Original Research Article

Overexpression of TRPM7 suppresses AngII-induced phenotypic changes in hypertensive human vascular smooth muscle cells

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Abstract

Purpose: To study the role of TRPM7 in suppressing AngII-induced phenotype changes in human vascular smooth muscle cells (HAVSMCs).
Methods: Relative levels of TRPM7, α-SMA and OPN in AngII-induced HAVSMCs were determined, and the regulatory effects of TRPM7 on levels of α-SMA and OPN, wound healing effect, 5-ethynyl-2'-deoxyuridine (EdU)-positive ratio, and viability in AngII-induced HAVSMCs were assessed. A total of 159 patients with essential hypertension attending The Second Xiangya Hospital, Central South University, Changsha, China, and 70 healthy controls were enrolled; after treatment with angiotensin receptor blocker (ARB), changes in SBP, DBP and plasma level of TRPM7 in hypertension patients were examined. Potential correlation between TRPM7 level with SBP and DBP was assessed.
Results: TRPM7 and α-SMA were downregulated, while OPN was upregulated in AngII-induced HAVSMCs, but this trend was partially reversed by losartan treatment ($p < 0.05$). Overexpression of TRPM7 reversed the expression changes of α-SMA and OPN in AngII-induced HAVSMCs. In addition, the overexpression of TRPM7 significantly inhibited the enhanced migratory and proliferative capacities in AngII-induced HAVSMCs ($p < 0.05$). After ARB treatment, SBP, DBP and plasma TRPM7 were significantly reduced in hypertension patients, while TRPM7 level positively correlated with SBP and DBP in hypertensive patients.
Conclusion: Overexpression of TRPM7 blocked AngII-induced phenotype changes in HAVSMCs under the pathological circumstance of hypertension.

Keywords: Hypertension, Transient receptor potential cation channel subfamily M member 7 (TRPM7), Phenotype changes

INTRODUCTION

Hypertension is a highly prevalent disease which causes damage to cardiac functions. Long-term hypertension results in myocardial ischemia, leading to atherosclerosis and myocardial fibrosis [1,2]. It is also a severe risk factor for brain and kidney diseases. Effective prevention and control of hypertension are therefore of great concern [3,4]. Vascular remodeling is one of the main pathological changes caused by hypertensive diseases. As the major cells of the blood vessel

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walls, vascular smooth muscle cells (VSMCs) are of significance during the occurrence and development of vascular remodeling. Under the normal circumstances, VSMCs maintain a contractile state, which is responsible for the regulation of blood pressure, as they mediate the contraction state of the blood vessels. However, under pathological conditions, VSMCs are transformed into the proliferate type, and their contraction ability markedly weakens [5]. An effective inhibition of VSMC transformation from the contractile type into proliferative type helps to prevent the occurrence of hypertension, and alleviate its development.

Human transient receptor potential cation channel subfamily M member 7 (TRPM7) is a 212 kD peptide consisting of 1864 amino acids [6]. Various gating mechanisms for TRPM7 channel in influencing pathological and physiological states have been highlighted [7-9]. It is reported that TRPM7 has a certain impact on morphology, adhesion, metastasis and angiogenesis of VSMCs [10]. A relevant study proposed that TRPM7 channel is involved in AngII-induced phenotypic changes in vascular smooth muscle of ascending aorta [11]. Inflammatory responses activate AngII, which favours hypertension development [12-16]. In this study, the aim was to mainly explore the potential impact of TRPM7 on AngII-induced phenotype changes of HAVSMCs.

METHODS

Cell culture

Human vascular smooth muscle cells were provided by Cell Bank (Shanghai, China). The cells were cultured in Roswell Park Memorial Institute 1640 (RPMI 1640) (HyClone, South Logan, UT, USA), and they contained 10 % fetal bovine serum (FBS) (HyClone, South Logan, UT, USA), 100 µg/mL penicillin and 100 mg/mL streptomycin. They were pre-treated with 10 µM Losartan for 1 h and with 1 µM AngII for another 48 h.

Quantitative real-time polymerase chain reaction (qRT-PCR)

TRIzol method (Invitrogen, Carlsbad, CA, USA) was used for cellular RNA extraction. Through reverse transcription of RNA, the extracted complementary deoxyribose nucleic acid (cDNA) was used for PCR via the SYBR Green method (TaKaRa, Tokyo, Japan). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal reference. The primer sequences were listed in Table 1.

Western blot

Cells were lysed for isolating cellular protein, and centrifuged at 14,000 x g for 15 minutes at 4 °C. Total protein concentration was determined by bicinchoninic acid (BCA) Protein Assay Kit (Pierce, Rockford, IL, USA). Proteins (30 ug) were added into per lane for the electrophoresis. After the electrophoresis, protein samples were loaded on polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Subsequently, non-specific antigens were blocked in 5 % skim milk for 2 h. Membranes were reacted with primary and secondary antibodies for indicated time. The gray value was analyzed using ImageJ software (version 1.38; National Institutes of Health, Bethesda, MA, USA).

