity. ORAI channels are indeed ubiquitously expressed in all tissues and are increasingly implicated in the regulation of a multitude of cellular functions. Dysregulation in ORAI channels, including in their expression, heteromeric or homomeric associations, post-translational modifications and interactions with other proteins, has been associated with cardiorespiratory disease. Despite that all three ORAI isoforms are ubiquitously expressed, future targeting of ORAI in patients with cardiorespiratory diseases is possible providing we have a thorough understanding of the molecular organization and regulation of this pathway in different tissues. Potential differences in regulation, oligomerization status and isoform involvement between different tissues could potentially be exploited to achieve specificity in therapeutic targeting. While side effects from systemic treatments would likely persist, careful understanding of ORAI channel regulation and dysregulation in a specific system combined with localized therapies may provide a therapeutic window to target ORAI in cardiorespiratory diseases. As will be discussed below, a common theme is the contribution of ORAI dysregulation to cellular remodeling in most of these diseases.

### 3. Systemic arterial hypertension

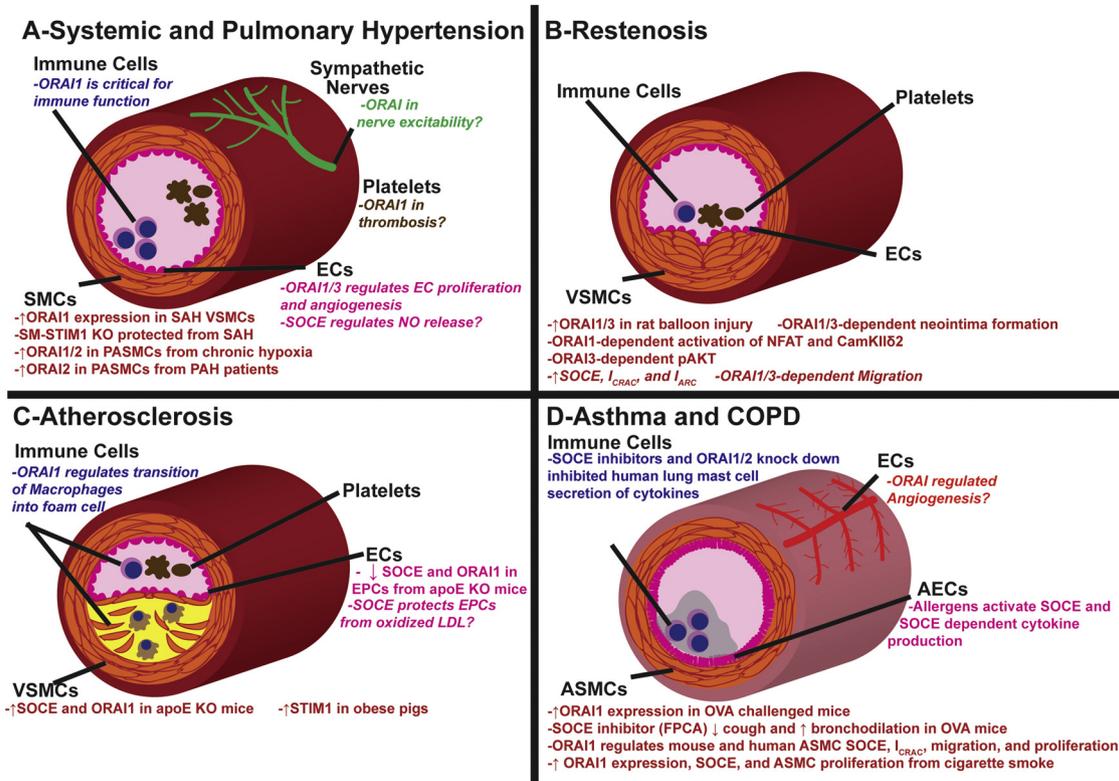
In the vasculature,  $\text{Ca}^{2+}$  signaling is essential for maintaining endothelial cell (EC) integrity, vascular smooth muscle cell (VSMC) tone, and modulating VSMC phenotype. Dysfunction in these  $\text{Ca}^{2+}$  signaling events have been well characterized in systemic arterial hypertension (SAH) (Fig. 3A). A key pathological feature of endothelial cells (ECs) in hypertension is the impaired bioavailability of nitric oxide (NO) and increased secretion of vasoconstrictive mediators and growth factors [66]. These vasoconstrictive mediators and growth factors then directly modulate VSMC tone and remodeling. ORAI1 and STIM1 protein expression, SOCE, and native  $I_{\text{CRAC}}$  have been characterized in endothelial cells [33,67]. Functionally, ORAI1 has been shown to be necessary for EC proliferation, angiogenesis, but not for EC barrier function in response to activation of G protein-coupled receptor (GPCR) agonists [33,68–70]. Decreased EC barrier function manifests with formation of interendothelial gaps, which enhance the permeability of the intimal layer to circulating cells and solutes and play a critical role in vascular disease [71]. Three different GPCR agonists (histamine, thrombin, and sphingosine-1-phosphate) that alter ECs permeability rely on STIM1 but function independently of  $\text{Ca}^{2+}$  release, SOCE, and ORAI1 [68,69]. Studies describing a functional role of endothelial ORAI channels in SAH, such as those using EC-specific knockout mice, are lacking. A

