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Canonical Transient Receptor Potential Channels and Their Link with Cardio/Cerebro-Vascular Diseases

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Abstract

The canonical transient receptor potential channels (TRPCs) constitute a series of nonselective cation channels with variable degrees of Ca^{2+} selectivity. TRPCs consist of seven mammalian members, TRPC1, TRPC2, TRPC3, TRPC4, TRPC5, TRPC6, and TRPC7, which are further divided into four subtypes, TRPC1, TRPC2, TRPC4/5, and TRPC3/6/7. These channels take charge of various essential cell functions such as contraction, relaxation, proliferation, and dysfunction. This review, organized into seven main sections, will provide an overview of current knowledge about the underlying pathogenesis of TRPCs in cardio/cerebrovascular diseases, including hypertension, pulmonary arterial hypertension, cardiac hypertrophy, atherosclerosis, arrhythmia, and cerebrovascular ischemia reperfusion injury. Collectively, TRPCs could become a group of drug targets with important physiological functions for the therapy of human cardio/cerebro-vascular diseases.

Key Words: Ca^{2+} signaling, Canonical transient receptor potential receptor, Cardiovascular disease, Cerebrovascular disease, Pathogenesis

INTRODUCTION

In 1969, Cosens and Manning (1969) discovered that *Drosophila* with mutations in a peculiar gene was defective and displayed transient light-induced receptor potentials (TRPs) in response to continuous light exposure, causing visual impairment in photoreceptor cells. This phenomenon was explained by a deletion in ion channels, and led to the discovery of "TRP genes" that were named TRP channels. To date, the TRP channels superfamily contains 28 members in mammals and is subdivided into six subfamilies: TRPA, TRPC, TRPML, TRPM, TRPN, TRPV and TRPP, all of which permeate cations (Montell, 2005). The canonical transient receptor potential channels (TRPCs) are the first encoded TRP gene family in mammals and are the most dominating non-voltage-gated, Ca^{2+}-permeable cation channels in various cells (Zhu *et al*., 1995). TRPCs fall into four groups in terms of their amino acid homology and similarities in function: TRPC1, TRPC2 (as a pseudogene in humans), TRPC4/5, and TRPC3/6/7 (Table 1) (Nilius and Voets, 2005; Minke, 2006). The seven subtypes have an invariant sequence in common in the C-terminal tail called a TRP box (Philipp *et al*., 2000) and include three to four ankyrin-like repetitive sequences in the N-terminus (Montell *et al*., 2002). Many subunits of TRPCs are able to coassemble. There exist heteromultimeric channels that consist of heterologously expressed and endogenous TRPC monomers (Nilius *et al*., 2007). Indeed, TRPC1, TPRC4 and TRPC5 can form heteromers. Similarly, TRPC3, TRPC6, and TRPC7 form heteromers. In terms of activation mechanisms, members of the TRPC3, TRPC6 and TRPC7 subtypes can be stimulated by diacylglycerol (DAG) (Hofmann *et al*., 1999), which is the phospholipase C (PLC)-derived production regulating their physiological activation. In contrast, the TRPC1/4/5 subgroups are completely insensitive to DAG, which is still a controversial mechanism (Venkatachalam *et al*., 2003).

Most TRPCs are inserted in the plasma membrane (PM) and can be hindered by blockers (Zhang *et al*., 2013). Generally speaking, G protein-coupled receptors (GPCRs) have important roles in the regulation of TRPCs. In some cases, lipid signals can regulate the signals from GPCRs to TRPCs (Kukkonen, 2011).

A cytosolic Ca^{2+} change may be induced by activation of specific GPCRs, including an initial transient increase resulting from release of calcium ions from the endoplasmic retic-

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Table 1. The properties of the TRPC family members

Category	Tissue distribution	Structure	Activation mechanism	Proposed regulation	Reference
TRPC1	Heart, Cartilage, Pituitary gland, Cerebellum, Caudate nucleus, Amygdala.	Six transmembrane spanning domains, TRP box in the C-terminus and three to four ankyrin-like repetitive sequences in the N-terminus	PKC-dependent phosphorylation	Store-operated, Store depletion	Riccio *et al*., 2002; Nilius and Voets, 2005; Minke, 2006; Xia *et al*., 2014
TRPC3	Pituitary gland, Cerebellum, Caudate nucleus, Putamen, Striatum.	Ibid ibidem	PKC-independent mechanism	DAG, Store-operated, Store depletion	Riccio *et al*., 2002; Welsh *et al*., 2002; Minke, 2006
TRPC4	Prostate, Bone. Parahippocampus.	Ibid ibidem	G-protein-coupled agonists	Store-operated, Store depletion?	Schaefer *et al*., 2000; Riccio *et al*., 2002; Plant and Schaefer, 2003
TRPC5	Cerebellum, Middle frontal gyrus, superior frontal gyrus	Ibid ibidem	G-protein-coupled agonists	Store-operated, Store depletion?	Schaefer *et al*., 2000; Riccio *et al*., 2002; Plant and Schaefer, 2003
TRPC6	Heart, Kidney, Adipose, Prostate, Cerebellum, Cingulate gyrus.	Ibid ibidem	PKC-independent mechanism	DAG, Receptor-operated	Riccio *et al*., 2002; Welsh *et al*., 2002; Winn *et al*., 2005; Xia *et al*., 2014
TRPC7	Pituitary gland, Kidney, Intestine, Prostate, Brain, Testis, Spleen, Cartilage.	Ibid ibidem	PKC-independent mechanism	DAG, Store depletion	Okada *et al*., 1999; Riccio *et al*., 2002
TRPC2	Only expressed in rodent,	Ibid ibidem	PLC-dependent mechanism	DAG, Store depletion?	Leypold *et al*., 2002; Stowers *et al*., 2002

"?" indicates that the proposed regulation is not completely confirmed.

Table 2. TRPC channels may participate in most cardio/cerebro-vascular diseases

Disease	Related TRPCs	Cells	Reference
Hypertension	TRPC1,TRPC3,TRPC6	SMCs, Monocytes	Dietrich *et al*., 2006; Chen *et al*., 2010; Dietrich *et al*., 2010; Inoue *et al*., 2009; Fuchs *et al*., 2010; Eder and Molkentin, 2011; Gopal *et al*., 2015
Pulmonary hypertension	TRPC1,TRPC3,TRPC6	PASMCs	Liu *et al*., 2007a, 2009; Edwards *et al*., 2010; Iwasaki *et al*., 2011; Liu *et al*., 2012; Loga *et al*., 2013; Malczyk *et al*., 2013; Maier *et al*., 2015
Cardiac hypertrophy	TRPC1,TRPC3,TRPC6, TRPC7	Cardiomyocytes	Piper *et al*., 2004; Montell, 2005; Minke, 2006; Onohara *et al*., 2006; Nakashima and Kumagai, 2007; Ohba *et al*., 2007; Rosenbaum *et al*., 2015
Atherosclerosis	TRPC1,TRPC3,TRPC4, TRPC5,TRPC6	Platelets, VSMCs, Monocytes/ Macrophages, Endothelial cells	Short *et al*., 1993; Satoh *et al*., 2007; Shan *et al*., 2008; Smedlund and Vazquez, 2008; Smedlund *et al*., 2010
Arrhythmia	TRPC3,TRPC6	Myocardial cells, Fibroblast	Wang *et al*., 2006; Takahashi *et al*., 2007; Thilo *et al*., 2009; Tauseef *et al*., 2016
Ischemia-reperfusion	TRPC3,TRPC6	Myocardial cells	Xu and Beech, 2001; Wu *et al*., 2010; Zhang *et al*., 2014

