

Functions of ANO1/TMEM16A, Ca²⁺-activated Cl⁻ channels in Regulation of Blood Pressure and Vascular Remodeling

Zhiqiang Qu, Bingxiang Wang, Zhen Zhang, Li Ma, Dan Li, Likun Zhuang, Jingwei Chi, Jiaxiu Liu

Zhiqiang Qu, Li Ma, Dan Li, Likun Zhuang, Jingwei Chi, Jiaxiu Liu, Institute of Clinical Medicine, Affiliated Hospital of Qingdao University, Qingdao 266071, China

Bingxiang Wang, Department of Physiology, Taishan Medical College, Taian 271000, China

Zhen Zhang, Department of Pathophysiology, Medical College of Qingdao University, Qingdao 266071, China

Correspondence to: Zhiqiang Qu, Center for Medical Research, Science Building, 1677 Wutaishan Rd., Huangdao Area, Qingdao 266555, China.

Email: 18661801173@163.com

Telephone: +86-18661801173

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ABSTRACT

Since the molecule of Ca²⁺-activated Cl⁻ channels (CaCC) had been identified as ANO1, major progresses have been made in recent studies on its roles in vascular functions. ANO1 has been confirmed to represent the CaCC channels in vascular smooth myocytes (VSMCs). Generally, ANO1 expresses more in resistance-size small arteries. When activated, it functions to control the VSMC membrane potentials by depolarization. As a result, the voltage-dependent Ca²⁺ channel is opened, leading to Ca²⁺ entry and VSMC contraction. Thus, the function of ANO1 is important for maintenance of normal blood pressure. Under influence of agonists, e.g., angiotensin II, ANO1 also participates in VSMC differentiation and remodeling which are mediated by KLF5, myocardin and SRF. The factors regulate the transcription of the *ANO1* gene and other genes for VSMC differentiation and proliferation.

Key words: Ca²⁺-activated Cl⁻ channels; ANO1; TMEM16A; Vascular contractility; Blood pressure; Vascular smooth myocytes; Cell proliferation; Vascular remodeling

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INTRODUCTION

Ca²⁺-activated Cl⁻ channels (CaCC) have been recognized for a long time as one of the most important anion channels in various physiological functions: electrolyte secretion of pulmonary and intestinal epithelia, motility of intestinal track, excitability of neurons and cardiomyocytes, vascular tone and sensations of olfactory, visual and gustatory organs^[1-3]. The CaCC channels are located to the apical membranes of epithelial cells in airways and intestines, and complete the secretion of Cl⁻ and HCO₃⁻ from epithelia into lumina. In digestive tracts, the channels as a pacemaker reside in the interstitial cells of Cajal, being responsible for the rhythm of intestinal movement. The CaCC channels are activated by the intracellular free Ca²⁺. When the channels are opened, the Cl⁻ influx or efflux participates in the formation of membrane resting potentials and the repolarization of action potential. Therefore, the normal function of the CaCC channels are important to the neuronal and myocardial excitability.

Since the endogenous CaCC channels in mammals were identified as the products expressed by ANO1 (anoctamin1) or TMEM16A (transmembrane member 16a) gene in 2008^[4-6], major progresses have been made in the study of various aspects of CaCC-mediated physiological functions and phenomena in mammals^[1-3,7]. Among the progresses, the channel identification outstandingly contributes to the elucidation of its roles in vascular functions^[3,8]. However, understanding of the mechanisms how ANO1 contributes to the vascular functions, e.g., regulation of blood pressure (BP) and vascular remodeling are not unanimous at present. In this review, the authors have summarized the recent discoveries that delineate the roles played by ANO1 in functions of blood vessels with different points of view.

