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Calcium Signaling Abnormality in Pulmonary Arterial Hypertension

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Abstract

Pulmonary arterial hypertension（PAH）is a severe and progressive disease that causes right heart failure when the condition progresses after being neglected in an early stage. The pathogenesis of PAH is generally characterized by vasoconstriction, dysregulated apoptosis, upregulated proliferation, migration, and pulmonary vascular remodeling in lung tissue. Although several vasodilating drugs are currently used for preventing the elevation of pulmonary arterial pressure, their therapeutic effects are still insufficient. Thus, there is an urgent need for novel therapeutic targets and drugs. In the past decades, the analysis of patients with idiopathic and familial PAH has revealed several genetic abnormalities that may cooperate with each other to cause pulmonary vascular proliferation and remodeling. On the other hand, recent studies that have employed genetic analyses and experimental models have suggested that the hypercontraction of the pulmonary artery induced by Ca^{2+} signaling abnormality may be involved in the pathogenesis of PAH. This review suggests the critical role of Ca^{2+} signaling abnormality in the development and progression of PAH, and the possibility that Ca^{2+}-permeable channels/transporters may represent novel therapeutic targets.

Key words: Pulmonary hypertension, Ca^{2+} signaling, Ion transporter, Vasoconstriction, Smooth muscle cell proliferation

Introduction

Pulmonary arterial hypertension（PAH）is a severe and progressive disease that causes right heart failure, although most patients are typically asymptomatic at the early stage of the disease. The pathogenesis of PAH is generally characterized by vasoconstriction, dysregulated apoptosis, upregulated proliferation, migration, and pulmonary vascular remodeling in lung tissue[1)]. Several vasodilating drugs, including endothelin receptor antagonists, phosphodiesterase 5 inhibitors, and soluble guanylate cyclase stimulants are currently used for preventing the elevation of the pulmonary arterial pressure; however, their therapeutic effects are still insufficient[2)]. Thus, there is an urgent need for novel therapeutic targets and drugs. In the past decade, research aiming at elucidating the pathophysiological mechanisms of PAH has been conducted, and some genetic abnormalities have been detected by the analysis of idiopathic and familial PAH patients[3),4)]. The identification of these genetic abnormalities led to the proposal of a theory called the "Multiple-Hits theory" whereby inflammation, viruses, hypoxia, and genetic abnormalities may cooperate with each other to cause epithelial injury, pulmonary arterial smooth muscle cell proliferation, and finally vascular remodeling[5)]. Recently, further genetic analyses revealed new genetic

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abnormalities, which are related to intracellular Ca^{2+} dysregulation, in familial PAH patients, suggesting that the hypercontraction of the pulmonary artery may be involved in the pathogenesis of PAH. This review will focus on the critical role of Ca^{2+} signaling abnormality in the development and progression of PAH.

Genetic abnormalities in PAH patients

In the past decade, a number of genetic abnormalities have been detected through the analysis of patients with idiopathic or familial PAH. The first genetic studies have shown that mutations in the gene of bone morphogenetic protein receptor type II (BMPR2) are present in approximately 70% of patients with familial PAH and in 10–25% of patients with idiopathic PAH[3),4)]. BMP4, one of the TGF-β super-families, binds to BMPR2 and exerts its action through the downstream activation of decapentaplegic homologue (SMAD). This cascade usually leads to anti-apoptotic dominance in endothelial cells and also to apoptotic dominance in vascular smooth muscle. The BMPR2 mutations identified in PAH patients were found to inhibit the activation of the downstream of SMAD in endothelial cells and the attenuation of the anti-apoptotic effect, resulting in endothelial dysfunction[6)]. In contrast, another report suggested that the mutations of BMPR2 inhibited apoptosis and promoted cellular proliferation in pulmonary arterial smooth muscle cells[7)]. Taken together, these findings suggest that BMPR2 mutations disrupt the endothelial cell function and enhance the response of growth factors to vascular smooth muscle cells. Subsequently, the BMPR2 mutations may promote vascular smooth muscle cell proliferation, which contributes to the pathological change and the induction of pulmonary artery pressure elevation. Thus, the TGF-β/BMPR2/SMAD pathway seems to play a critical role in the pathogenesis of PAH. Following this first report, genetic mutations in activin receptor-like kinase 1 (ALK1), endoglin, SMAD9, and caveolin 1 were also found in patients with idiopathic and familial PAH[8)]. These proteins are thought to mainly be associated with cell growth and abnormalities of the proteins cause tumorigenesis-like cell activity.