study showed that ORAI3 is necessary for in vitro and in vivo endothelial tube formation in response to vascular endothelial growth factor (VEGF) and that VEGF induces movement of ORAI3 to the PM of ECs through production of  $\text{LTC}_4$  and arachidonic acid [72]. Another group showed that arachidonic acid stimulates NO release by ECs through activation of  $\text{Ca}^{2+}$  entry through TRPV4 channels, subsequent activation of calmodulin, and  $\text{Ca}^{2+}$ /calmodulin-dependent endothelial NO synthase, eNOS [73]. Although further studies are needed to clarify the specific role of ORAI3 in ECs, these studies suggest that TRPV4, ORAI3 (and potentially  $I_{\text{ARC}}$ ) have a protective role by maintaining EC function.

ORAI channels have been more extensively studied in VSMCs [74,75]. Unlike other excitable muscle tissues such as skeletal and cardiac muscle, smooth muscle exhibits tremendous plasticity of phenotype. In healthy normotensive vasculature, VSMCs are tonically constricted and serve to maintain vascular tone. Vascular tone is primarily the result of contractility activated by increased cytosolic  $\text{Ca}^{2+}$  originating from PM-located L-type  $\text{Ca}^{2+}$  Channels (LTCCs) and ryanodine receptors (RyRs) expressed in the SR/ER. Healthy contractile VSMCs are quiescent and normally do not proliferate or migrate towards the vascular lumen [76]. However, aberrant extracellular cues including cytokines, growth factors, hormones, inflammatory mediators, or mechanical stressors causes these quiescent VSMCs to undergo a phenotypic switch to a more proliferative, migratory, or “synthetic” phenotype. Synthetic VSMCs lose their ability to contract due to transcriptional downregulation of contractile proteins including LTCCs, RyRs, smooth muscle myosin heavy chain (smMHC), and smooth muscle  $\alpha$ -actin [76,77]. In exchange, synthetic VSMCs increase expression of proteins that control proliferation and migration and contribute to vascular disease. The phenotypic switch from quiescent to synthetic VSMCs plays a prominent role in hypertension, atherosclerosis, diabetic vascular diseases, artery stenosis, and aneurysms [78]. Interestingly, this switch in phenotype can also be modeled in cell culture by placing freshly dissociated quiescent VSMCs in culture media containing serum [79].

In quiescent VSMCs, STIM and ORAI protein expression, SOCE, and  $I_{\text{CRAC}}$  are barely detectable [36]. Thus, there is a lack of evidence to suggest SOCE has a physiological role in contractile smooth muscle in the systemic vasculature. In contrast, STIM1, ORAI1, SOCE and  $I_{\text{CRAC}}$  are greatly up-regulated in synthetic VSMCs [36,79]. The peptide hormones urotensin-II and angiotensin II, which are well-known mediators of hypertension, and other pro-proliferative and pro-migratory mediators are thought to drive the expression of ORAI1 in VSMC remodeling [80–82]. ORAI1 is necessary for VSMC proliferation and migration, and ORAI1 (but not ORAI2 or ORAI3) is necessary for platelet-derived growth factor (PDGF)-induced SOCE and VSMC migration [83]. ORAI1 associates with other  $\text{Ca}^{2+}$  Channels, and the expression of the Sodium ( $\text{Na}^+$ )- $\text{Ca}^{2+}$  exchanger (NCX) and plasma membrane  $\text{Ca}^{2+}$  ATPase (PMCA) seems to be dependent on ORAI1 in VSMCs [84]. Takahashia et al. showed that in VSMC, phosphorylation of the pro-proliferative transcription factor CREB is dependent on STIM1 [85]. Importantly, smooth muscle-specific STIM1 knockout mice [86] are partially protected against endothelial dysfunction and development of hypertension after angiotensin II infusion [87]. These mice showed significantly blunted systolic blood pressure, ER stress, and cardiac fibrosis in response to angiotensin II infusion [87]. Similar studies using smooth muscle specific ORAI knockout mice have not been reported.