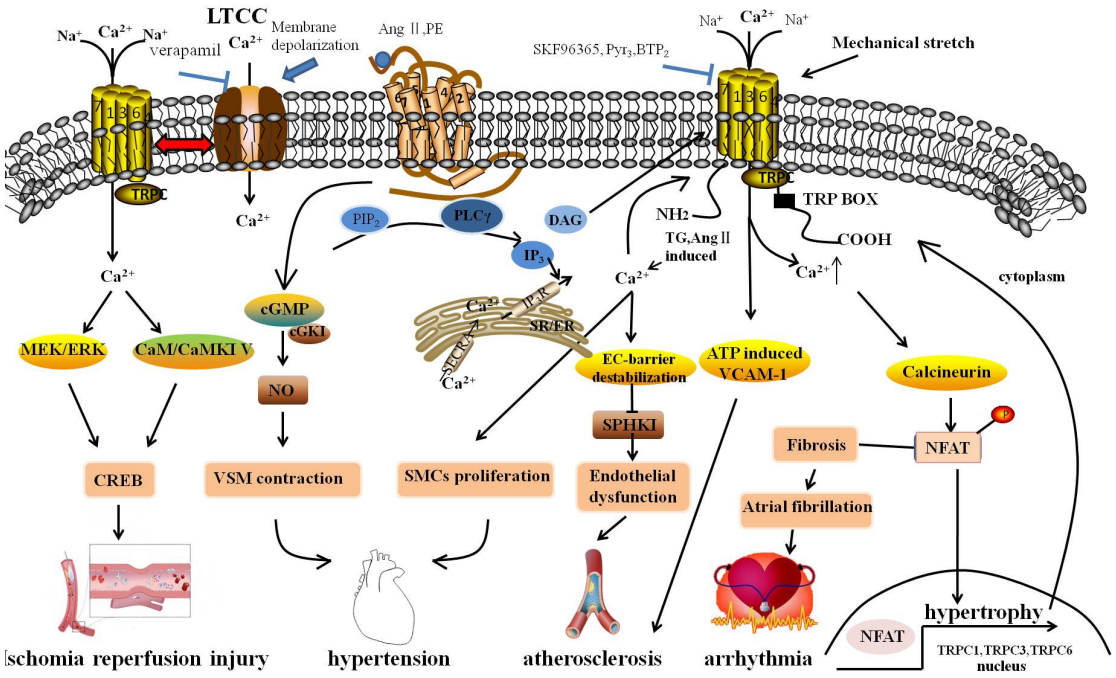


Fig. 1. Molecular mechanism underlying cardiovascular diseases associated with the changing of intracellular Ca^{2+} through TRPCs. GPCRs, releasing DAG and IP3 via PIP2 with the subsequent activation of PLC, were stimulated by Ang II and PE, which were hypertrophic stimuli. DAG stimulated ROCs, including TRPC3 and TRPC6, resulting in extracellular Ca^{2+} influx. IP3 activated SOCE in response to depletion of intracellular Ca^{2+} stores by Ca^{2+} release in the SR/ER and subsequently activated TRPCs. The sustained TRPC-mediated Ca^{2+} entry directly activated the calcineurin-NFAT pathway, subsequently resulting in the activation of hypertrophic gene expression, including TRPC1, TRPC3 and TRPC6. Simultaneously, after activating, NFAT might activate TRPC gene expression through a positive feedback mechanism. TRPCs interacted with the LTCC through membrane depolarization, playing a role in regulation of cardiac pacemaking, conduction, ventricular activity, and contractility. Mechanical stretch caused arrhythmia through the activation of SACs to elevate cytosolic Ca^{2+} levels. Fibroblast regulated by Ca^{2+}-permeable TRPCs might be associated with AF, and fibroblast proliferation and differentiation are a central feature in AF-promoting remodeling. TRPCs maintained adherens junction plasticity and enabled EC-barrier destabilization by suppressing SPHK1 expression to induce endothelial hyperpermeability, leading to atherosclerosis. In addition, the omission of extracellular Ca^{2+} with channel blockers (SKF96365, Pyr3) reduced monocyte adhesion and ATP-induced VCAM-1 and also relieved the progress of atherosclerosis. The rise of cytosolic $[Ca^{2+}]i$ promoted SMC proliferation. TRPC channels associated with vascular remodeling caused hyperplasia of SMCs. Moreover, TRPCs participated in blood pressure regulation due to receptor-mediated and pressure-induced changes in VSMC cytosolic Ca^{2+}. Signaling via cGKI in vascular smooth muscle, by which endothelial NO regulated vascular tone, caused VSMC contraction. Activated TRPCs can activate downstream effectors and CREB proteins that have many physiological functions; TRPCs activated in neurons are linked to numerous stimuli, including growth factors, hormones, and neuronal activity through the Ras/MEK/ERK and CaM/CaMKIV pathways. GPCRs, G protein-coupled receptor; Ang II, Angiotensin II; PE, phenylephrine; ROCs, receptor-operated channels; SOCE, store-operated Ca^{2+} entry; LTCC, L-type voltage-gated calcium channel; SACs, stretch-activated ion channels; AF, atrial fibrillation; SPHK1, sphingosine kinase 1; VCAM-1, Vascular cell adhesion molecule-1; SMCs, smooth muscle cells; VSMC, vascular smooth muscle cells; cGKI, cGMP-dependent protein kinase I; CREB, cAMP/Ca^{2+}- response element-binding.

ulum (ER)/sarcoplasmic reticulum (SR) and a subsequent sustained plateau phase via receptor-operated channels (ROCs) (Berridge *et al*., 2003). This latter manner of Ca^{2+} entry is named "receptor-operated Ca^{2+} entry" (ROCE) (Soboloff *et al*., 2005; Inoue *et al*., 2009). Another manner of Ca^{2+} entry has been termed "store-operated Ca^{2+} entry" (SOCE) via store-operated channels (SOCs) (Shi *et al*., 2016). SOCE occurs linked to depletion of intracellular Ca^{2+} stores (Putney, 1986; Ng and Gurney, 2001). Ca^{2+} refills depleted intracellular Ca^{2+} storages, directly accessing the SR/ER via SOCE. Although the exact functional relationship between TRPC and SOCE/ROCE is still indistinct, it is clear that TRPCs are the main channels of SOCs and ROCs. In recent years, SOCs and ROCs have gained increased attention for their role in mediating Ca^{2+} influx in response to cell function and disease. Previous studies suggested that TRPC family members, except TRPC2, are detectable at the mRNA level in the whole heart, vascular system, cerebral arteries, smooth muscle cells (SMCs) and endothelial cells (ECs) (Yue *et al*., 2015). TRPCs may participate in most cardio/cerebro-vascular diseases (Table 2) and play important roles in reactive Ca^{2+}-signaling in the cardio/cerebro-vascular system (Fig. 1).

Role of TRPCs in hypertension

Hypertension is a chronic cardiovascular disease characterized by persistently elevated blood pressure and is a major risk factor for coronary artery disease, stroke, heart failure, and peripheral vascular disease. In recent years, numerous studies have focused on the relationship between primary hypertension and TRPCs (Fuchs *et al*., 2010). In pathological states, some signaling factors are involved in the transition of SMCs into the proliferative phenotype, leading to an excessive growth of SMCs (Beamish *et al*., 2010). Abnormal overgrowth of SMCs is implicated in various vascular diseases,

including hypertension (Beamish *et al*., 2010). Previous studies have convincingly suggested that several TRPC members are involved in hyperplasia of SMCs. TRPC1/3/6 all have been involved in enhanced proliferation and phenotype switching of SMCs (Dietrich *et al*., 2005; Takahashi *et al*., 2007; Koenig *et al*., 2013). Kumar *et al*. (2006) suggested that TRPC1 was upregulated in rodent vascular injury models and in human neointimal hyperplasia after vascular damage. In coronary artery SMCs, upregulation of TRPC1 results in angiotensin-II (Ang II)-mediated human coronary artery SMC proliferation (Takahashi *et al*., 2007). Moreover, other studies found that the visible whole-cell currents were triggered by passive depletion of Ca^{2+} storages in vascular smooth muscle cells (VSMCs) in wild type mice, but not in *Trpc1*$^{-/-}$ mice (Shi *et al*., 2012), suggesting TRPC1 contributed to the alteration of whole-cell currents in VSMCs (Shi *et al*., 2012).

In addition, TRPC3 also plays a pivotal role in Ca^{2+} signaling and a pathophysiological role in hypertension. The previous studies suggested TRPC3 levels were elevated in patients with hypertension as well as in the pressure-overload rat and the spontaneous hypertensive rat (SHR) models (Liu *et al*., 2009; Onohara *et al*., 2006; Thilo *et al*., 2009). In monocytes, DAG-, thapsigargin- and Ang II-induced Ca^{2+} influxes were elevated in response to pathological state in SHR. However, further studies proved that downregulating TRPC3 by siRNA or applying with Pyrazole-3 (Pyr3), a highly selective inhibitor of TRPC3, reduced DAG-, thapsigargin- and Ang II-induced Ca^{2+} influx in monocytes from SHR (Liu *et al*., 2007a; Chen *et al*., 2010), prevented stent-induced arterial remodeling, and inhibited SMC proliferation (Yu *et al*., 2004; Schleifer *et al*., 2012). Similarly, compared with normotensive patients, increased expression of TRPC3 and a subsequent increase in SOCE has been noticed in monocytes from hypertension patients (Liu *et al*., 2006, 2007b). These data show a positive association between blood pressure and TRPC3, indicating an underlying role for TRPC3 in hypertension.

TRPC6 is a ubiquitous TRPC isoform expressed in the whole vasculature, which plays a pivotal role in blood pressure regulation because of its physiological importance in both receptor-mediated and pressure-induced increases of cytosolic Ca^{2+} in VSMCs (Toth *et al*., 2013). Studies suggested that cGMP-dependent protein kinase I (cGKI), which was implicated in the regulation of smooth muscle relaxation, inhibited the activity of TRPCs in SMCs (Kwan *et al*., 2004; Takahashi *et al*., 2008; Chen *et al*., 2009; Dietrich *et al*., 2010) and regulated vascular tone via endothelial nitric oxide (NO) (Loga *et al*., 2013). However, the knockout of TRPC6 might injure endothelial cGKI signaling and vasodilator tone in the aorta (Loga *et al*., 2013). Although deletion of TRPC6 decreases SMC contraction and depolarization induced by pressure in arteries, the basal mean arterial pressure in *Trpc6*$^{-/-}$ mice is about more than 7 mm Hg higher than that in wild type mice (Welsh *et al*., 2002; Dietrich *et al*., 2005), indicating that TRPC6 participated in smooth muscle contraction. Similarly, in deoxycorticosterone acetate (DOCA)-salt-hypertensive rats, overexpression of TRPC6 strengthened agonist mediated VSMC contractility companied with increased mean blood pressure (Bae *et al*., 2007). Additionally, mineralocorticoid receptor-induced TRPC6 mRNA level was elevated in the aldosterone-treated rat A7r5 VSMCs, suggesting that heightened TRPC6 expression importantly participates in increased VSM reactivity (Bae *et al*., 2007).