On the other hand, a heterozygous missense variant of the KCNK3 gene, which encodes the potassium channel subfamily K member 3, was identified by the whole-exome analysis of familial PAH patients[9)]. In an experimental mouse model, the knockout of the TWIK2 gene, which encodes KCNK6, led to the development of spontaneous pulmonary hypertension[10)]. In addition, Xia *et al*. reported that in transient receptor potential cation channel C6 (TRPC6) and/or TRPC1 knockout mice, an increase in right ventricular systolic pressure after 3 weeks of hypoxia was suppressed in comparison to WT mice[11)]. Furthermore, Na^+/Ca^{2+} exchanger type-1 (NCX1) has been shown to be upregulated in pulmonary arterial smooth muscle cells isolated from patients with idiopathic PAH[12)]. These reports suggest that the dysregulation of ion channels/transporters in the pulmonary artery may be involved in the pathogenesis of PAH.

Calcium signaling in vascular smooth muscle cells

Ca^{2+} signaling in vascular smooth muscle cells plays important roles in various cellular functions, including gene transcription, vasoconstriction, and cellular proliferation[13)]. The level of intracellular Ca^{2+} is regulated by a balance between the Ca^{2+} influx into the cytoplasm and the Ca^{2+} efflux from the cytoplasm through the combined functions of the Ca^{2+}-permeable channels/transporters (Fig. 1).

During the initiation of Ca^{2+} signaling, the Ca^{2+} influx is generated from the external source of Ca^{2+}, by activating various channels including voltage-dependent Ca^{2+} channels (VDCCs), store-operated channels (SOCs), and transient receptor potential cation (TRP) channels in vascular smooth muscle cells. The most prominent plasma membrane Ca^{2+} entry channels are VDCCs, which are mainly expressed in excitable cells and which generate the rapid Ca^{2+} influxes that control the fast cellular processes. In addition to these more clearly defined channel-opening mechanisms, there are many other channel types, such as SOCs and stretch-activated channels. The TRP channel family may contribute to the opening of these Ca^{2+} channels[14)-16)]. In vascular smooth muscle cells, Ca^{2+} signaling is also formed from the internal source of Ca^{2+} through the activation of the inositol trisphosphate receptors (IP_3Rs) or ryanodine receptors (RYRs), which are sensitive to Ca^{2+} (Fig.1). The RYRs operate as a Ca^{2+} induced-Ca^{2+} release (CICR) process, which is related to the rapid increase in the Ca^{2+} levels during muscle contraction. In the case of the IP3Rs, the main regulation factors are IP_3 and Ca^{2+}. The binding of IP_3 increases the sensitivity of the receptor to Ca^{2+}, resulting in the promotion of intracellular Ca^{2+} mobilization.