Cytosolic  $\text{Ca}^{2+}$  has been reported to be elevated in hypertensive rat models and patients [88,89]. The role of ORAI in SAH was first studied in 2009 using male spontaneous hypertensive rats (SHR) [90]. Compared to normotensive Wistar Kyoto (WKY) rats, SHR rats had higher protein and mRNA expression of both ORAI1 and STIM1 in the aorta. Treatment of SHR aortic rings with 100  $\mu\text{M}$  of either SOCE inhibitor 2-APB or  $\text{Gd}^{3+}$  or with STIM1 or ORAI1 neutralizing antibodies had reduced force generation to similar levels as WKY rats. However, these data should be interpreted with caution as neutralizing antibodies can



**Fig. 3. Role of ORAI channels in cellular remodeling in cardiorespiratory disease.** Summary of functions of ORAI channels in A) SAH and PAH, B) restenosis, C) atherosclerosis, D) asthma and COPD are listed under each cell type. Marked in bold have been studied exclusively in that disease, while those marked in italic have been speculated based on other tissues or diseases. A question mark indicates that the role of ORAI in this cell type is unknown.

have non-specific effects and the concentrations of 2-APB or  $Gd^{3+}$  used are quite high and were shown in previous studies to interfere with many channels, including  $IP_3$  receptor  $Ca^{2+}$  release channels and various plasma membrane TRP channels [38,91]. A similar study with WKY rats subjected to 30 days of chronic ethanol consumption demonstrated that chronic ethanol caused increased systolic blood pressure (SBP), SOCE, and STIM1 protein expression isolated from aortic tissue [92]. The SOCE inhibitors  $Gd^{3+}$  and SKF 96365 have also been reported to inhibit SBP in rats [93]. However, follow up studies are necessary because the concentrations of SOCE inhibitors used in these studies are relatively high and may have off-target effects. The isolated aortic rings are also a collection of a variety of cell types, and it is unclear which cell type these inhibitors are targeting.

Epidemiological evidence supports the idea that males are more susceptible to hypertension than females [94]. Giachini et al. observed that male SHR aortic rings which generate more contractile force, had enhanced ORAI1 and STIM1 protein and mRNA expression in aortic tissue compared to female SHR rats [95]. Treatment of male SHR aortic rings with ORAI1 or STIM1 neutralizing antibodies restored force contraction to similar levels as SHR females. An ovariectomy in female SHR also increased aortic ORAI1 protein expression and force contraction in aortic rings to similar levels as male SHR, suggesting that estrogen or progesterone can regulate ORAI protein expression and protect against SAH [95]. Interestingly, estrogen has been shown to increase ORAI3 but not ORAI1 protein expression in estrogen-positive breast cancer cells [48]. By virtue of ORAI3 being uniquely involved as an essential component of heteromeric ARC channels, ORAI3 might be required for activating downstream signaling pathways distinct from those activated downstream SOCE [14]. Determination of ORAI3 protein expression and ARC channel activity in female versus male SHR rats has not been undertaken.

Sympathetic nerve fibers maintain vascular homeostasis; they innervate arteriolar VSMCs and secrete norepinephrine (NE) to stimulate