THE CACC CHANNELS ARE IMPORTANT FACTORS TO CONTROL VASCULAR CONTRACTION

The CaCC currents and channels have been recognized for three

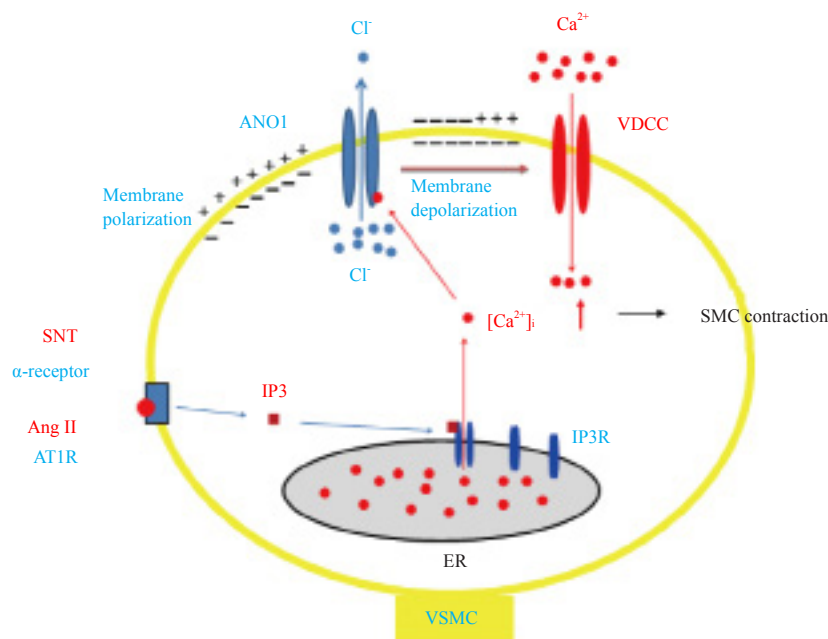


Figure 1 Role of ANO1 in regulation of intracellular Ca^{2+} of VSMC. Ang II: Angiotensin II; ANO1: Anoctamin-1; AT1R: Angiotensin II receptor type 1; SMC: Smooth myocyte; VSMC: Vascular smooth myocyte; VDCC: Voltage-dependent Ca^{2+} channel.

decades as one of determining factors to drive vascular tone and contraction^[9,10]. Gating of voltage-dependent Ca^{2+} channels (VDCCs) in vascular smooth muscle cells is controlled by the plasma membrane potential which is formed by the cooperative activities of K^+ channels and CaCC channels (Figure 1). At resting, membranous K^+ channels open, allows K^+ efflux and depolarize the membrane, which eventually forms resting potential. At the moment, due to the low cytoplasmic Ca^{2+} concentration, CaCC channels remain closed. When intracellular environment changes, e.g. sympathetic neurons are excited and release sympathetic neuronal transmitters (SNT) which via α -receptors activate the PLC/IP3/IP3-receptors pathway or circulating angiotensin II (Ang II) acts on its receptor, AT1R (angiotensin II receptor type 1), the Ca^{2+} in endoplasmic reticulum (ER) is released into cytoplasm when IP3 receptors are opened. Cytoplasmic Ca^{2+} activates CaCC channels and makes them open. By the driving force of Cl^- concentration gradient, cytoplasmic Cl^- flows out of cells and depolarizes the plasma membrane. The depolarized membrane potential opens VDCC and extracellular Ca^{2+} heavily flows into cells and triggers the SMC contraction (Figure 1). One can easily find that the CaCC channels not only sense the changes of intracellular Ca^{2+} concentration and regulate the SMC membrane potentials but also influence the intracellular Ca^{2+} concentrations *via* VDCC. Therefore, the CaCC channels play important roles in maintenance of intracellular Ca^{2+} homeostasis and VSMC contraction.

DOMINANT DISTRIBUTION OF ANO1 PROTEINS TO VASCULAR SMOOTH MUSCLE CELLS (VSMC)

Early studies demonstrated that CaCC currents were distributed to VSMC instead of endothelia. At present, several studies have demonstrated that ANO1 proteins represent the endogenous CaCC channels in VSMC by way of ANO1 transcript knockdown approach. Being consistent with the early studies, ANO1 distribution has been presently found dominant to the VSMC.

Greenwood group examined the expression profile and protein translation of ANO1 in SMCs of mouse portal vein, thoracic aorta and carotid artery by qualitative recording of whole-cell CaCC currents, quantitative PCR and western blot analysis^[11]. Patch-clamp experiments revealed that ANO1-GFP-induced CaCC currents in HEK 293 cells and in vascular myocytes displayed biophysical and pharmacological similarities. An ANO1 specific antibody indicated that ANO1 protein was distributed diffusely throughout the cytoplasm as well as near the plasma membrane of isolated SMCs and identified in the smooth muscle layers of vessels. The antibody also recognized a 120 kDa band on the western blot membrane. ANO1 has also been demonstrated to be the major constituent of the vascular CaCC channels in rat pulmonary artery SMCs (PASMCS) with molecular knockdown approach and electrophysiology^[12].

In further experiments, using molecular biology and patch-clamp electrophysiology, Jaggar group confirmed that it was ANO1 in SMCs of resistance-size rat cerebral arteries that produced the CaCC currents^[13]. Cell surface biotinylation and immunofluorescence of arteries indicated that ANO1 channels are located primarily within the SMC plasma membrane. Knockdown of ANO1 using siRNA further supported that ANO1 channels which were inserted into the plasma membrane generated CaCC currents in cerebral artery SMCs.