Role of TRPCs in pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is characterized by a thickening of the pulmonary arterial walls, which can cause right heart failure (Yu *et al*., 2004). Increased pulmonary vascular resistance is a primary factor in the progression of PAH. Ca^{2+} entry from the extracellular space, acting as a crucial mediator, is implicated in vasoconstriction (through its pivotal effect on pulmonary artery smooth muscle cells (PASMCs) contraction) and vascular remodeling (through its stimulatory effect on PASMC proliferation) (Kuhr *et al*., 2012; Weber *et al*., 2015). The most frequently expressed isoforms of TRPC in VSMCs are TRPC1, TRPC4, and TRPC6; TRPC3, TRPC5, and TRPC7 are less frequently detected (Inoue *et al*., 2006; Maier *et al*., 2015). Studies showed that Ca^{2+} entry improved the level of cytosolic Ca^{2+} through SOCs and ROCs (which is formed by TPRCs), and sufficient Ca^{2+} in the SR induced VSMC proliferation (Birnbaumer *et al*., 1996; Golovina *et al*., 2001; Bergdahl *et al*., 2003; Satoh *et al*., 2007; Seo *et al*., 2014).

TRPC1, TRPC4 and TRPC6 are involved in hypoxic pulmonary vasoconstriction, which is related to increased SOCE. Additionally, SOCE contributes to basal intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) and the proliferation and migration of PASMCs in rat (Lu *et al*., 2008). Malczyk *et al*. (2013) demonstrated that TRPC1 played an important role in hypoxia-induced PAH, as hypoxia-induced PAH is alleviated in *Trpc1*$^{-/-}$ mice. Xia *et al*. (2014) found that TRPC1/6 are crucial for the regulation of neo-muscularization, vasoreactivity, and vasomotor tone of pulmonary vasculatures; the combined actions of the two channels have a distinctly larger influence using *Trpc1*$^{-/-}$, *Trpc6*$^{-/-}$ and *Trpc1*$^{-/-}$/*Trpc6*$^{-/-}$ mice. Significantly, another study confirmed the upregulation of TRPC1/6 expression in murine chronic hypoxia PAH models (Wang *et al*., 2006). Silence of TRPC1 and TRPC6 specifically attenuated thapsigargin- and 1-oleoyl-2-acetyl-sn-glycerol (OAG)-induced cation entries, respectively, indicating that TRPC1-mediated SOCE and TRPC6-mediated ROCE are upregulated by chronic hypoxia (Lin *et al*., 2004). TRPC4 is also involved in PAH. In monocrotaline-induced PAH rats, TRPC1 and TRPC4 protein levels were both increased significantly, resulting in enhanced vasoconstriction to endothelin-1 (ET-1) (Liu *et al*., 2012). In addition, siRNA specifically targeting TRPC4 reduced increases in TRPC4 expression and capacitative calcium entry (CCE) amplitude and inhibited ATP-induced PASMC proliferation (Zhang *et al*., 2004).

The expression and function of TRPCs are variously regulated by molecules in PAH. Wang *et al*. (2015) implied that both bone morphogenetic protein-4 (BMP4) and hypoxia inducible factor-1α (HIF-1α) upregulated TRPC1 and TRPC6, leading to elevated basal $[Ca^{2+}]i$ in PASMCs, driving the development of chronic hypoxia-induced PAH (Wang *et al*., 2015). Another study found that TRPC expression was found absent in mice partially deficient for HIF-1α (Wang *et al*., 2006). In human PASMCs, siRNA of the HIF-1α reduced hypoxia-induced BMP4 expression and knockout of either HIF-1α or BMP4 abrogated hypoxia-induced basal cytosolic Ca^{2+} increase and TRPC expression (Zhang *et al*., 2014; Wang *et al*., 2015). Also, TRPCs have been recognized as reactive oxygen species (ROS)-activated channels and it is suggested that they are critical for hypoxia associated with vascular regulatory procedures in lung tissue.

TRPCs could be regulated by pharmacological intervention

during PAH. The treatment of experimental PAH with sildenafil and sodium tanshinone IIA sulfonate suppresses TRPC1/6 expression (Lu *et al*., 2010; Wang *et al*., 2013a). SAR7334, an inhibitor of TRPC6, suppresses native TRPC6 activity *in vivo* (Maier *et al*., 2015) and opens new opportunities for the investigation of TRPC function. In the lung and PASMC from idiopathic PAH patients, the mRNA and protein expression levels of TRPC6 were much higher than that from normotensive or secondary PAH patients. Also, inhibition of TRPC6 expression markedly attenuated idiopathic PAH-PASMC proliferation (Yu *et al*., 2004). As a consequence, the participation of TRPC1/4/6 are crucial for PAH.

These results suggest that overexpression of TRPC may partially contribute to the increased PASMC proliferation, hinting at a promising therapeutic strategy for PAH patients.

Role of TRPCs in cardiac hypertrophy

Cardiac hypertrophy serves as a common pathway in cardiovascular diseases. It is the most important pathological foundation resulting in cardiogenic death. Although one study showed that the knockout of some TRPC genes did not result in abnormality in normal mice hearts (Yue *et al*., 2015). TRPCs have been demonstrated to play an important role in the pathological progress of cardiac hypertrophy through the mediation of ion channel activities and downstream signaling. Dysregulation of TRPCs may lead to maladaptive cardiac hypertrophy.

Numerous studies have shown that TRPC expression and activity are up-regulated in pathological cardiac hypertrophy (Bush *et al*., 2006; Kuwahara *et al*., 2006; Ohba *et al*., 2007; Seth *et al*., 2009). Cardiac hypertrophy induced by transverse aortic constriction (TAC) was improved in *Trpc1*$^{-/-}$ mice. Meanwhile, downregulation of TRPC1 reduced SOCE and prevented ET-1-, Ang II-, and phenylephrine (PE)-induced cardiac hypertrophy, indicating that deletion of TRPC1 avoided harmful influences in response to increased cardiac stresses in *Trpc1*$^{-/-}$ mice (Ohba *et al*., 2007). Also verified that TRPC1-mediated Ca^{2+} entry stimulated hypertrophic signaling in cardiomyocytes (Seth *et al*., 2009). Similarly, cardiac pathological hypertrophy could be caused by stimulation of pressure overload or overexpression of the TRPC3 gene in cardiomyocytes from TRPC3 transgenic mice, and could be selectively inhibited by Pyr3 (Nakayama *et al*., 2006; Kiyonaka *et al*., 2009). Also, TRPC6 has been proposed as a critical target of anti-hypertrophic effects elicited via the cardiac ANP/BNP-GC-A pathway (Kinoshita *et al*., 2010). However, a recent study showed *Trpc6*$^{-/-}$ mice resulted in an obvious augment in the cardiac mass/tibia length (CM/TL) ratio after Ang II, while the *Trpc3*$^{-/-}$ mice showed no alteration after Ang II injection. However, the protective effect against hypertrophy of pressure overload was detected in *Trpc3*$^{-/-}$/*Trpc6*$^{-/-}$ mice rather than in *Trpc3*$^{-/-}$ or *Trpc6*$^{-/-}$ mice alone (Seo *et al*., 2014). Similarly, the newly developed selective TRPC3/6 dual blocker showed an obvious inhibition to myocyte hypertrophy signaling activated by Ang II, ET-1 and PE in a dose-dependent manner in HEK293T cells as well as in neonatal and adult cardiomyocytes (Seo *et al*., 2014).

Although the TRPCs role in myocardial hypertrophy is controversial, it is generally believed that calcineurin-nuclear factor of activated T-cells (Cn/NFAT) is a critical factor of microdomain signaling in the heart to control pathological hypertrophy. Studies found that transgenic mice that express dominant-negative myocyte-specific TRPC3, TRPC6 or TRPC4 attenuated the reactivity following either neuroendocrine-like or pressure overload-induced pathologic cardiac hypertrophy through Cn/NFAT stimulation *in vivo*, demonstrating that blockades of TRPCs are necessary adjusters of hypertrophy (Dietrich *et al*., 2006; Wu *et al*., 2010; Eder and Molkentin, 2011).

Undoubtedly, TRPCs play an important role in cardiac hypertrophy and can be regarded as new therapeutic target in the development of new drugs.

Role of TRPCs in atherosclerosis

Atherosclerosis is commonly considered a chronic disease with dominant accumulation of lipids and inflammatory cells of the arterial wall throughout all stages of the disease (Tabas *et al*., 2010). Several types of cells such as VSMCs, ECs, monocytes/macrophages, and platelets are involved in the pathological mechanisms of atherosclerosis.

It has been reported that the participation of proliferative phenotype of VSMCs is a consequential part in atherosclerosis. Cytoplasmic Ca^{2+} dysregulation via TRPC1 can mediate VSMC proliferation (Edwards *et al*., 2010). Studies have established that TRPC1 is implicated in coronary artery disease (CAD), during which the expression of TRPC1 mRNA and protein are elevated (Cheng *et al*., 2008; Edwards *et al*., 2010). Kumar *et al*. (2006) showed the upregulated TRPC1 in hyperplastic VSMCs was related to cell cycle activity and enhanced Ca^{2+} entry using a model of vascular injury in pigs and rats. In addition, the inhibition of TRPC1 effectively attenuates neointimal growth in veins (Kumar *et al*., 2006). These results indicate that upregulation of TRPC1 in VSMCs is a general feature of atherosclerosis.