Cell transfection

Cells were inoculated in 35 mm^2 dishes and cultured to 50 % confluence. 2 µg plasmid and 4 µL of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) were applied in each dish. Six hours later, fresh medium was replaced.

Wound healing assay

Wound healing assay was performed according to the manufacturer's instructions, and the cells were then briefly inoculated in a 6-well plate and cultured for 24 h.

Table 1: Primer sequences used for PCR

Gene		Primer sequence
TRPM7	Forward	5'-GCAA ATGACTCCACTCTC-3'
	Reverse	5'-GATTCTCTCTTCACTCCCAG-3'
α-SMA	Forward	5'-AGCGTGGCTACCCTTCGTGAC-3'
	Reverse	5'-GCTCGTTGCCGATGGTGATGAC-3'
OPN	Forward	5'-TGAGTCTGGAAATAACTAATGTGTTTGA-3'
	Reverse	5'-GAACATAGACATAACCCTGAAGCTTTT-3'
GAPDH	Forward	5'-ACAG CAACAGGGTGGTGGAC-3'
	Reverse	5'-TTTGAGG GTGCAGCGAACTT-3'

An artificial wound was created using the 200 µL pipette tip, and then wound healing was observed at 0 and 48 h respectively under the microscope (Olympus, Tokyo, Japan).

5-Ethynyl-2'- deoxyuridine (EdU) assay

Cells were inoculated in a 24-well plate at a density of 2×10^4 cells per well, and then incubated in 4 % methanol for 30 min, followed by 10-min permeabilization in 0.5 % TritonX-100, and 30-min reaction in 400 µL of 1 × ApollorR. Afterwards, the cells were dyed in Hoechst 33342 for another 30 min. EdU-positive cells and Hoechest-labelled nuclei were captured under the microscope ((Olympus, Tokyo, Japan).

Cell counting kit-8 (CCK-8) assay

Cells were inoculated in a 96-well plate at a density of 2×10^3 cells/well. At the appointed time points, absorbance value at 450 nm of each sample was recorded using the CCK-8 kit (Dojindo Laboratories, Kumamoto, Japan) for the plotting of the viability curves.

Patients enrolled in the study

A total of 80 males and 79 females with essential hypertension who underwent treatment or physical examination in our hospital were enrolled. Patients combined with valvular heart disease, congenital heart disease, cardiomyopathy, acute and chronic viral or bacterial infections, asthma, tumor or connective tissue disease were excluded. Subjects with history of severe hypertension (≥ 180/110 mmHg) or secondary hypertension were excluded as well. Subjects taking any drugs that may affect blood pressure, including non-steroidal anti-inflammatory drugs, glucocorticoids, potent CYP3A4 inhibitors or potassium supplements were also excluded from the study [17]. In the meantime, 70 age-matched healthy controls in the same period were selected. Patients/subjects gave their informed consent to the study, and the investigation was approved by the hospital ethics committee of The Second Xiangya Hospital, Central South University, Changsha, China, and conducted in accordance with the guidelines of Declaration of Helsinki.

Blood sample collection

Peripheral blood (10 mL) was obtained from the elbows of all subjects and collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes. Plasma was harvested from the upper layer after centrifugation at 3000 rpm for 10 min, and stored at - 80 °C for subsequent determination of the plasma TRPM7 level.

Statistical analysis

SPSS statistical analysis software (version 26.0) was used for all statistical analysis. Data are expressed as mean ± SD (standard deviation). The *t*-test was used for analyzing differences between two groups. Pearson correlation analysis was conducted to assess the relationship between TRPM7 level with DBP and SBP. $p < 0.05$ indicated the significant difference.

RESULTS

TRPM7 was downregulated in AngII-induced phenotype changes of HAVSMCs

The mRNA levels of TRPM7 (Figure 1 A) and α-SMA (Figure 1 B) were downregulated, while OPN (Figure 1 C) was upregulated in AngII-induced HAVSMCs, suggesting that AngII successfully induced phenotype changes in HAVSMCs. Interestingly, the above trends were partially reversed by Losartan treatment. Changes in protein levels of TRPM7, α-SMA and OPN were consistent with those of mRNA levels (Figure 1 D).
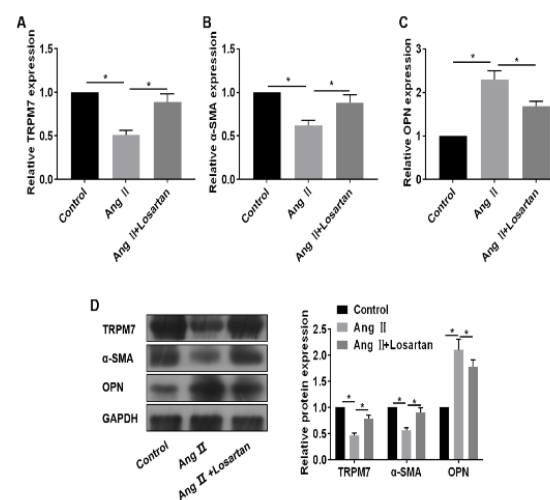