Downregulating CaCC currents in rat resistance-size mesenteric small arteries by *in vivo* transfection of ANO1 siRNA, Matchkov group demonstrated that knockdown of ANO1 resulted in inhibition of both the “classical” and the cGMP-dependent CaCC currents^[14]. Downregulation of the latter may be due to the somehow reduced expression of bestrophins for unknown reasons. With isolated arterial segments, the authors observed that knock down of ANO1 reduced agonists (noradrenaline and vasopressin)- and K^+ -induced contractions measured by isometric force, suppressed agonists-induced membrane depolarization and inhibited agonists-induced rise in $[\text{Ca}^{2+}]_i$ (intracellular Ca^{2+} concentration) and Ca^{2+} entry. This study strengthened the concept that the “classical” CaCC currents regulate SMC membrane potentials which, in turn, gate VDCC and Ca^{2+} entry.

ANO1 LOCATED TO DIFFERENT VESSEL BEDS MAY PLAY DIFFERENT ROLES IN REGULATION OF VASCULAR CONTRACTILITY

It has been a well-known consensus that activation of CaCC channels by intracellular Ca^{2+} in VSMC plays a role in depolarization of plasma membrane which activates VDCC and induces Ca^{2+} influx and VSMC contraction^[9]. CaCC channels in VSMCs have been confirmed as an important player in production of vascular contractility. Since the molecular identity of the endogenous CaCC channels was confirmed as ANO1, Huebner and Schroeder group has made one of the most remarkable progresses to reveal how ANO1 participates in the regulation of BP^[15]. The group firstly examined the CaCC current density of arterial SMCs at different loci and found substantial CaCCs in VSMCs of the mouse aorta and carotid arteries while CaCC currents were found to be small or absent in VSMCs of medium-sized vessels such as mesenteric arteries and larger retinal arterioles but the currents were particularly large in small vessels of the retina, brain and skeletal muscle where contractile intermediate cells or pericytes gradually replace VSMCs. With conditional knockout of ANO1, the group successfully demonstrated that CaCC currents were eliminated from these cell types in all small vessels studied. Consistently, the ANO1-targeted mice had lower systemic BP, a lower hypertensive response to vasoconstrictor, Ang II and reduced responsiveness of the aorta, small retinal arterioles and hind limb arteries to the vasoconstriction-inducing drug U46619. The results strongly support that the physiological function of the CaCC channels, ANO1 is promoting VSMC contraction.

In line with the above, Qu group recently confirmed the similar role of ANO1 in the spontaneously hypertensive rat (SHR) model. ANO1 was found to be overexpressed in the aortic, carotid and hindlimb arteries, and branches of mesenteric arteries (resistance-size vessels)^[16]. The higher CaCC currents of VSMCs of SHRs than control WKY rats were mediated by ANO1, which was demonstrated by an ANO1 inhibitor, T16A_{inh}-A01, and RNAi. Knockdown of ANO1 by siRNA *in vivo* transfection prevented hypertension onset and attenuation of ANO1 channel activity by T16A_{inh}-A01 *in vivo* reduced BP in SHRs. The phenylephrine-constricted mesenteric arterial rings from SHRs were much less responsive to T16A_{inh}-A01 than WKY rats. When the endogenous ANO1 in arteries of SHRs were knocked down, the ED₅₀ of T16A_{inh}-A01 for SHRs decreased to the similar level to that for WKY rats. The data indicate that the increased ANO1 expression and activity induced hypertension in SHRs, being consistent with the physiological role of CaCC channels.

Supporting the above results, in a monocrotaline-induced pulmonary hypertension (PH) rat model, Leblanc group found that the augmented ANO1-encoded CaCC activity in PASMCs from intralobar pulmonary arteries of the model is associated with PH^[17].

However, Guan and Zhou group revealed a surprising phenomenon with a renohypertensive rat model where the activity of CaCC in SMCs of cerebral basilar arteries was decreased and the CaCC current density or ANO1 protein in the SMC layer of basilar arteries was negatively correlated with BP levels during hypertension of the model. The authors stated that both the channel activity and expression of ANO1 were downregulated by CaMKII kinase while the enzyme activity in the basilar artery was increasing with renohypertensive progression^[18]. Unfortunately, the authors did not provide any evidence whether the lowered ANO1 expression affected the contractility of cerebral basilar arteries and how ANO1 regulated the BP in the renohypertensive rat model.

Collectively, the function of ANO1 in different loci of blood vessel beds may differently affect the vessel contractility.

HOW DOES ANO1 PARTICIPATE IN SELF-REGULATION OF CEREBRAL ARTERIAL CONTRACTION?

Participation of ANO1 in regulation of VSMC contractility is not only mediated by humoral factors but also by mechanosensitive mechanism which controls the intravascular pressure of arterioles and the blood flow volume through organs^[19]. The mechanosensitive mechanism has been well-known to be contributed by cation channels, *e.g.*, Ca^{2+} , K^{+} and TRP channels^[20,21]. However, the study on the involvement of anion channels in the process had been retarded due to unknown identity of CaCC channels which was regarded as an important factor in the process. Jaggar group recently made a major contribution to the elucidation of the role ANO1 played in the myogenic response of the cerebral arteries after ANO1 was identified as the endogenous CaCC channel^[19].