$\alpha_1$  adrenergic receptors on VSMCs to induce vasoconstriction. Hyperactivity of the sympathetic nervous system is a well-known characteristic of hypertension [96]. However, the potential role of ORAI or STIM in sympathetic nerve function is currently unknown. Inflammation has also emerged as a crucial player in the pathogenesis of hypertension [97]. Numerous markers of inflammation are augmented in hypertension including an elevation of the acute phase protein C-reactive peptide (CRP), Interleukin-6 (IL-6), and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) [98]. These inflammatory cytokines recruit leukocytes to the vasculature to further propagate inflammation, endothelial dysfunction, and VSMC remodeling [99,100]. ORAI has been thoroughly described in the context of immunity [40,101–103]. ORAI1 channels are the main source of cytosolic  $Ca^{2+}$  required for activation of the calcineurin-NFAT pathway, which is critical for clonal expansion and secretion of cytokines by immune cells [104]. SOCE is required for T cell, neutrophil, B cell, and natural killer cell function [105]. Neutrophil recruitment to sites of inflamed endothelium is an important step in vascular disease. SOCE mediated by ORAI1 is necessary for neutrophil polarization and arrest at sites of inflamed endothelium [106]. Clinical studies have demonstrated an association between treatment with immunosuppressants (azathioprine, mycophenolic acid, and anti-TNF- $\alpha$  therapy) and a lower SBP [107,108], suggesting that targeting ORAI1 may reduce inflammation to the vasculature and counteract hypertension. T regulatory cells (Tregs), which are circulating immune cells that suppress inflammation in both immune and nonimmune cells through anti-inflammatory cytokines such as Interleukin-10 (IL-10) and transforming growth factor  $\beta$  (TGF $\beta$ ), are reduced in hypertension [109–111]. Adoptive transfer of Tregs into angiotensin II-infused mice enhanced vasodilator response and reduced SBP, vascular stiffness, and aortic macrophage recruitment [112,113], supporting the protective role of Tregs in hypertension. Any potential differences in the mechanisms of activation and regulation of  $I_{CRAC}$  between Tregs and effector T cells that could be exploited for selective

therapy of hypertension remain uncertain.

#### 4. Restenosis and thrombosis

Restenosis is the pathological remodeling and re-narrowing of arteries (most often coronary arteries) after percutaneous angioplasty and stenting, which occurs in 1–3% of patients and often leads to acute myocardial infarction (MI) or acute cardiac arrest [114,115]. Restenosis is the result of neointima formation, which results from VSMC proliferation and migration into the lumen of vessels (Fig. 3B). ORAI1 and STIM1 are upregulated in animal models of neointimal hyperplasia, including the rat carotid balloon injury model [83,116] and the carotid ligation model in mice [116]. Knockdown of either STIM1 or ORAI1 using shRNA-encoding lentiviral constructs inhibited VSMC neointimal hyperplasia [116] and this coincided with decreased expression of CamKII $\delta$  isoform and NFAT nuclear translocation [116]. Both CamKII $\delta$  and NFAT are important for transcribing pro-proliferative and pro-migratory genes [117,118]. Similar results were obtained with STIM1 *in vivo* knockdown [116,119,120], as well as with STIM1 smooth muscle-specific knockout mice subjected to ligation injury [121]. Gonzalez-Cobos et al. showed that ORAI3 was also upregulated in VSMC after balloon injury and is necessary for driving neointimal hyperplasia [122]. In neointimal VSMC, ORAI3 association with ORAI1 produces a heteromeric channel that was not activated upon ER Ca<sup>2+</sup> store depletion but by cytosolic LTC<sub>4</sub> produced from arachidonic acid metabolism downstream receptor activation [15,55,123]. Subsequent studies revealed that this LTC<sub>4</sub>-activated heteromeric ORAI1/ORAI3 Ca<sup>2+</sup> channel is in fact I<sub>ARC</sub>, encoded by the same populations of ORAI1 and ORAI3 in both VSMCs and HEK293 cells [124].

The initial pathological injury in restenosis is denudation of the ECs in the intimal layer. ECs are important for secreting factors like growth factors and NO to maintain the integrity of the basement membrane and to preserve VSMCs in the quiescent state [125]. Loss of this endothelial layer causes a sudden increase in VSMC remodeling [126]. As described above, ORAI and SOCE are essential for endothelial cell proliferation and angiogenesis [33]. The role of EC ORAI channels in restenosis is currently unknown. Whether promoting the expression of ORAI in ECs can salvage the formation of the intimal layer and prevent intimal hyperplasia is an intriguing idea that remains untested. Likewise, endothelial progenitor cells (EPCs) are circulating hemopoietic cells, which can migrate to injured intimal layers. At these sites, EPCs differentiate into mature ECs. Accelerating EPC migration and differentiation can accelerate intimal healing and may prevent restenosis [127]. SOCE has been measured in EPCs, and the SOCE inhibitor BTP-2 caused reduced EPC proliferation [128]. ORAI1 and SOCE were decreased in EPCs during atherosclerosis, which is another disease contributed by endothelial dysfunction [129]. In the context of restenosis, it might be worthwhile to determine ORAI expression and SOCE activation in EPCs as restoring or promoting ORAI in EPCs may have beneficial effects in restenosis.