The vascular endothelium is a polyfunctional organ, and ECs can generate extensive factors to mediate cellular adhesion, smooth muscle cell proliferation, thromboresistance, and vessel wall inflammation. Vascular endothelial dysfunction is the earliest detectable manifestation of atherosclerosis, which is associated with the malfunction of multiple TRPCs (Poteser *et al*., 2006). Tauseef *et al*. (2016) showed that TRPC1 maintained adherens junction plasticity and enabled EC-barrier destabilization by suppressing sphingosine kinase 1 (SPHK1) expression to induce endothelial hyperpermeability. Also, Poteser *et al*. (2006) demonstrated that porcine aorta endothelial cells, which co-expressed a redox-sensitive TRPC3 and TRPC4 complex, could give rise to cation channel activity. Furthermore, mice transfected with TRPC3 showed increased size and cellularity of advanced atherosclerotic lesions (Smedlund *et al*., 2015). In addition, studies further supported the relevance of EC migration to the healing of arterial injuries, suggesting TRPC5 and TRPC6 were activated by hypercholesterolemia, which impairs endothelial healing *in vitro* and *in vivo* (Rosenbaum *et al*., 2015; Chaudhuri *et al*., 2016).

Monocyte activation, adhesion to the endothelium, and transmigration into the sub-endothelial space are critical for early pathogenesis of atherosclerosis. The roles of TRPCs have been identified in the macrophage efferocytosis and survival, two crucial events in atherosclerosis lesion development (Tano *et al*., 2012). It has been shown that high D-glucose or peroxynitrite-induced oxidative stress significantly increased the expression of TRPCsin human monocytes (Wuensch *et al*., 2010). Vascular cell adhesion molecule-1 (VCAM-1) is important in monocyte recruitment to the endothelium as a critical factor in the development of atherosclerotic lesions. Smedlund *et al*. suggested that inhibition of TRPC3 expression

could significantly attenuate ATP-induced VCAM-1 and monocyte adhesion (Smedlund and Vazquez, 2008; Smedlund *et al*., 2010), indicating TRPC3 is involved in atherosclerosis lesion development. The platelet also plays important roles in cardiovascular diseases, especially in atherosclerosis, by participating in the formation of thrombosis and the induction of inflammation (Wang *et al*., 2016). Liu *et al*. (2008) investigated platelets in type II diabetes mellitus (DM) patients and found a time-dependent and concentration-dependent amplification of TRPC6 expression on the platelet membrane after challenge with high glucose. These results indicate that the incremental expression and activation of TRPC6 in platelets of DM patients may result in the risk of increasing atherosclerosis.

In summary, the pathophysiological relevance of TRPCs in several critical progresses has been linked to atherosclerosis.

Role of TRPCs in arrhythmia

Arrhythmia is a group of conditions in which the electrical activity of the heart is irregular, either too fast (above 100 beats per minute, called tachycardia) or too slow (below 60 beats per minute, called bradycardia). Several experiments have shed light on TRPC-regulated Ca^{2+} entry in arrhythmia. Sabourin *et al*. (2011) found that the existence of TRPC1,3,4,5,6 and 7 in the atria and ventricle, via association with the L-type voltage-gated calcium channel (LTCC), plays a role in the modulation of cardiac pacemaking, conduction, ventricular activity, and contractility during cardiogenesis. Mechanical stretch is one of the causes of cardiac arrhythmia. It has been demonstrated that mechanical transformation of ventricular myocytes can modulate TRPC6. The process can be inhibited by GsMTx-4, which is a peptide isolated from tarantula venom and a specific inhibitor of stretch-activated channels (SAC) (Dyachenko *et al*., 2009; Anderson *et al*., 2013; Gopal *et al*., 2015).

One of the most common arrhythmias is atrial fibrillation (AF) (Nattel, 2011; Wakili *et al*., 2011). By researching fibroblast regulation by Ca^{2+}-permeable TRPC3, Harada *et al*. (2012) found that AF increased expression of TRPC3 by activating NFAT-mediated downregulation of microRNA-26. Further, they found that AF induced TRPC3-dependent increase of fibroblast proliferation and differentiation, likely by mediating the Ca^{2+} entry that stimulates extracellular signal-regulated kinase signaling. TRPC3 blockade prevented AF substrate development in a dog model of electrically maintained AF *in vivo* (Harada *et al*., 2012). In conclusion, by promoting fibroblast pathophysiology, TRPC3 is likely to play an important role in AF.

Role of TRPCs in ischemia reperfusion injury

Tissue injury led by ischemia reperfusion is the main cause of cell apoptosis and necrosis leading to myocardial infarction, stroke, and other deadly diseases. After focal cerebral ischemia, brain injury results from a suite of pathological progresses, including inflammation, excitotoxicity, and apoptosis. Researchers have indicated that an increase in cytosolic Ca^{2+} is a critical step in initiating myocardial cell apoptosis and necrosis responding to ischemia reperfusion (Carafoli, 2002; Brookes *et al*., 2004). Several Ca^{2+} entry pathways, including the CCE and the Na^{+}/Ca^{2+} exchanger channel, have been implicated in mediating myocardial cell Ca^{2+} overload (Carafoli, 2002; Brookes *et al*., 2004; Piper *et al*., 2004). An increasing number of studies show that members of the TRPC proteins are involved in regulating CCE. Given this growing evidence linking TRPC proteins to CCE in myocardial cells subjected to ischemia reperfusion injury, Liu *et al*. (2016) tested the assumption that increased expression of TRPC3 in myocardial cells results in increased sensitivity to the injury after ischemia reperfusion, and found that the treatment of CCE inhibitor SKF96365 markedly improved cardiomyocytes viability in response to overexpressed TRPC3. In contrast, the LTCC inhibitor verapamil had no effect (Shan *et al*., 2008; Liu *et al*., 2016). These data strongly indicate that CCE mediated through TRPCs may lead to Ca^{2+}-induced cardiomyocyte apoptosis caused by ischemia reperfusion injury.

Intracellular Ca^{2+} overload is also the major reason of neuronal death after cerebral ischemia. TRPC6 protein is hydrolyzed by the activation of calpain induced by intracellular Ca^{2+} overload in the neurons after ischemia, which precedes ischemic neuronal cell death. The inhibition of proteolytic degeneration of TRPC6 protein by blocking calpain prevented ischemic neuronal death in an animal model of stroke (Du *et al*., 2010). Studies found that the upregulated TRPC6 could activate downstream effectors cAMP/Ca^{2+}-response element-binding (CREB) proteins, which are activated in neurons linked to a number of stimuli including growth factors, hormones, and neuronal activity through the Ras/MEK/ERK and CaM/CaMKIV pathways (Shaywitz and Greenberg, 1999; Tai *et al*., 2008; Du *et al*., 2010). It was also demonstrated that enhanced CREB activation activated neurogenesis, avoided myocardial infarct expansion, and reduced the penumbra region of cerebral ischemia and infarct volumes (Zhu *et al*., 2004). Thus, TRPC6 neuroprotection relied on CREB activation. Similarly, Lin *et al*. (2013) demonstrated that resveratrol prevented cerebral ischemia/reperfusion injury through the TRPC6-MEK-CREB and TRPC6-CaMKIV-CREB pathway.

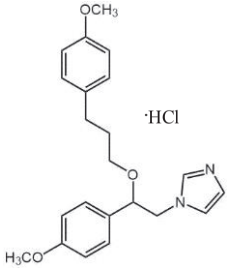
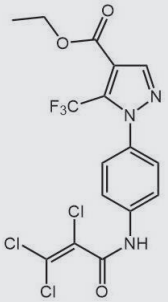
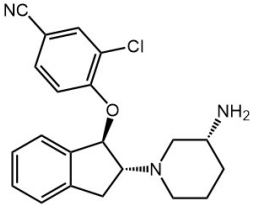
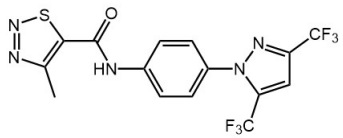
The aforementioned results provide further evidence that TRPC3 and TRPC6 play roles in the mediation of cardiomyocyte function and suggest that TRPC3 and TRPC6 may contribute to increased tolerance to ischemia reperfusion injury.

DISCUSSION

Mechanisms including elevated activation or expression of TRPCs that partake in mediating Ca^{2+} influx activated by GPCRs offer the chance to interfere with Ca^{2+}-dependent signaling processes, thus playing a significant role in cardio/cerebro-vascular diseases. The primary regulatory paradigm for most of these activities takes charge of total cytosolic Ca^{2+} or the propagation of intracellular Ca^{2+} signaling events that regulate cellular activity. Strong evidence indicates that TRPCs conduce to mechanical and agonist-induced SMC or fibroblast proliferation, cardiomyocytes apoptosis, and endothelium dysfunction. TRPCs were also present in Ang II-induced endothelium-dependent vasodilation and elevated contractility, regulation of vascular angiogenesis to participate in hypertension, pulmonary arterial hypertension, cardiac hypertrophy, atherosclerosis, arrhythmia, and ischemia reperfusion injury. These new findings permit a more comprehensive assessment of the molecular and cellular importance of TRPCs in physiology and pathophysiology.