With an ANO1 inhibitory antibody and siRNA, the group skillfully demonstrated that hyposmolarity or cell swelling-induced whole-cell Cl^{-} currents and the Cl^{-} currents activated by the stretch on the cell-attached membrane induced by negative pressure through recording pipettes, were produced by ANO1 expressed in cerebral arterial SMCs. Since ANO1 knockdown reduced intravascular pressure-induced depolarization of endothelium-denuded arteries and vasoconstriction, the group concluded that it was activation of ANO1 that triggered membranous depolarization and myogenic response. The results obtained with nimodipine (a VDCC inhibitor) or thapsigargin depleting intracellular Ca^{2+} stores, both of which did not alter swelling-activated ANO1 currents, further supported the conclusion^[19].

Furthermore, with Gd^{3+} and SKF-96365, nonselective cation channel blockers, the study suggests that mechanical stretch on smooth myocytic membrane may activate nonselective cation channels and induce extracellular Ca^{2+} influx which forms local high cytoplasmic Ca^{2+} environment around ANO1 and opens the CaCC channel which leads to membrane depolarization. The latter was proved by removal of extracellular Ca^{2+} and replacement of intracellular EGTA with BAPTA, a fast Ca^{2+} chelator. The myogenic contraction ensues after VDCC activation by the depolarization and VDCC-induced large amplitude of Ca^{2+} influx (Figure 2)^[19].

EFFECT OF ANO1 ON VSMC PROLIFERATION AND REMODELING

ANO1 has been found capable of promoting cell proliferation in many cell types including malignant cells^[7]. Consistently, in mesenteric and aortic arterial SMCs, Qu group observed that the protein level and activity of cellular ANO1 positively correlated with VSMC proliferation. Moreover, Ang II upregulated ANO1 expression via AT1R in primary cultures of VSMCs. Therefore, the group believes that ANO1 may mediate Ang II-dependent vascular remodeling during hypertensive development in SHRs^[16]. Interestingly, in human atrial fibroblasts contributing to myocardial tissue remodeling, Ang II activated the CaCC channel ANO1 via AT1R instead of upregulating ANO1 expression^[22].

Nonetheless, in the SMCs of cerebral basilar arteries of the renohypertensive rat model, Guan and Zhou group realized that Ang II and ANO1 inhibited their effects reciprocally^[18]. The group demonstrated that Ang II repressed ANO1 expression of

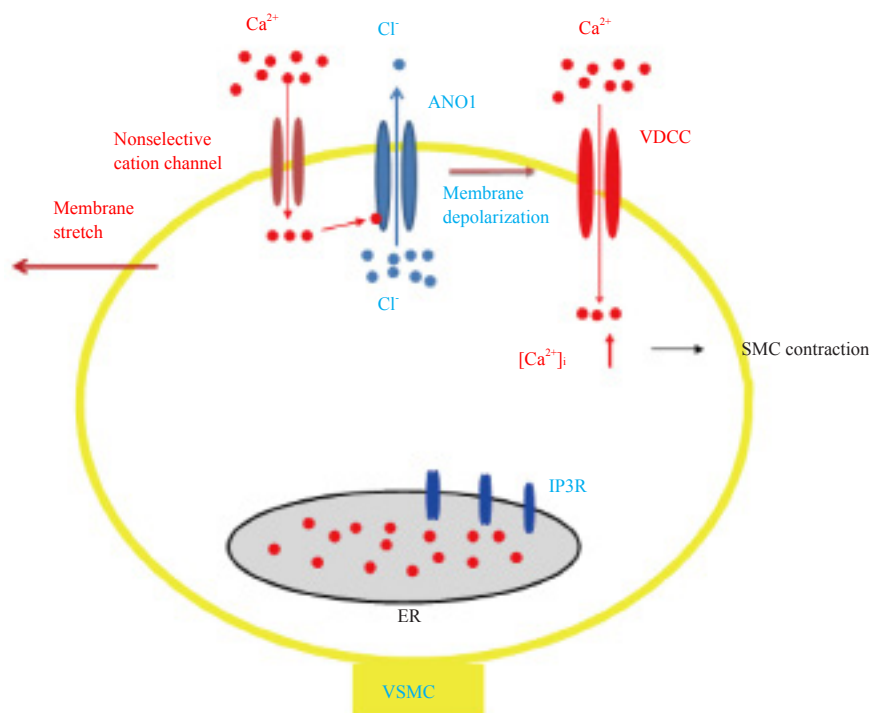


Figure 2 ANO1-mediated myogenic contraction of VSMC. ANO1: Anoctamin-1; SMC: Smooth myocyte; VSMC: Vascular smooth myocyte; VDCC: Voltage-dependent Ca²⁺ channel.

basilar arterial SMCs while knockdown of ANO1 facilitated but its overexpression inhibited Ang II-induced cell cycle transition and cell proliferation determined by flow cytometry and BrdU incorporation. ANO1 affected cell cycle progression mainly through regulating the expression of cyclin D1 and cyclin E. However, the intermediate links between ANO1 and latter ones remain unknown.