In most cases of percutaneous angioplasty and stenting, thrombosis is an early and major contributor to myocardial infarction. A necessary step in thrombosis is the binding of agonists like thrombin, thromboxane A<sub>2</sub> (TXA<sub>2</sub>), and ADP to PLC-coupled receptors, which causes an elevation in cytosolic Ca<sup>2+</sup> in platelets [130]. Through the ORAI1 chimera mouse model (an irradiated wildtype mouse infused with the bone marrow of an ORAI1 knockout mouse), ORAI1 channels were shown to be necessary for SOCE in response to thapsigargin, ADP, thrombin, and TXA<sub>2</sub> in platelets [131]. However, platelet aggregation was similar to controls when stimulated with thrombin, ADP, CRP, and collagen, although stimulation with low concentrations of CRP, convulxin, and collagen showed reduced platelet aggregation in ORAI1 chimera mice. Similarly, the activation and exposure of the platelet activation markers glycoprotein IIb/IIIa and P-selectin were identical between ORAI1 chimeras and controls when stimulated with ADP and thrombin; yet, the activation and exposure of these markers were

reduced in ORAI1 chimeras when stimulated with CRP and convulxin. Under shear perfusion on a collagen-coated surface, ORAI1 chimera failed to form stable thrombi. Unlike thrombin and ADP, which activate PLC $\beta$ , CRP, convulxin (CVX), and collagen activate PLC $\gamma$  through the receptor Glycoprotein VI. Perhaps ORAI1 differentially modulates platelet activation and aggregation based on the specific receptor activated. In addition, ORAI1 chimera mice were protected from cerebral ischemia [131]. The same group reported similar findings with STIM1 chimera mice [132]. Treating mice with the SOCE inhibitors 2-APB, Synta66, and GSK-7975A also reduced thrombosis from cerebral ischemia [133]. Another group observed that chimeric mice bearing the mutation found in SCID patients, ORAI1<sup>R93W</sup>, had reduced SOCE in platelets. Only low concentrations of the agonists PAR4p and CVX induced platelet activation and exposure of glycoprotein IIb/IIIa and P-selectin. The platelet activation marker phosphatidylserine (PS) was also reduced in ORAI1<sup>R93W</sup> chimera mice [134]. However, Bergmeier et al. found platelet aggregation to be identical between ORAI1<sup>R93W</sup> chimera mice and controls in response to all agonists. One gain of function (GOF) STIM1 mutant mice (STIM1<sup>D84G</sup>) showed premature platelet activation and macrothrombocytopenia [135] while another GOF mutant STIM1<sup>R304W</sup> had impaired platelet activation possibly due to reduced STIM1 expression in platelets in these mutant mice [132,136]. While these results are quite confusing, what might be the consensus from these studies is that ORAI1 is important for SOCE in platelets but perhaps not for physiological coagulation. However, from the cerebral ischemia studies [131,133], SOCE may play a role in pathological coagulation such as during restenosis, which warrants a comprehensive study. This also suggests there may be a therapeutic window for SOCE inhibitors in thrombosis. The other ORAI homologs are also expressed in platelets but have not been studied in the context of pathological thrombosis.

There is a known inflammatory component to thrombosis as neutrophils, monocytes, and lymphocytes are quickly recruited to the injured site once a thrombus has formed [137]. As mentioned above for the case of SAH, these active immune cells further stimulate VSMC remodeling and endothelial dysfunction [99,100,138]. Inhibition of ORAI in immune cells is expected to prevent immune cell differentiation and activation [105], suggesting that ORAI in immune cells might be a contributor to restenosis.