Many questions remain to be elucidated. Therefore, researchers should keep a watchful eye on how the novel effects of TRPCs can be committed to human cardio/cerebrovascular diseases and clarify the clinical relevance of TRPC

Table 3. The essential information about inhibitors of TRPC channels or interdependent channels

Inhibitor	Chemical structure	Targeting channels	Predicted effects	Action mechanism	Reference
SKF96365		TRPC1, TRPC2, TRPC3, TRPC4, TRPC5, TRPC6, TRPC7	Selectively decrease receptor-mediated calcium entry (RMCE) in human platelets, neutrophils and endothelial cells	Inhibit receptor-mediated Ca^{2+} entry and voltage-gated Ca^{2+} entry	Merritt *et al.*, 1990; Farooqi *et al.*, 2013
Pyrazole-3 (Pyr3)		TRPC3	Prevent stent-induced arterial remodeling and inhibit SMC proliferation	Inhibit TRPC3 by binding to the extracellular side of the receptor	Rowell *et al.*, 2010; Christian and Maik, 2011; Koenig *et al.*, 2013
SAR7334		TRPC3, TRPC6, TRPC7	Effect on acute hypoxic pulmonary vasoconstriction and systemic blood pressure	Inhibit TRPC3, TRPC6, TRPC7-mediated Ca^{2+} influx into cells	Maier *et al.*, 2015
GsMTx-4	Gly-Cys-Leu-Glu-Phe-Trp-Trp-Lys-Cys-Asn-Pro-Asn-Asp-Asp-Lys-Cys-Cys-Arg-Pro-Lys-Leu-Lys-Cys-Ser-Lys-Leu-Phe-Lys-Leu-Cys-Asn-Phe-Ser-Phe-NH2	Stretch-activated ion channels and NaV1.7 Na^+ channels and TRPC1, TRPC6	Potential therapeutic targets for cardiac arrhythmias	Inhibit Na^+ voltage-gated channels and cation-selective mechanosensitive channels	Franz and Bode, 2003; Bowman *et al.*, 2007; Rowell *et al.*, 2010
BTP2		TRPCs and Store-operated Ca^{2+} influx and Ca^{2+} release-activated Ca^{2+} channels	Suppresses cytokine production (IL-2, IL-4, IL-5, IFN-γ, etc.) and proliferation in T cells *in vitro*	Inhibit anti-CD3 antibody-induced sustained Ca^{2+} influx	Ohga *et al.*, 2008; Rowell *et al.*, 2010

activities. An improved understanding of the underlying mechanisms of cardiovascular and cerebrovascular diseases may assist in the design of new therapies and the identification of more selective pharmacological agonists and antagonists (Table 3) for TRPCs or interdependent channels as well as promote exciting chances to develop new therapies that prevent or treat cardio/cerebro-vascular diseases.

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REFERENCES

Anderson, M., Kim, E. Y., Hagmann, H., Benzing, T. and Dryer, S. E. (2013) Opposing effects of podocin on the gating of podocyte TRPC6 channels evoked by membrane stretch or diacylglycerol. *Am. J. Physiol., Cell Physiol.* **305**, C276-C289.

Bae, Y. M., Kim, A., Lee, Y. J., Lim, W., Noh, Y. H., Kim, E. J., Kim, J., Kim, T. K., Park, S. W., Kim, B., Cho, S. I., Kim, D. K. and Ho, W. K. (2007) Enhancement of receptor-operated cation current and TRPC6 expression in arterial smooth muscle cells of deoxycorticosterone acetate-salt hypertensive rats. *J. Hypertens.* **25**, 809-817.

Beamish, J. A., He, P., Kottke-Marchant, K. and Marchant, R. E. (2010) Molecular regulation of contractile smooth muscle cell phenotype: implications for vascular tissue engineering. *Tissue Eng. Part B Rev.* **16**, 467-491.

Bergdahl, A., Gomez, M. F., Dreja, K., Xu, S. Z., Adner, M., Beech, D. J., Broman, J., Hellstrand, P. and Sward, K. (2003) Cholesterol depletion impairs vascular reactivity to endothelin-1 by reducing store-operated Ca^{2+} entry dependent on TRPC1. *Circ. Res.* **93**, 839-847.

Berridge, M. J., Bootman, M. D. and Roderick, H. L. (2003) Calcium signalling: dynamics, homeostasis and remodelling. *Nat. Rev. Mol. Cell Biol.* **4**, 517-529.

Birnbaumer, L., Zhu, X., Jiang, M., Boulay, G., Peyton, M., Vannier, B., Brown, D., Platano, D., Sadeghi, H., Stefani, E. and Birnbaumer, M. (1996) On the molecular basis and regulation of cellular capacitative calcium entry: roles for Trp proteins. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 15195-15202.

Bowman, C. L., Gottlieb, P. A., Suchyna, T. M., Murphy, Y. K. and Sachs, F. (2007) Mechanosensitive ion channels and the peptide inhibitor GsMTx-4: history, properties, mechanisms and pharmacology. *Toxicon* **49**, 249-270.

Brookes, P. S., Yoon, Y., Robotham, J. L., Anders, M. W. and Sheu, S. S. (2004) Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am. J. Physiol., Cell Physiol.* **287**, C817-C833.

Bush, E. W., Hood, D. B., Papst, P. J., Chapo, J. A., Minobe, W., Bristow, M. R., Olson, E. N. and McKinsey, T. A. (2006) Canonical transient receptor potential channels promote cardiomyocyte hypertrophy through activation of calcineurin signaling. *J. Biol. Chem.* **281**, 33487-33496.

Carafoli, E. (2002) Calcium signaling: a tale for all seasons. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 1115-1122.

Chaudhuri, P., Rosenbaum, M. A., Sinharoy, P., Damron, D. S., Birnbaumer, L. and Graham, L. M. (2016) Membrane translocation of TRPC6 channels and endothelial migration are regulated by calmodulin and PI3 kinase activation. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 2110-2115.

Chen, J., Crossland, R. F., Noorani, M. M. and Marrelli, S. P. (2009) Inhibition of TRPC1/TRPC3 by PKG contributes to NO-mediated vasorelaxation. *Am. J. Physiol. Heart Circ. Physiol.* **297**, H417-H424.

Chen, X., Yang, D., Ma, S., He, H., Luo, Z., Feng, X., Cao, T., Ma, L., Yan, Z., Liu, D., Tepel, M. and Zhu, Z. (2010) Increased rhythmicity in hypertensive arterial smooth muscle is linked to transient receptor potential canonical channels. *J. Cell. Mol. Med.* **14**, 2483-2494.

Cheng, K. T., Liu, X., Ong, H. L. and Ambudkar, I. S. (2008) Functional requirement for Orai1 in store-operated TRPC1-STIM1 channels. *J. Biol. Chem.* **283**, 12935-12940.

Christian, H. and Maik, G. (2011) Pharmacological Modulation of Diacylglycerol-Sensitive TRPC3/6/7 Channels. *Curr. Pharm. Biotechnol.* **12**, 35-41.

Cosens, D. J. and Manning, A. (1969) Abnormal electroretinogram from a *Drosophila* mutant. *Nature* **224**, 285-287.

Dietrich, A., Chubanov, V., Kalwa, H., Rost, B. R. and Gudermann, T. (2006) Cation channels of the transient receptor potential superfamily: their role in physiological and pathophysiological processes of smooth muscle cells. *Pharmacol. Ther.* **112**, 744-760.

Dietrich, A., Kalwa, H. and Gudermann, T. (2010) TRPC channels in vascular cell function. *Thromb. Haemost.* **103**, 262-270.

Dietrich, A., Mederos, Y. S. M., Gollasch, M., Gross, V., Storch, U., Dubrovska, G., Obst, M., Yildirim, E., Salanova, B., Kalwa, H., Essin, K., Pinkenburg, O., Luft, F. C., Gudermann, T. and Birnbaumer, L. (2005) Increased vascular smooth muscle contractility in *Trpc6*$^{-/-}$ mice. *Mol. Cell. Biol.* **25**, 6980-6989.

Du, W., Huang, J., Yao, H., Zhou, K., Duan, B. and Wang, Y. (2010) Inhibition of TRPC6 degradation suppresses ischemic brain damage in rats. *J. Clin. Invest.* **120**, 3480-3492.

Dyachenko, V., Husse, B., Rueckschloss, U. and Isenberg, G. (2009) Mechanical deformation of ventricular myocytes modulates both TRPC6 and Kir2.3 channels. *Cell Calcium* **45**, 38-54.

Eder, P. and Molkentin, J. D. (2011) TRPC channels as effectors of cardiac hypertrophy. *Circ. Res.* **108**, 265-272.

Edwards, J. M., Neeb, Z. P., Alloosh, M. A., Long, X., Bratz, I. N., Peller, C. R., Byrd, J. P., Kumar, S., Obukhov, A. G. and Sturek, M. (2010) Exercise training decreases store-operated Ca^{2+} entry associated with metabolic syndrome and coronary atherosclerosis. *Cardiovasc. Res.* **85**, 631-640.

Farooqi, A. A., Riaz, A. M. and Bhatti, S. (2013) TRPC signaling mechanisms and therapeutic opportunities: trapdoors are monitored by gatekeepers. *Pak. J. Pharm. Sci.* **26**, 847-852.

Franz, M. R. and Bode, F. (2003) Mechano-electrical feedback underlying arrhythmias: the atrial fibrillation case. *Prog. Biophys. Mol. Biol.* **82**, 163-174.

Fuchs, B., Dietrich, A., Gudermann, T., Kalwa, H., Grimminger, F. and Weissmann, N. (2010) The role of classical transient receptor potential channels in the regulation of hypoxic pulmonary vasoconstriction. *Adv. Exp. Med. Biol.* **661**, 187-200.

Golovina, V. A., Platoshyn, O., Bailey, C. L., Wang, J., Limsuwan, A., Sweeney, M., Rubin, L. J. and Yuan, J. X. (2001) Upregulated TRP and enhanced capacitative Ca^{2+} entry in human pulmonary artery myocytes during proliferation. *Am. J. Physiol. Heart Circ. Physiol.* **280**, H746-H755.

Gopal, S., Sogaard, P., Multhaupt, H. A., Pataki, C., Okina, E., Xian, X., Pedersen, M. E., Stevens, T., Griesbeck, O., Park, P. W., Pocock, R. and Couchman, J. R. (2015) Transmembrane proteoglycans control stretch-activated channels to set cytosolic calcium levels. *J. Cell Biol.* **210**, 1199-1211.