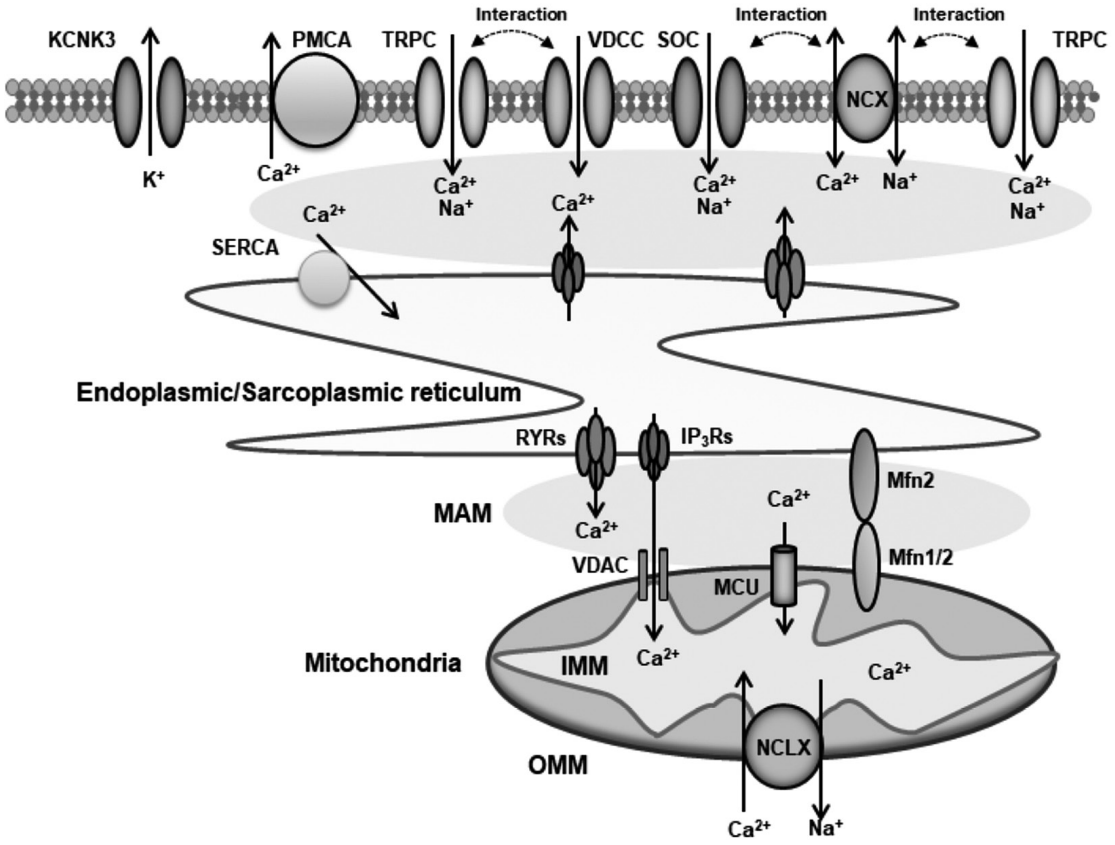

Figure 1. Schematic model of intracellular Ca^{2+} regulation mechanisms in pulmonary arterial smooth muscle cells.

The intracellular Ca^{2+} is regulated by a balance between Ca^{2+} influx to the cytoplasm and Ca^{2+} efflux from the cytoplasm through combined actions of ion channels/transporters. Recent experimental findings suggest that abnormal Ca^{2+} regulation in pulmonary arterial smooth muscle cells may be involved in the development and progression of pulmonary arterial hypertension.

VDCC: Voltage-dependent Ca^{2+} channel, TRPC: Transient receptor potential channel, PMCA: Plasma membrane Ca^{2+} ATPase, SOC: Store-operated Ca^{2+} channels, NCX: Na^+/Ca^{2+} exchanger, KCNK3: Potassium channel subfamily K member 3, IP_3Rs: IP_3 receptors, RYRs: Ryanodine receptors, SERCA: Sarcoplasmic reticulum Ca^{2+}-ATPase, Mfn: Mitofusin, VDAC: Voltage-dependent anion channel, NCLX: Mitochondrial Na^+/Ca^{2+} exchanger, MCU: Mitochondrial calcium uniporter, OMM: Outer mitochondrial membrane, IMM: Inner mitochondrial membrane, MAM: Mitochondria-associated ER membranes.

During the termination of Ca^{2+} signaling, the Ca^{2+} influx is counteracted by the Ca^{2+} efflux reactions mediated by Ca^{2+} pumps and Ca^{2+} transporters to remove Ca^{2+} from the cytoplasm. There are four main mechanisms by which Ca^{2+} is removed from the cytoplasm: (1) plasma-membrane Ca^{2+}-ATPase (PMCA), (2) Na^+/Ca^{2+} exchanger (NCX), (3) sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA), and (4) the mitochondrial Ca^{2+} uniporter (MCU) (Fig.1).