To uncover the mechanism how ANO1 is involved in the Ang II-induced vascular differentiation and remodeling, Wen group recently studied the molecular regulation of *ANO1* gene transcription^[23]. With a luciferase reporter assay and molecular manipulation of human *ANO1* gene promoter, the group first studied the relation of myocardin (a transcriptional coactivator expressed exclusively in SMCs and closely related with SMC differentiation) with serum response factor (SRF) which recognizes a binding site on the *ANO1* promoter in human aortic SMCs and found that myocardin promoted ANO1 expression by forming a complex with SRF. In turn, the promoted ANO1 expression upregulated expression of myocardin and VSMC marker genes (*SM22 α* and *SM α -actin*), thus forming a positive feedback loop that induced cell differentiation. Meanwhile, ANO1 decreased proliferation-relating PCNA (proliferating cell nuclear antigen) and cyclin D1 levels possibly through myocardin and inhibited cell proliferation (Figure 3A). Unfortunately, how ANO1 upregulated myocardin transcription or translation remains mechanically unsolved yet.

In further study, the group elucidated the mechanism that Ang II inhibits ANO1 expression. Because Krüppel-like factor 5 (KLF5) is known to mediate Ang II-induced gene expression, VSMC proliferation and cardiovascular remodeling^[24], the authors investigated whether KLF5 mediated Ang II-induced suppression of ANO1 expression^[23]. At three levels, the mediation was elaborately demonstrated with cultured SMCs, *KLF5*^{-/-} mice, *ANO1* promoter-carrying luciferase reporters and coimmunoprecipitation. Consequently, AngII inhibited ANO1 expression *via* KLF5 in cultured human aortic SMCs; infusion of Ang II into mice caused a marked reduction in vascular ANO1

expression and remodeling but the Ang II-induced effects were largely reversed in *KLF5*^{-/-} mice; KLF5 competed with SRF to interact with myocardin, limited myocardin binding to SRF and inhibited the synergistic activation of the ANO1 promoter by myocardin and SRF; a coimmunoprecipitation assay showed that Ang II, in dose- and time-dependent manners, promoted the interaction of KLF5 with myocardin and disrupted the association of myocardin with SRF. A schematic delineation of the relation between all factors is shown in Figure 3B.

It is noteworthy that overexpression of ANO1 largely blocked Ang II-induced upregulation of cyclin D1 and PCNA, and BrdU incorporation which are related with cell proliferation and abrogated Ang II-induced downregulation of *SM22 α* and *SM α -actin* which are related with cell differentiation. These findings strongly suggested that ANO1 is an intermediate link, *standing* between the initial sensor (AT1R) and the effectors (proliferating factors). ANO1 itself may not be an effector for cell proliferation and differentiation.

The studies evidently demonstrated that Ang II induced KLF5 expression and KLF5 associated with myocardin and disrupted the myocardin-SRF complex, subsequently leading to inhibition of ANO1 transcription.

Another note worthy phenomenon is that it seems that ANO1 expression and function are differentially regulated in blood vessel beds of different loci. ANO1 also behaved similarly in cancer cells or tissues, playing different roles in proliferation in different cancer cell types, *e.g.*, ANO1 promotes cell proliferation in many cancer tissues^[7] but in one liver cancer cell line it could dramatically prohibit cell proliferation (data not shown).

FACTORS PHYSICALLY BINDING WITH ANO1

ANO1 is a membranous protein located to plasma membranes of VSMCs where it functions to regulate the membrane potentials. Greenwood group discovered that ANO1 overlapped with the lipid