Harada, M., Luo, X., Qi, X. Y., Tadevosyan, A., Maguy, A., Ordog, B., Ledoux, J., Kato, T., Naud, P., Voigt, N., Shi, Y., Kamiya, K., Murohara, T., Kodama, I., Tardif, J. C., Schotten, U., Van Wagoner, D. R., Dobrev, D. and Nattel, S. (2012) Transient receptor potential canonical-3 channel-dependent fibroblast regulation in atrial fibrillation. *Circulation* **126**, 2051-2064.

Hofmann, T., Obukhov, A. G., Schaefer, M., Harteneck, C., Gudermann, T. and Schultz, G. (1999) Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. *Nature* **397**, 259-263.

Inoue, R., Jensen, L. J., Jian, Z., Shi, J., Hai, L., Lurie, A. I., Henriksen, F. H., Salomonsson, M., Morita, H., Kawarabayashi, Y., Mori, M., Mori, Y. and Ito, Y. (2009) Synergistic activation of vascular TRPC6 channel by receptor and mechanical stimulation via phospholipase C/diacylglycerol and phospholipase A2/omega-hydroxylase/20-HETE pathways. *Circ. Res.* **104**, 1399-1409.

Inoue, R., Jensen, L. J., Shi, J., Morita, H., Nishida, M., Honda, A. and Ito, Y. (2006) Transient receptor potential channels in cardiovascu-

lar function and disease. *Circ. Res.* **99**, 119-131.
Iwasaki, Y. K., Nishida, K., Kato, T. and Nattel, S. (2011) Atrial fibrillation pathophysiology: implications for management. *Circulation* **124**, 2264-2274.
Kinoshita, H., Kuwahara, K., Nishida, M., Jian, Z., Rong, X., Kiyonaka, S., Kuwabara, Y., Kurose, H., Inoue, R., Mori, Y., Li, Y., Nakagawa, Y., Usami, S., Fujiwara, M., Yamada, Y., Minami, T., Ueshima, K. and Nakao, K. (2010) Inhibition of TRPC6 channel activity contributes to the antihypertrophic effects of natriuretic peptides-guanylyl cyclase-A signaling in the heart. *Circ. Res.* **106**, 1849-1860.
Kiyonaka, S., Kato, K., Nishida, M., Mio, K., Numaga, T., Sawaguchi, Y., Yoshida, T., Wakamori, M., Mori, E., Numata, T., Ishii, M., Takemoto, H., Ojida, A., Watanabe, K., Uemura, A., Kurose, H., Morii, T., Kobayashi, T., Sato, Y., Sato, C., Hamachi, I. and Mori, Y. (2009) Selective and direct inhibition of TRPC3 channels underlies biological activities of a pyrazole compound. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 5400-5405.
Koenig, S., Schernthaner, M., Maechler, H., Kappe, C. O., Glasnov, T. N., Hoefler, G., Braune, M., Wittchow, E. and Groschner, K. (2013) A TRPC3 blocker, ethyl-1-(4-(2,3,3-trichloroacrylamide)phenyl)-5-(trifluoromethyl)-1H-pyrazole-4-c arboxylate (Pyr3), prevents stent-induced arterial remodeling. *J. Pharmacol. Exp. Ther.* **344**, 33-40.
Kuhr, F. K., Smith, K. A., Song, M. Y., Levitan, I. and Yuan, J. X. (2012) New mechanisms of pulmonary arterial hypertension: role of Ca^{2+} signaling. *Am. J. Physiol. Heart Circ. Physiol.* **302**, H1546-H1562.
Kukkonen, J. P. (2011) A menage a trois made in heaven: G-protein-coupled receptors, lipids and TRP channels. *Cell Calcium* **50**, 9-26.
Kumar, B., Dreja, K., Shah, S. S., Cheong, A., Xu, S. Z., Sukumar, P., Naylor, J., Forte, A., Cipollaro, M., McHugh, D., Kingston, P. A., Heagerty, A. M., Munsch, C. M., Bergdahl, A., Hultgardh-Nilsson, A., Gomez, M. F., Porter, K. E., Hellstrand, P. and Beech, D. J. (2006) Upregulated TRPC1 channel in vascular injury *in vivo* and its role in human neointimal hyperplasia. *Circ. Res.* **98**, 557-563.
Kuwahara, K., Wang, Y., McAnally, J., Richardson, J. A., Bassel-Duby, R., Hill, J. A. and Olson, E. N. (2006) TRPC6 fulfills a calcineurin signaling circuit during pathologic cardiac remodeling. *J. Clin. Invest*. **116**, 3114-3126.
Kwan, H. Y., Huang, Y. and Yao, X. (2004) Regulation of canonical transient receptor potential isoform 3 (TRPC3) channel by protein kinase G. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 2625-2630.
Leypold, B. G., Yu, C. R., Leinders-Zufall, T., Kim, M. M., Zufall, F. and Axel, R. (2002) Altered sexual and social behaviors in trp2 mutant mice. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 6376-6381.
Lin, M. J., Leung, G. P., Zhang, W. M., Yang, X. R., Yip, K. P., Tse, C. M. and Sham, J. S. (2004) Chronic hypoxia-induced upregulation of store-operated and receptor-operated Ca^{2+} channels in pulmonary arterial smooth muscle cells: a novel mechanism of hypoxic pulmonary hypertension. *Circ. Res.* **95**, 496-505.
Lin, Y., Chen, F., Zhang, J., Wang, T., Wei, X., Wu, J., Feng, Y., Dai, Z. and Wu, Q. (2013) Neuroprotective effect of resveratrol on ischemia/reperfusion injury in rats through TRPC6/CREB pathways. *J. Mol. Neurosci.* **50**, 504-513.
Liu, D., Maier, A., Scholze, A., Rauch, U., Boltzen, U., Zhao, Z., Zhu, Z. and Tepel, M. (2008) High glucose enhances transient receptor potential channel canonical type 6-dependent calcium influx in human platelets via phosphatidylinositol 3-kinase-dependent pathway. *Arterioscler. Thromb. Vasc. Biol.* **28**, 746-751.
Liu, D., Scholze, A., Zhu, Z., Krueger, K., Thilo, F., Burkert, A., Streffer, K., Holz, S., Harteneck, C., Zidek, W. and Tepel, M. (2006) Transient receptor potential channels in essential hypertension. *J. Hypertens.* **24**, 1105-1114.
Liu, D., Yang, D., He, H., Chen, X., Cao, T., Feng, X., Ma, L., Luo, Z., Wang, L., Yan, Z., Zhu, Z. and Tepel, M. (2009) Increased transient receptor potential canonical type 3 channels in vasculature from hypertensive rats. *Hypertension* **53**, 70-76.
Liu, D. Y., Scholze, A., Kreutz, R., Wehland-von-Trebra, M., Zidek, W., Zhu, Z. M. and Tepel, M. (2007a) Monocytes from spontaneously hypertensive rats show increased store-operated and second messenger-operated calcium influx mediated by transient receptor potential canonical Type 3 channels. *Am. J. Hypertens.* **20**, 1111-1118.
Liu, D. Y., Thilo, F., Scholze, A., Wittstock, A., Zhao, Z. G., Harteneck, C., Zidek, W., Zhu, Z. M. and Tepel, M. (2007b) Increased store-operated and 1-oleoyl-2-acetyl-sn-glycerol-induced calcium influx in monocytes is mediated by transient receptor potential canonical channels in human essential hypertension. *J. Hypertens.* **25**, 799-808.
Liu, H., Yang, L., Chen, K. H., Sun, H. Y., Jin, M. W., Xiao, G. S., Wang, Y. and Li, G. R. (2016) SKF-96365 blocks human ether-a-go-go-related gene potassium channels stably expressed in HEK 293 cells. *Pharmacol. Res.* **104**, 61-69.
Liu, X. R., Zhang, M. F., Yang, N., Liu, Q., Wang, R. X., Cao, Y. N., Yang, X. R., Sham, J. S. and Lin, M. J. (2012) Enhanced store-operated Ca^{2+} entry and TRPC channel expression in pulmonary arteries of monocrotaline-induced pulmonary hypertensive rats. *Am. J. Physiol., Cell Physiol.* **302**, C77-C87.
Loga, F., Domes, K., Freichel, M., Flockerzi, V., Dietrich, A., Birnbaumer, L., Hofmann, F. and Wegener, J. W. (2013) The role of cGMP/cGKI signalling and Trpc channels in regulation of vascular tone. *Cardiovasc. Res.* **100**, 280-287.
Lu, W., Ran, P., Zhang, D., Peng, G., Li, B., Zhong, N. and Wang, J. (2010) Sildenafil inhibits chronically hypoxic upregulation of canonical transient receptor potential expression in rat pulmonary arterial smooth muscle. *Am. J. Physiol., Cell Physiol.* **298**, C114-C123.
Lu, W., Wang, J., Shimoda, L. A. and Sylvester, J. T. (2008) Differences in STIM1 and TRPC expression in proximal and distal pulmonary arterial smooth muscle are associated with differences in Ca^{2+} responses to hypoxia. *Am. J. Physiol. Lung Cell Mol. Physiol.* **295**, L104-L113.
Maier, T., Follmann, M., Hessler, G., Kleemann, H. W., Hachtel, S., Fuchs, B., Weissmann, N., Linz, W., Schmidt, T., Lohn, M., Schroeter, K., Wang, L., Rutten, H. and Strubing, C. (2015) Discovery and pharmacological characterization of a novel potent inhibitor of diacylglycerol-sensitive TRPC cation channels. *Br. J. Pharmacol.* **172**, 3650-3660.
Malczyk, M., Veith, C., Fuchs, B., Hofmann, K., Storch, U., Schermuly, R. T., Witzenrath, M., Ahlbrecht, K., Fecher-Trost, C., Flockerzi, V., Ghofrani, H. A., Grimminger, F., Seeger, W., Gudermann, T., Dietrich, A. and Weissmann, N. (2013) Classical transient receptor potential channel 1 in hypoxia-induced pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* **188**, 1451-1459.
Merritt, J. E., Armstrong, W. P., Benham, C. D., Hallam, T. J., Jacob, R., Jaxa-Chamiec, A., Leigh, B. K., McCarthy, S. A., Moores, K. E. and Rink, T. J. (1990) SK&F 96365, a novel inhibitor of receptor-mediated calcium entry. *Biochem. J.* **271**, 515-522.
Minke, B. (2006) TRP channels and Ca^{2+} signaling. *Cell Calcium* **40**, 261-275.
Montell, C. (2005) Drosophila TRP channels. *Pflugers Arch.* **451**, 19-28.
Montell, C., Birnbaumer, L., Flockerzi, V., Bindels, R. J., Bruford, E. A., Caterina, M. J., Clapham, D. E., Harteneck, C., Heller, S., Julius, D., Kojima, I., Mori, Y., Penner, R., Prawitt, D., Scharenberg, A. M., Schultz, G., Shimizu, N. and Zhu, M. X. (2002) A unified nomenclature for the superfamily of TRP cation channels. *Mol. Cell* **9**, 229-231.
Nakashima, H. and Kumagai, K. (2007) Reverse-remodeling effects of angiotensin II type 1 receptor blocker in a canine atrial fibrillation model. *Circ. J.* **71**, 1977-1982.
Nakayama, H., Wilkin, B. J., Bodi, I. and Molkentin, J. D. (2006) Calcineurin-dependent cardiomyopathy is activated by TRPC in the adult mouse heart. *FASEB J.* **20**, 1660-1670.
Nattel, S. (2011) From guidelines to bench: implications of unresolved clinical issues for basic investigations of atrial fibrillation mechanisms. *Can. J. Cardiol.* **27**, 19-26.
Ng, L. C. and Gurney, A. M. (2001) Store-operated channels mediate Ca^{2+} influx and contraction in rat pulmonary artery. *Circ. Res.* **89**, 923-929.
Nilius, B., Owsianik, G., Voets, T. and Peters, J. A. (2007) Transient receptor potential cation channels in disease. *Physiol. Rev.* **87**, 165-217.
Nilius, B. and Voets, T. (2005) TRP channels: a TR(I)P through a world of multifunctional cation channels. *Pflugers Arch.* **451**, 1-10.
Ohba, T., Watanabe, H., Murakami, M., Takahashi, Y., Iino, K., Kuromitsu, S., Mori, Y., Ono, K., Iijima, T. and Ito, H. (2007) Upregula-