#### 5. Atherosclerosis

The pathogenesis of atherosclerosis begins as a subendothelial accumulation of lipids in the vasculature known as “fatty streaks.” Fatty streaks stimulate the migration of monocytes and macrophages into the plaques between the ECs and VSMCs (Fig. 3C). At these fatty streaks, these immune cells engulf excessive amounts of lipids and become “foam cells,” which propagate a chronic inflammatory environment in the vasculature. This chronic inflammation activates VSMC remodeling and further induces endothelial dysfunction, which ultimately cause narrowing of the vascular lumen [139]. The apolipoprotein E (apoE) knockout mouse model spontaneously develops hyperlipidemia and atherosclerosis. Assche et al. reported apoE knockout mice have higher ATP induced SOCE in endothelial denuded aortic rings. A substantial SOCE was measured in four month old apoE KO mice, which are too young to develop significant atherosclerotic plaques [140]. This suggests SOCE and VSMC remodeling might be an early step in atherosclerosis. Another group reported ORAI1 protein and mRNA expression to be upregulated in aortic tissue isolated from apoE knockout mice when fed a high fat diet. Knockdown of ORAI1 with siRNA or use of the SOCE inhibitor, SKF96365, decreased atherosclerotic plaque size [141]. Again these studies have not specifically examined one particular cell type and warrants further clarification. Similarly, STIM1 and ORAI1 protein expression and SOCE were reported to be elevated in VSMCs isolated from pigs fed a high-calorie diet. Interestingly, exercise had reduced STIM1 expression and SOCE [142], which suggests that diet

and exercise modulate SOCE and STIM1 protein expression. Further studies are necessary to identify the mechanisms of how risk factors of atherosclerosis regulate STIM and ORAI1 expression (and those of other STIM/ORAI isoforms) and their role in smooth muscle remodeling during atherosclerosis.

Endothelial dysfunction is an early step in the development of atherosclerotic plaques. As is the case for SAH and restenosis, STIM and ORAI are important for maintaining EC integrity, proliferation, and angiogenesis. It is unclear what role EC proliferation and angiogenesis may play in atherosclerosis [143]. Similar to systemic hypertension and restenosis, ECs are clearly essential for maintaining VSMCs in the quiescent state through mediators like NO [125]. EPCs are also important for regenerating healthy intima, and apoE knockout mice have decreased EPC proliferation and migration [129]. The EPCs from apoE knockout mice also have reduced SOCE, STIM1, and ORAI1 protein expression [129]. EPC proliferation and migration was also attenuated with the SOCE inhibitors 2-APB, ML-9, and shRNA targeting STIM1. The same group also reported that SOCE serves to protect EPCs from decreased cellular proliferation induced by oxidized LDL [144]. Oxidized LDL increased STIM1 protein expression, increased intracellular  $Ca^{2+}$  in EPCs through SOCE and induced autophagy in EPCs through activation of calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2) and inactivation of mammalian target of rapamycin (mTOR). This mechanism was proposed to ultimately spare EPCs from deleterious effects of oxidized LDL [144].

Because ORAI1 is necessary for neutrophil migration into inflamed endothelium [106], neutrophils and monocytes migration could be a contributor to atherosclerosis, although this idea remain untested. ORAI has been proposed as necessary for the formation of foam cells from lipid-laden macrophages [141]. Liang et al. observed that oxidized LDL activated  $Ca^{2+}$  entry through ORAI1 in macrophages. However, the molecular mechanisms of how oxidized LDL activates ORAI1 remain unknown. These authors proposed that oxidized LDL activation of ORAI1-mediated  $Ca^{2+}$  entry activates calcineurin, which leads to the expression of scavenger receptor A through c-Jun N-terminal kinase (JNK) and p38 kinase. The upregulated expression of scavenger receptor A would cause further uptake of LDL and promote the transition to a foam cell [141].

## 6. Pulmonary arterial hypertension

Although a relatively rare disease, pulmonary arterial hypertension (PAH) causes major complications including dyspnea and heart failure. Like SAH, much of the pathogenesis of PAH is idiopathic; however, the two diseases often have distinct etiologies. The pathological hallmark of PAH are smooth muscle and endothelial proliferation and migration, as well as thrombosis (Fig. 3A) [145]. While the endothelium only plays a supporting role in SAH, endothelial dysfunction is believed to drive PAH. The role of endothelial ORAI in PAH remain unknown. Pulmonary artery smooth muscle (PASM) remodeling in PAH is quite similar to VSMC remodeling in the systemic arteries. ORAI1 mediated SOCE has been measured in isolated PSMCs [146], and Fernandez et al. observed ORAI2 protein expression and SOCE to be increased in cultured synthetic PSMCs. In contrast, acutely isolated PSMCs expressed contractile proteins like smooth muscle 22- $\alpha$  actin and myosin heavy chain [147]. Others observed ORAI1 and ORAI2 (but not ORAI3) expression and increased SOCE in PSMCs under chronic hypoxia [148,149]. This upregulation under hypoxia was also unique to PSMCs since it was not observed with coronary VSMCs. The upregulation of ORAI2 (but not ORAI1) was dependent on the transcription factor hypoxia-inducible factor 1 $\alpha$  (HIF1 $\alpha$ ) [149]. PSMCs isolated from patients with PAH also had increased expression of ORAI2 and STIM2 [150]. Further studies are necessary to dissect the relative role of ORAI2 versus ORAI1 in PASM remodeling in PAH mouse models and how HIF1 $\alpha$  regulates the expression of these proteins. ORAI3 protein expression has been measured in PSMCs [149]; however, ORAI3 role