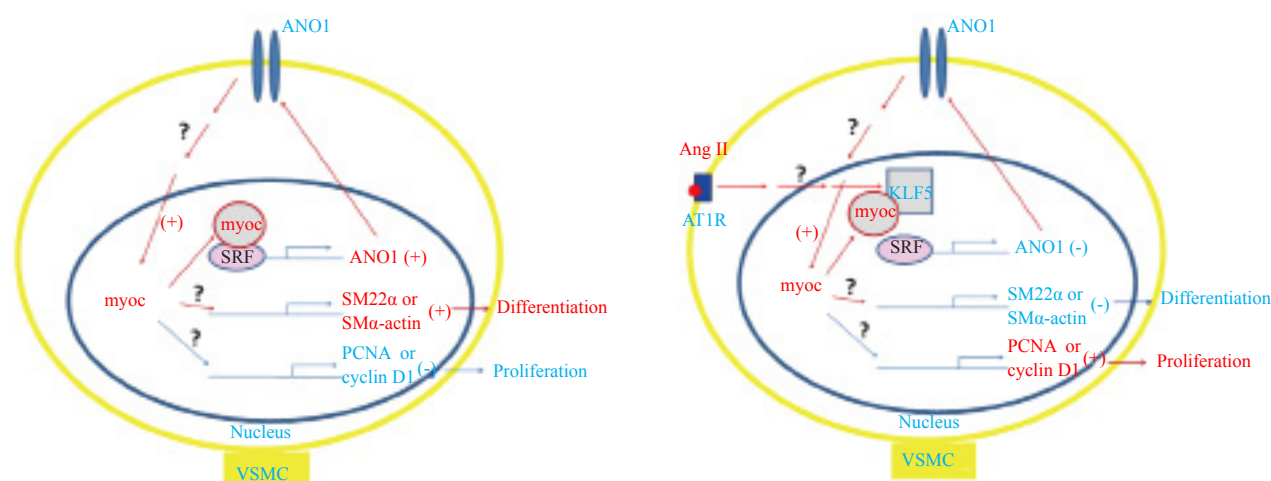


Figure 3 Ang II/KLF5/Myocardin-SRF axis and ANO1 expression. myoc, myocardin.

raft markers of VSMC membranes, caveolin and flotillin-2 in the fractions of discontinuous sucrose gradient ultracentrifugation, suggesting that ANO1 possibly existed in the caveolae of lipid raft^[25]. Lipid raft is a macromolecular complex comprising a multitude of proteins. M- β CD (methyl- β -cyclodextrin, a cholesterol chelating agent capable of enhancing aqueous solubility of cholesterol and influencing integrity of the membranous lipid raft), augmented the CaCC currents and altered the membranous distribution of caveolin, flotillin-2 and ANO1 when applied to the SMC isolated from mouse portal veins to delete the membranous cholesterol. It remains unknown what factors interact with ANO1 inside the lipid raft of SMC membranes.

The group further found that phosphatidylinositol^[4,5] bisphosphate [PI(4,5)P₂] bound directly to ANO1 protein on the plasma membrane of isolated rat PSMCs^[26]. PI(4,5)P₂ is a bio-active integral component of cell membranes. Agents that reduced PI(4,5)P₂ levels by phospholipase C activation, PI4K (phosphatidylinositol 4-kinase) inhibition or PI(4,5)P₂ scavenging and absorption, all increased CaCC activity of PSMCs whereas PI(4,5)P₂ inhibited the CaCC currents, suggesting that PI(4,5)P₂ was a negative regulator of ANO1 channels.

ANO1 has been demonstrated to physically interact with various protein factors in other types of cells, *e.g.*, calmodulin, EGFR, TRPV1, TRPV4, VDCC and cytoskeleton protein actin and moesin, *etc.*^[27-33]. In cerebral basilar arteries, the result that ANO1 channel activity was negatively regulated by CaMKII activity may be related with binding of calmodulin with the channel^[18].

The physical and functional interactions between ANO1 and VDCC were found in presynaptic terminals of murine photoreceptors. Not only does ANO1 physically associate with the α 1 subunit of VDCC *in vitro* and in the retina but the functional interaction between ANO1 and VDCC auxiliary α 2 δ 4 subunit was also demonstrated in the retina of *Cacna2d4* mutant mice, in which the VDCC auxiliary α 2 δ 4 subunit carried a nonsense mutation disrupting the VDCC channel function. Because of the disruption, synaptic terminals of mutant photoreceptors were disarranged and synaptic proteins as well as ANO1 channels lost their characteristic localization. Simultaneously, CaCC currents are impaired in rod photoreceptors.

It remains to be examined whether the physical interaction of ANO1 and VDCC also occurs in VSMC. It is well-known that VDCC channels in VSMC exhibit close functional association with CaCC channels. Therefore, it is worthy to characterize their

physical interaction. Also, it will be a valuable experiment to delineate the mechanism of ANO1 in regulation of vascular tone and contraction with the *Cacna2d4* mutant mice.

Since cytochalasin D disrupted the actin cytoskeleton and changed the CaCC biophysical characteristics of ANO1 in SMCs of murine portal vein, it was suggested that ANO1 may interact with abundant actin cytoskeleton^[27]. Along with the fact that ANO1 interacts with moesin, another cytoskeleton protein^[28], it seems that ANO1 channel function requires support from the cytoskeleton proteins in VSMCs.