tion of TRPC1 in the development of cardiac hypertrophy. *J. Mol. Cell. Cardiol.* **42**, 498-507.
Ohga, K., Takezawa, R., Arakida, Y., Shimizu, Y. and Ishikawa, J. (2008) Characterization of YM-58483/BTP2, a novel store-operated Ca^{2+} entry blocker, on T cell-mediated immune responses *in vivo*. *Int. Immunopharmacol.* **8**, 1787-1792.
Okada, T., Inoue, R., Yamazaki, K., Maeda, A., Kurosaki, T., Yamakuni, T., Tanaka, I., Shimizu, S., Ikenaka, K., Imoto, K. and Mori, Y. (1999) Molecular and functional characterization of a novel mouse transient receptor potential protein homologue TRP7. Ca^{2+}-permeable cation channel that is constitutively activated and enhanced by stimulation of G protein-coupled receptor. *J. Biol. Chem*. **274**, 27359-27370.
Onohara, N., Nishida, M., Inoue, R., Kobayashi, H., Sumimoto, H., Sato, Y., Mori, Y., Nagao, T. and Kurose, H. (2006) TRPC3 and TRPC6 are essential for angiotensin II-induced cardiac hypertrophy. *EMBO J.* **25**, 5305-5316.
Philipp, S., Wissenbach, U. and Flockerzi, V. (2000) Molecular biology of calcium channels. In Calcium Signaling (J. W. J. Putney, Ed.), pp. 321-342. CRC Press, Boca Raton.
Piper, H. M., Abdallah, Y. and Schafer, C. (2004) The first minutes of reperfusion: a window of opportunity for cardioprotection. *Cardiovasc. Res.* **61**, 365-371.
Plant, T. D. and Schaefer, M. (2003) TRPC4 and TRPC5: receptor-operated Ca^{2+}-permeable nonselective cation channels. *Cell Calcium* **33**, 441-450.
Poteser, M., Graziani, A., Rosker, C., Eder, P., Derler, I., Kahr, H., Zhu, M. X., Romanin, C. and Groschner, K. (2006) TRPC3 and TRPC4 associate to form a redox-sensitive cation channel. Evidence for expression of native TRPC3-TRPC4 heteromeric channels in endothelial cells. *J. Biol. Chem.* **281**, 13588-13595.
Putney, J. W., Jr. (1986) A model for receptor-regulated calcium entry. *Cell Calcium* **7**, 1-12.
Riccio, A., Medhurst, A. D., Mattei, C., Kelsell, R. E., Calver, A. R., Randall, A. D., Benham, C. D. and Pangalos, M. N. (2002) mRNA distribution analysis of human TRPC family in CNS and peripheral tissues. *Brain Res. Mol. Brain Res.* **109**, 95-104.
Rosenbaum, M. A., Chaudhuri, P. and Graham, L. M. (2015) Hypercholesterolemia inhibits re-endothelialization of arterial injuries by TRPC channel activation. *J. Vasc. Surg.* **62**, 1040-1047.e2.
Rowell, J., Koitabashi, N. and Kass, D. A. (2010) TRP-ing up heart and vessels: canonical transient receptor potential channels and cardiovascular disease. *J. Cardiovasc. Transl. Res.* **3**, 516-524.
Sabourin, J., Robin, E. and Raddatz, E. (2011) A key role of TRPC channels in the regulation of electromechanical activity of the developing heart. *Cardiovasc. Res.* **92**, 226-236.
Satoh, S., Tanaka, H., Ueda, Y., Oyama, J., Sugano, M., Sumimoto, H., Mori, Y. and Makino, N. (2007) Transient receptor potential (TRP) protein 7 acts as a G protein-activated Ca^{2+} channel mediating angiotensin II-induced myocardial apoptosis. *Mol. Cell. Biochem.* **294**, 205-215.
Schaefer, M., Plant, T. D., Obukhov, A. G., Hofmann, T., Gudermann, T. and Schultz, G. (2000) Receptor-mediated regulation of the nonselective cation channels TRPC4 and TRPC5. *J. Biol. Chem.* **275**, 17517-17526.
Schleifer, H., Doleschal, B., Lichtenegger, M., Oppenrieder, R., Derler, I., Frischauf, I., Glasnov, T. N., Kappe, C. O., Romanin, C. and Groschner, K. (2012) Novel pyrazole compounds for pharmacological discrimination between receptor-operated and store-operated Ca^{2+} entry pathways. *Br. J. Pharmacol.* **167**, 1712-1722.
Seo, K., Rainer, P. P., Shalkey Hahn, V., Lee, D. I., Jo, S. H., Andersen, A., Liu, T., Xu, X., Willette, R. N., Lepore, J. J., Marino, J. P., Jr., Birnbaumer, L., Schnackenberg, C. G. and Kass, D. A. (2014) Combined TRPC3 and TRPC6 blockade by selective small-molecule or genetic deletion inhibits pathological cardiac hypertrophy. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 1551-1556.
Seth, M., Zhang, Z. S., Mao, L., Graham, V., Burch, J., Stiber, J., Tsiokas, L., Winn, M., Abramowitz, J., Rockman, H. A., Birnbaumer, L. and Rosenberg, P. (2009) TRPC1 channels are critical for hypertrophic signaling in the heart. *Circ. Res.* **105**, 1023-1030.
Shan, D., Marchase, R. B. and Chatham, J. C. (2008) Overexpression of TRPC3 increases apoptosis but not necrosis in response to ischemia-reperfusion in adult mouse cardiomyocytes. *Am. J. Physiol., Cell Physiol*. **294**, C833-C841.
Shaywitz, A. J. and Greenberg, M. E. (1999) CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu. Rev. Biochem.* **68**, 821-861.
Shi, J., Ju, M., Abramowitz, J., Large, W. A., Birnbaumer, L. and Albert, A. P. (2012) TRPC1 proteins confer PKC and phosphoinositol activation on native heteromeric TRPC1/C5 channels in vascular smooth muscle: comparative study of wild-type and *Trpc1*$^{-/-}$ mice. *FASEB J.* **26**, 409-419.
Shi, J., Miralles, F., Birnbaumer, L., Large, W. A. and Albert, A. P. (2016) Store depletion induces Galphaq-mediated PLCbeta1 activity to stimulate TRPC1 channels in vascular smooth muscle cells. *FASEB J.* **30**, 702-715.
Short, A. D., Bian, J., Ghosh, T. K., Waldron, R. T., Rybak, S. L. and Gill, D. L. (1993) Intracellular Ca^{2+} pool content is linked to control of cell growth. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 4986-4990.
Smedlund, K., Tano, J. Y. and Vazquez, G. (2010) The constitutive function of native TRPC3 channels modulates vascular cell adhesion molecule-1 expression in coronary endothelial cells through nuclear factor κB signaling. *Circ. Res*. **106**, 1479-1488.
Smedlund, K. and Vazquez, G. (2008) Involvement of native TRPC3 proteins in ATP-dependent expression of VCAM-1 and monocyte adherence in coronary artery endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* **28**, 2049-2055.
Smedlund, K. B., Birnbaumer, L. and Vazquez, G. (2015) Increased size and cellularity of advanced atherosclerotic lesions in mice with endothelial overexpression of the human TRPC3 channel. *Proc. Natl. Acad. Sci. U.S.A.* **112**, E2201-E2206.
Soboloff, J., Spassova, M., Xu, W., He, L. P., Cuesta, N. and Gill, D. L. (2005) Role of endogenous TRPC6 channels in Ca^{2+} signal generation in A7r5 smooth muscle cells. *J. Biol. Chem.* **280**, 39786-39794.
Stowers, L., Holy, T. E., Meister, M., Dulac, C. and Koentges, G. (2002) Loss of sex discrimination and male-male aggression in mice deficient for TRP2. *Science* **295**, 1493-1500.
Tabas, I., Tall, A. and Accili, D. (2010) The impact of macrophage insulin resistance on advanced atherosclerotic plaque progression. *Circ. Res.* **106**, 58-67.
Tai, Y., Feng, S., Ge, R., Du, W., Zhang, X., He, Z. and Wang, Y. (2008) TRPC6 channels promote dendritic growth via the CaMKIV-CREB pathway. *J. Cell Sci.* **121**, 2301-2307.
Takahashi, S., Lin, H., Geshi, N., Mori, Y., Kawarabayashi, Y., Takami, N., Mori, M. X., Honda, A. and Inoue, R. (2008) Nitric oxide-cGMP-protein kinase G pathway negatively regulates vascular transient receptor potential channel TRPC6. *J. Physiol.* **586**, 4209-4223.
Takahashi, Y., Watanabe, H., Murakami, M., Ohba, T., Radovanovic, M., Ono, K., Iijima, T. and Ito, H. (2007) Involvement of transient receptor potential canonical 1 (TRPC1) in angiotensin II-induced vascular smooth muscle cell hypertrophy. *Atherosclerosis* **195**, 287-296.
Tano, J. Y., Lee, R. H. and Vazquez, G. (2012) Macrophage function in atherosclerosis: potential roles of TRP channels. *Channels (Austin)* **6**, 141-148.
Tauseef, M., Farazuddin, M., Sukriti, S., Rajput, C., Meyer, J. O., Ramasamy, S. K. and Mehta, D. (2016) Transient receptor potential channel 1 maintains adherens junction plasticity by suppressing sphingosine kinase 1 expression to induce endothelial hyperpermeability. *FASEB J.* **30**, 102-110.
Thilo, F., Loddenkemper, C., Berg, E., Zidek, W. and Tepel, M. (2009) Increased TRPC3 expression in vascular endothelium of patients with malignant hypertension. *Mod. Pathol.* **22**, 426-430.
Toth, P., Csiszar, A., Tucsek, Z., Sosnowska, D., Gautam, T., Koller, A., Schwartzman, M. L., Sonntag, W. E. and Ungvari, Z. (2013) Role of 20-HETE, TRPC channels, and BKCa in dysregulation of pressure-induced Ca^{2+} signaling and myogenic constriction of cerebral arteries in aged hypertensive mice. *Am. J. Physiol. Heart Circ. Physiol.* **305**, H1698-H1708.
Venkatachalam, K., Zheng, F. and Gill, D. L. (2003) Regulation of canonical transient receptor potential (TRPC) channel function by diacylglycerol and protein kinase *C*. *J. Biol. Chem.* **278**, 29031-