in PAH is unknown. The vasoconstrictor serotonin, which induces PASM remodeling [151], has been shown to activate a store-independent  $Ca^{2+}$  channel that resembled the pharmacological profile of  $I_{ARC}$  [152], suggesting that serotonin might regulate PASM remodeling through activation of  $I_{ARC}$  and ORAI3 [122].

## 7. Asthma

Asthma is a chronic inflammatory disease in the airways that affects 25.7 million American per year [153]. Inflammatory T-helper type 2 ( $T_H2$ ) cells inappropriately infiltrate the airways and secrete cytokines like IL-4, IL-5, and IL-13, which activate B cells, basophils, mast cells, and eosinophils to secrete inflammatory mediators like histamine, trypsin, thrombin, and bradykinin (Fig. 3D). These inflammatory mediators act on PLC-coupled receptors and stimulate airway hyper-responsiveness (AHR) to physiological stimuli [154]. Similar to the vascular diseases discussed above, this chronic inflammation also causes significant structural remodeling in the airways. This airway remodeling includes goblet cell hyperplasia, airway smooth muscle cell (ASMC) proliferation and migration, fibrosis, and angiogenesis [75,155]. Airway remodeling causes a significant loss of pulmonary function and is most prevalent in severe and difficult to treat asthmatics [156,157].

ORAI1 and STIM1 protein expression were upregulated in airway smooth muscle tissue isolated from the asthmatic mouse model challenged with the allergen ovalbumin [158].  $\beta_1$  integrin and TGF $\beta$  are inflammatory mediators that are elevated in asthmatic airways, and these mediators have been shown to modulate the expression of ORAI1 in the mouse airways and rat ASMCs respectively, although the mechanism is unclear [159,160]. ORAI protein expression, SOCE, and  $I_{CRAC}$  have also been measured in human airway smooth muscle cells (ASMCs) [158,161]. Secreted PDGF from inflammation also activated ORAI1 dependent  $I_{CRAC}$  in human ASMCs. Knockdown of ORAI1 in human ASMCs inhibited PDGF-induced proliferation and migration [162]. Similarly, ORAI1 and STIM1 were necessary for mouse ASMC SOCE,  $I_{CRAC}$ , proliferation, and migration [158]. Animal studies of asthma using ASMC tissue-specific ORAI knockout mice have not been reported. Sutovska et al. showed that asthmatic mice treated with the SOCE inhibitor 3-fluoropyridine-4-carboxylic acid (FPCA) had significant bronchodilation and cough suppression [163]. Treatment of mouse ASMCs with the SOCE inhibitors GSK7975A and GSK5498A inhibited methacholine induced-oscillations and lung slice contractions [138], while voltage-gated  $Ca^{2+}$  channel inhibitors were not effective. Although ORAI3-mediated  $I_{ARC}$  has not been directly measured in ASMCs, arachidonic acid-induced  $Ca^{2+}$  oscillations were detected in cultured ASMCs. These oscillations were unaffected by SOCE inhibitors but were inhibited by knockdown of ORAI3 with siRNA [164].