SUMMARY

With more and more progresses made in the study about the roles of ANO1 in vascular functions since the molecule of the CaCC channel was identified, ANO1 is getting close to taking the center court as a player for vascular functions. ANO1 has been confirmed to represent the CaCC channels in VSMCs. It functions to control the membrane potentials by depolarization when activated. As a result, ANO1 is capable of affecting VDCC gating, Ca²⁺ entry and VSMC contraction. Under influence of agonists, *e.g.*, Ang II, ANO1, through unknown-yet pathways, participates in vascular differentiation and remodeling with help of some nuclear factors, *e.g.*, KLF5, myocardin and SRF which regulate transcription of the *ANO1* gene.

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CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

REFERENCES

- Hartzell HC, Yu K, Xiao Q, Chien LT, Qu Z. Anoctamin/TMEM16 family members are Ca²⁺-activated Cl⁻ channels. *The Journal of physiology* 2009; **587**(Pt 10): 2127-2239.
- Pedemonte N, Galiotta LJ. Structure and function of TMEM16 proteins (anoctamins). *Physiological reviews* 2014; **94**(2): 419-459.
- Hubner CA, Schroeder BC, Ehmke H. Regulation of vascular tone

- and arterial blood pressure: role of chloride transport in vascular smooth muscle. *Pflugers Archiv: European journal of physiology* 2015; **467**(3): 605-614.
4. Caputo A, Caci E, Ferrera L, Pedemonte N, Barsanti C, Sondo E, *et al.* TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity. *Science* 2008; **322**(5901): 590-594.
 5. Yang YD, Cho H, Koo JY, Tak MH, Cho Y, Shim WS, *et al.* TMEM16A confers receptor-activated calcium-dependent chloride conductance. *Nature* 2008; **455**(7217): 1210-1215.
 6. Schroeder BC, Cheng T, Jan YN, Jan LY. Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. *Cell* 2008; **134**(6): 1019-1029.
 7. Qu Z, Yao W, Yao R, Liu X, Yu K, Hartzell C. The Ca(2+) -activated Cl(-) channel, ANO1 (TMEM16A), is a double-edged sword in cell proliferation and tumorigenesis. *Cancer medicine* 2014; **3**(3): 453-461.
 8. Matchkov VV, Secher Dam V, Bodtkjer DM, Aalkjaer C. Transport and function of chloride in vascular smooth muscles. *Journal of vascular research* 2013; **50**(1): 69-87.
 9. Large WA, Wang Q. Characteristics and physiological role of the Ca(2+)-activated Cl- conductance in smooth muscle. *Am J Physiol* 1996; **271**(2 Pt 1): C435-C454.
 10. Hartzell C, Putzier I, Arreola J. Calcium-activated chloride channels. *Annual review of physiology* 2005; **67**: 719-758.
 11. Davis AJ, Forrest AS, Jepps TA, Valencik ML, Wiwchar M, Singer CA *et al.* Expression profile and protein translation of TMEM16A in murine smooth muscle. *American journal of physiology Cell physiology* 2010; **299**(5): C948-C959.
 12. Manoury B, Tamuleviciute A, Tammaro P. TMEM16A/anoctamin 1 protein mediates calcium-activated chloride currents in pulmonary arterial smooth muscle cells. *The Journal of physiology* 2010; **588**(Pt 13): 2305-2314.
 13. Thomas-Gatewood C, Neeb ZP, Bulley S, Adebisi A, Bannister JP, Leo MD *et al.* TMEM16A channels generate Ca(2+)-activated Cl(-) currents in cerebral artery smooth muscle cells. *American journal of physiology Heart and circulatory physiology* 2011; **301**(5): H1819-H1827.
 14. Dam VS, Boedtkjer DM, Nyvad J, Aalkjaer C, Matchkov V. TMEM16A knockdown abrogates two different Ca(2+)-activated Cl (-) currents and contractility of smooth muscle in rat mesenteric small arteries. *Pflugers Archiv: European journal of physiology* 2014; **466**(7): 1391-1409.
 15. Heinze C, Seniuk A, Sokolov MV, Huebner AK, Klementowicz AE, Szijarto IA, *et al.* Disruption of vascular Ca2+-activated chloride currents lowers blood pressure. *The Journal of clinical investigation* 2014; **124**(2): 675-686.
 16. Wang B, Li C, Huai R, Qu Z. Overexpression of ANO1/TMEM16A, an arterial Ca2+-activated Cl- channel, contributes to spontaneous hypertension. *J Mol Cell Cardiol* 2015; **82**:22-32.