29040.

Wakili, R., Voigt, N., Kaab, S., Dobrev, D. and Nattel, S. (2011) Recent advances in the molecular pathophysiology of atrial fibrillation. *J. Clin. Invest.* **121**, 2955-2968.

Wang, J., Fu, X., Yang, K., Jiang, Q., Chen, Y., Jia, J., Duan, X., Wang, E. W., He, J., Ran, P., Zhong, N., Semenza, G. L. and Lu, W. (2015) Hypoxia inducible factor-1-dependent up-regulation of BMP4 mediates hypoxia-induced increase of TRPC expression in PASMCs. *Cardiovasc. Res.* **107**, 108-118.

Wang, J., Jiang, Q., Wan, L., Yang, K., Zhang, Y., Chen, Y., Wang, E., Lai, N., Zhao, L., Jiang, H., Sun, Y., Zhong, N., Ran, P. and Lu, W. (2013a) Sodium tanshinone IIA sulfonate inhibits canonical transient receptor potential expression in pulmonary arterial smooth muscle from pulmonary hypertensive rats. *Am. J. Respir. Cell Mol. Biol.* **48**, 125-134.

Wang, J., Weigand, L., Lu, W., Sylvester, J. T., Semenza, G. L. and Shimoda, L. A. (2006) Hypoxia inducible factor 1 mediates hypoxia-induced TRPC expression and elevated intracellular Ca^{2+} in pulmonary arterial smooth muscle cells. *Circ. Res.* **98**, 1528-1537.

Wang, Z. T., Wang, Z. and Hu, Y. W. (2016) Possible roles of platelet-derived microparticles in atherosclerosis. *Atherosclerosis* **248**, 10-16.

Weber, E. W., Han, F., Tauseef, M., Birnbaumer, L., Mehta, D. and Muller, W. A. (2015) TRPC6 is the endothelial calcium channel that regulates leukocyte transendothelial migration during the inflammatory response. *J. Exp. Med.* **212**, 1883-1899.

Welsh, D. G., Morielli, A. D., Nelson, M. T. and Brayden, J. E. (2002) Transient receptor potential channels regulate myogenic tone of resistance arteries. *Circ. Res.* **90**, 248-250.

Winn, M. P., Conlon, P. J., Lynn, K. L., Farrington, M. K., Creazzo, T., Hawkins, A. F., Daskalakis, N., Kwan, S. Y., Ebersviller, S., Burchette, J. L., Pericak-Vance, M. A., Howell, D. N., Vance, J. M. and Rosenberg, P. B. (2005) A mutation in the TRPC6 cation channel causes familial focal segmental glomerulosclerosis. *Science* **308**, 1801-1804.

Wu, X., Eder, P., Chang, B. and Molkentin, J. D. (2010) TRPC channels are necessary mediators of pathologic cardiac hypertrophy. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 7000-7005.

Wuensch, T., Thilo, F., Krueger, K., Scholze, A., Ristow, M. and Tepel, M. (2010) High glucose-induced oxidative stress increases transient receptor potential channel expression in human monocytes. *Diabetes* **59**, 844-849.

Xia, Y., Yang, X. R., Fu, Z., Paudel, O., Abramowitz, J., Birnbaumer, L. and Sham, J. S. (2014) Classical transient receptor potential 1 and 6 contribute to hypoxic pulmonary hypertension through differential regulation of pulmonary vascular functions. *Hypertension* **63**, 173-180.

Xu, S. Z. and Beech, D. J. (2001) TrpC1 is a membrane-spanning subunit of store-operated Ca^{2+} channels in native vascular smooth muscle cells. *Circ. Res.* **88**, 84-87.

Yu, Y., Fantozzi, I., Remillard, C. V., Landsberg, J. W., Kunichika, N., Platoshyn, O., Tigno, D. D., Thistlethwaite, P. A., Rubin, L. J. and Yuan, J. X. (2004) Enhanced expression of transient receptor potential channels in idiopathic pulmonary arterial hypertension. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 13861-13866.

Yue, Z., Xie, J., Yu, A. S., Stock, J., Du, J. and Yue, L. (2015) Role of TRP channels in the cardiovascular system. *Am. J. Physiol. Heart Circ. Physiol.* **308**, H157-H182.

Zhang, S., Remillard, C. V., Fantozzi, I. and Yuan, J. X. (2004) ATP-induced mitogenesis is mediated by cyclic AMP response element-binding protein-enhanced TRPC4 expression and activity in human pulmonary artery smooth muscle cells. *Am. J. Physiol., Cell Physiol.* **287**, C1192-C1201.

Zhang, Y., Lu, W., Yang, K., Xu, L., Lai, N., Tian, L., Jiang, Q., Duan, X., Chen, M. and Wang, J. (2013) Bone morphogenetic protein 2 decreases TRPC expression, store-operated Ca^{2+} entry, and basal $[Ca^{2+}]i$ in rat distal pulmonary arterial smooth muscle cells. *Am. J. Physiol., Cell Physiol.* **304**, C833-C843.

Zhang, Y., Wang, Y., Yang, K., Tian, L., Fu, X., Wang, Y., Sun, Y., Jiang, Q., Lu, W. and Wang, J. (2014) BMP4 increases the expression of TRPC and basal $[Ca^{2+}]i$ via the p38MAPK and ERK1/2 pathways independent of BMPRII in PASMCs. *PLoS ONE* **9**, e112695.

Zhu, D. Y., Lau, L., Liu, S. H., Wei, J. S. and Lu, Y. M. (2004) Activation of cAMP-response-element-binding protein (CREB) after focal cerebral ischemia stimulates neurogenesis in the adult dentate gyrus. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 9453-9457.

Zhu, X., Chu, P. B., Peyton, M. and Birnbaumer, L. (1995) Molecular cloning of a widely expressed human homologue for the *Drosophila* trp gene. *FEBS Lett.* **373**, 193-198.