Calcium signaling abnormality in the pathogenesis of PAH

VDCCs are traditional targets for the study on PAH. In particular, the pathological interaction between voltage-gated potassium (Kv) channels and VDCCs has been widely investigated. Hypoxia has been reported to cause the downregulation of the Kv channels in pulmonary arterial smooth muscle cells and to induce membrane depolarization[17]. Membrane depolarization may cause the opening of VDCCs and an increase in the intracellular Ca^{2+} concentration[18]. Recently, a heterozygous missense

variant of the KCNK3 gene were detected in patients with familial PAH[9]. This is the first report to identify ion channel mutations in PAH patients from a genetic analysis. The loss of function in the KCNK3 mutants induces a decrease in the membrane K^+ current and the depolarization of the membrane potential. The membrane depolarization in pulmonary arterial smooth muscle cells causes pulmonary vasoconstriction though the acceleration of VDCCs. As a result of the pulmonary vasoconstriction, the thickening of the pulmonary artery may cause the development of PAH. In a monocrotaline (MCT) rat model, which is a typical animal model of PAH, it was also confirmed that the expression of KCNK3 was decreased in the pulmonary artery[19]. Interestingly, PAH in MCT rats was improved with the treatment of ONO-RS-082, a KCNK3 agonist.[19] Thus, KCNK3 is thought to be useful as a therapeutic target for PAH.

Another report indicated that TRPC6, which is one of Ca^{2+}-permeable channels, was upregulated in pulmonary arterial smooth muscle cells isolated from patients with idiopathic PAH[20]. Actually, these pulmonary arterial smooth muscle cells were found to have a higher proliferation ability than control cells[21]. The upregulation of the TRP channels may increase the influx of Ca^{2+} into the cytoplasm, resulting in cellular proliferation and vasoconstriction. Furthermore, NCX, which is a Ca^{2+}-permeable transporter, was upregulated in pulmonary arterial smooth muscle cells isolated from patients with idiopathic PAH[12]. In the arteries, NCX seems to work in a reverse mode (Ca^{2+} influx mode)[22]; thus, the cytosolic Ca^{2+} concentration may be increased in pulmonary arterial smooth muscle cells isolated from patients with idiopathic PAH[12]. These experimental findings indicate that Ca^{2+} signaling abnormalities in the pulmonary arterial smooth muscle cells may be involved in the pathogenesis of PAH (Fig. 1).

Continued pulmonary arterial contraction and proliferation cause persistent pulmonary vasoconstriction and vascular remodeling[23]. Enhanced Ca^{2+} signaling promotes pulmonary arterial proliferation by activating Ca^{2+}/calmodulin (CaM)-dependent protein kinase (CaMK) and Ca^{2+}/CaM-dependent protein phosphatase (calcineurin) and downstream transcription factors, such as cAMP response element binding protein (CREB) and nuclear factor of activated T cells (NFAT), respectively, which are necessary for cell growth[24),25]. Enhanced Ca^{2+} signaling also induces Ca^{2+}-dependent gene transcription in vascular smooth muscle cells[26]. In addition, cytosolic Ca^{2+} affects the gene expression by interacting with protein kinase C and CaM, and activates the proteins involved in the cell cycle (*i.e.,* cyclins and cyclin dependent kinases)[27]. Ca^{2+} is actually required for cell cycle progression and cellular proliferation because the removal of extracellular Ca^{2+} and the depletion of intracellularly conserved Ca^{2+} inhibits the proliferation of pulmonary arterial smooth muscle cells[28]. Enhanced Ca^{2+} signaling in pulmonary arterial smooth muscle cells also causes continued pulmonary vasoconstriction[26),29], which may be involved in the elevated pulmonary artery pressure that is observed in patients with PAH.

Concluding remarks

Recent studies have shown that abnormal Ca^{2+} signaling in pulmonary arterial smooth muscle cells may be involved in the development and progression of PAH. Although a detailed investigation is still required, this Ca^{2+}-dependent mechanism is necessary to understand the pathogenesis of PAH, and suggests that Ca^{2+}-permeable channels/transporters might be novel therapeutic targets for PAH.

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