 17. Forrest AS, Joyce TC, Huebner ML, Ayon RJ, Wiwchar M, Joyce J *et al.* Increased TMEM16A-encoded calcium-activated chloride channel activity is associated with pulmonary hypertension. *Am J Physiol Cell Physiol* 2012; **303**(12): C1229-C1243.
 18. Wang M, Yang H, Zheng LY, Zhang Z, Tang YB, Wang GL *et al.* Downregulation of TMEM16A calcium-activated chloride channel contributes to cerebrovascular remodeling during hypertension by promoting basilar smooth muscle cell proliferation. *Circulation* 2012; **125**(5): 697-707.
 19. Bulley S, Neeb ZP, Burris SK, Bannister JP, Thomas-Gatewood CM, Jangsangthong W *et al.* TMEM16A/ANO1 channels contribute to the myogenic response in cerebral arteries. *Circ Res* 2012; **111**(8): 1027-1036.
 20. Hill MA, Zou H, Potocnik SJ, Meininger GA, Davis MJ. Invited review: arteriolar smooth muscle mechanotransduction: Ca(2+) signaling pathways underlying myogenic reactivity. *J Appl Physiol (1985)* 2001; **91**(2): 973-983.
 21. Earley S, Brayden JE. Transient receptor potential channels and vascular function. *Clin Sci (Lond)* 2010; **119**(1): 19-36.
 22. El Chemaly A, Norez C, Magaud C, Bescond J, Chatelier A, Fares N *et al.* ANO1 contributes to angiotensin-II-activated Ca2+-dependent Cl- current in human atrial fibroblasts. *J Mol Cell Cardiol* 2014; **68**: 12-19.
 23. Zhang XH, Zheng B, Yang Z, He M, Yue LY, Zhang RN *et al.* TMEM16A and myocardin form a positive feedback loop that is disrupted by KLF5 during Ang II-induced vascular remodeling. *Hypertension* 2015; **66**(2): 412-421.
 24. Liu Y, Wen JK, Dong LH, Zheng B, Han M. Kruppel-like factor (KLF) 5 mediates cyclin D1 expression and cell proliferation via interaction with c-Jun in Ang II-induced VSMCs. *Acta Pharmacol Sin* 2010; **31**(1): 10-18.
 25. Sones WR, Davis AJ, Leblanc N, Greenwood IA. Cholesterol depletion alters amplitude and pharmacology of vascular calcium-activated chloride channels. *Cardiovasc Res* 2010; **87**(3): 476-484.
 26. Pritchard HA, Leblanc N, Albert AP, Greenwood IA. Inhibitory role of phosphatidylinositol 4,5-bisphosphate on TMEM16A-encoded calcium-activated chloride channels in rat pulmonary artery. *Br J Pharmacol* 2014; **171**(18): 4311-4321.
 27. Ohshiro J, Yamamura H, Suzuki Y, Imaizumi Y. Modulation of TMEM16A-channel activity as Ca(2+)-activated Cl(-) conductance via the interaction with actin cytoskeleton in murine portal vein. *J Pharmacol Sci* 2014; **125**(1): 107-111.
 28. Perez-Cornejo P, Gokhale A, Duran C, Cui Y, Xiao Q, Hartzell HC *et al.* Anoctamin 1 (Tmem16A) Ca2+-activated chloride channel stoichiometrically interacts with an ezrin-radixin-moesin network. *Proc Natl Acad Sci U S A* 2012; **109**(26): 10376-10381.
 29. Jung J, Nam JH, Park HW, Oh U, Yoon JH, Lee MG. Dynamic modulation of ANO1/TMEM16A HCO3(-) permeability by Ca2+/calmodulin. *Proc Natl Acad Sci U S A* 2013; **110**(1): 360-365.
 30. Bill A, Gutierrez A, Kulkarni S, Kemp C, Bonenfant D, Voshol H *et al.* ANO1 interacts with EGFR and correlates with sensitivity to EGFR-targeting therapy in head and neck cancer. *Oncotarget* 2015; **6**(11): 9173-9188.
 31. Takayama Y, Uta D, Furue H, Tominaga M. Pain-enhancing mechanism through interaction between TRPV1 and anoctamin 1 in sensory neurons. *Proc Natl Acad Sci U S A* 2015; **112**(16): 5213-5218.
 32. Caputo A, Piano I, Demontis GC, Bacchi N, Casarosa S, Della Santina L *et al.* TMEM16A is associated with voltage-gated calcium channels in mouse retina and its function is disrupted upon mutation of the auxiliary alpha2delta4 subunit. *Front Cell Neurosci* 2015; **9**: 422.
 33. Takayama Y, Shibasaki K, Suzuki Y, Yamanaka A, Tominaga M. Modulation of water efflux through functional interaction between TRPV4 and TMEM16A/anoctamin 1. *FASEB J* 2014; **28**(5): 2238-2248.

Peer reviewer: Domenico Di Raimondo, MD, PhD, U.O.C. Medicina Vascolare, Dipartimento Biomedico di Medicina Interna e Specialistica, Università degli Studi di Palermo, Piazza delle Cliniche n° 2, 90127, Palermo, Italy.