The lumen of the airways is lined with epithelial cells, which serve as a barrier to pathogens, chemicals, and inflammatory cells from the environment. A special type of epithelial cell known as goblet cells secrete mucus in the airways, which acts as a lubricant and an innate immune defense mechanism for the airways. In airway remodeling, the epithelium cycles from epithelium damage, repair, and metaplasia. Indeed, goblet cell metaplasia and hyperplasia are pathological hallmarks of airway remodeling [165]. Epithelial cells are also important for maintaining ASMCs in the quiescent state, and epithelial cell injury induces the secretion of cytokines such as IL-6, IL-8, matrix metalloproteinase 9 (MMP-9), which stimulate ASMC proliferation [166,167]. Jairaman et al. has investigated the role of ORAI in airway epithelial cells and reported that allergens such as house dust mite extracts were able to activate ORAI1-dependent SOCE through the protease-activated receptor type 2 (PAR2) in a bronchial epithelial cell line. SOCE inhibitors caused decreased allergen-induced cytokine production from epithelial cells [168].

Ashmole et al. showed that human lung mast cells, an important cell type in development of asthma, express ORAI1, ORAI2, and ORAI3

mRNA and protein and develop  $I_{CRAC}$  upon  $FC\epsilon R1$  activation. The SOCE inhibitors GSK-7975A and Synta-66 inhibited mast cell secretion of histamine,  $LTC_4$ , IL-5, IL-13, TNF- $\alpha$ , and IL-8 [169]. Knockdown or dominant negative strategies against ORAI1 or ORAI2 inhibited human lung mast cell SOCE and  $LTC_4$  secretion [170]. In vivo studies on the contribution of ORAI channels from a specific cell type (e.g. mast cells,  $T_H2$  cells, Tregs or endothelial cells) to development of asthma using tissue-specific knockout mice are currently lacking.

## 8. Chronic obstructive pulmonary disorder (COPD)

COPD is highly prevalent and estimated to affect over 384 million people worldwide. The dyspnea and cough in COPD are progressive and very difficult to treat since many patients become resistant to corticosteroids [171]. ASMC remodeling due to chronic inflammation is central to the pathogenesis of COPD. Much of the mechanisms previously discussed involving ORAI channels have not been studied in COPD. Unlike asthma, Cigarette smoke is the primary etiology of COPD. Wylam et al. observed that cigarette smoke enhanced the expression of ORAI1 as well as increase ORAI1-dependent SOCE in cultured ASMCs. Cigarette smoke also enhanced ASMC growth [172]. Since COPD has many similar pathological features to asthma and other vascular diseases, ORAI channels involvement in the pathogenesis of COPD warrants investigation.

## 9. Concluding remarks

The cardiorespiratory diseases discussed above are highly prevalent diseases that cause significant morbidity and mortality. A major theme of these diseases is cellular remodeling, which coincides with a remodeling of the  $Ca^{2+}$  signaling machinery. Among these, ORAI  $Ca^{2+}$  channels, which are activated either as a result of ER/SR  $Ca^{2+}$  store depletion ( $I_{CRAC}$ ) or independently of store depletion by inflammatory metabolites ( $I_{ARC}$ ), have emerged as major regulators of cellular remodeling through their ability to modulate transcriptional programs that drive proliferation, hypertrophy, migration and secretion of cytokines [16,53,75]. We have just begun to understand the role ORAI channels play in various cell types involved in cardiorespiratory diseases. Studies on isolated cells, including smooth muscle, endothelial, and immune cells have been useful in understanding the contribution of ORAI channels to  $Ca^{2+}$  signaling in response to various disease-relevant receptor agonists. These cellular studies have also shed light on the contribution of various STIM and ORAI isoforms to cell function. However, there is much more to understand and future studies need to address the downstream targets and mechanisms modulated by ORAI activation as well as the upstream regulators leading to changes in ORAI expression and function during disease. Much of the previous work has focused on the canonical ORAI1 isoform, and future studies should dissect the contribution of other ORAI isoforms, including ORAI2, ORAI3, and ORAI1 $\beta$ . This includes their ability to heteromultimerize and to be subject to post-translational and regulatory modifications. The contribution of various ORAI isoforms to the makeup of channels with unique biophysical properties could offer hope for specific targeting of these channels in future therapy of cardiorespiratory disease. ORAI1 glycosylation among other post-translational modifications have been shown to modulate channel function [173], and perhaps different modifications occur for other ORAI isoforms that can drive cellular remodeling in cardiorespiratory diseases. However, one hindrance to such studies is the current lack of specific and reliable antibodies against ORAI2 and ORAI3. Most importantly, more studies with animal models of disease, especially those using cell type-specific knockout mice of different ORAI isoforms, are needed to demonstrate the translational potential of this important class of  $Ca^{2+}$  channels in cardiorespiratory disease.

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