Figure 1: TRPM7 was downregulated in AngII-induced phenotype changes in HAVSMCs. HAVSMCs were treated with blank control, AngII or AngII + Losartan. (A-C) mRNA levels of TRPM7 (A), α-SMA (B) and OPN (C) in HAVSMCs. (D) Protein levels of TRPM7, α-SMA and OPN in HAVSMCs

Overexpression of TRPM7 alleviated AngII-induced phenotype changes in HAVSMCs

Interestingly, AngII-induced downregulation of TRPM7 and α-SMA, as well as upregulation of

OPN were partially reversed by transfection of pcDNA-TRPM7 (Figure 2). It indicates that TRPM7 exerted significant effect during AngII-induced phenotype changes of HAVSMCs.
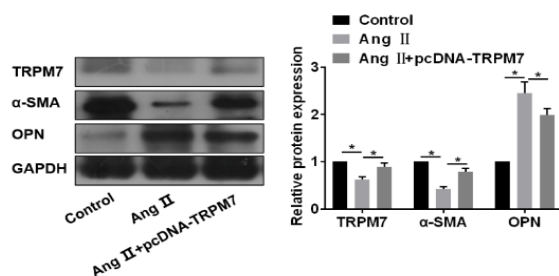

Figure 2: Overexpression of TRPM7 alleviated AngII-induced phenotype changes in HAVSMCs. Protein levels of TRPM7, α-SMA and OPN in HAVSMCs treated with blank control, AngII or AngII+transfection of pcDNA-TRPM7

Overexpression of TRPM7 inhibited AngII-induced enhancement of migratory and proliferative potential in HAVSMCs

Wound healing percentage was markedly elevated after AngII induction, which was reversed by overexpression of TRPM7 in HAVSMCs (Figure 3 A). Besides, the increased EdU-positive ratio (Figure 3 B) and viability (Figure 3 C) in AngII-treated HAVSMCs were partially reserved after transfection of pcDNA-TRPM7. Therefore, TRPM7 could affect proliferative and migratory abilities, thereby alleviating the development of hypertension.
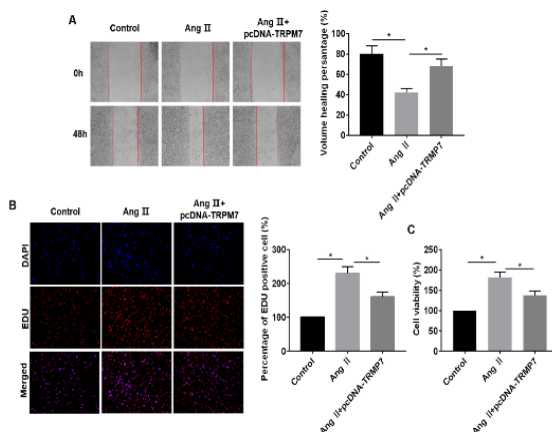

Figure 3: Overexpression of TRPM7 inhibited AngII-induced enhancement of migratory and proliferative potential in HAVSMCs. HAVSMCs were treated with blank control, AngII or AngII + transfection of pcDNA-TRPM7. (A) Percentage of wound healing in HAVSMCs; (B) EdU positive ratio in HAVSMCs (magnification: 400×); (C) Viability in HAVSMCs

TRPM7 was positively correlated to blood pressure

ARB treatment significantly reduced the levels of SBP, DBP and plasma TRPM7 (Figure 4 A - C) in hypertension patients. Pearson correlation analyses uncovered that TRPM7 level was positively correlated with DBP (R^2 = 0.5999, p < 0.0001, Figure 4 D) and SBP (R^2 = 0.6265, p < 0.0001, Figure 4 E). This study finds that TRPM7 has a certain impact on the development of hypertension.
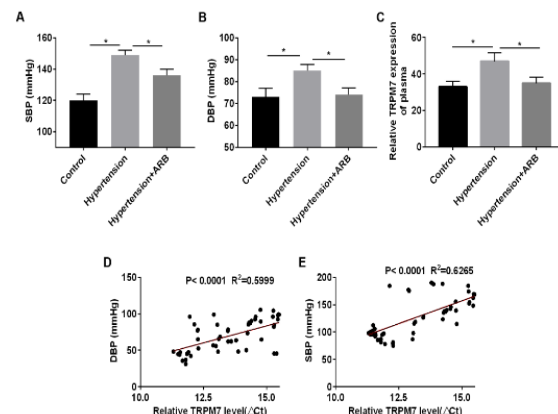

Figure 4: TRPM7 was positively correlated with blood pressure. (A-C) SBP (A), DBP (B) and plasma level of TRPM7 (C) in healthy controls, hypertension patients and those treated with ARB. (D, E) Positive correlations between TRPM7 with DBP (D) and SBP (E) in hypertension patients

DISCUSSION

SMCs are highly differentiated cells that maintain vascular tension and blood pressure. Under internal or external stimuli, VSMCs are transformed to become proliferate, manifesting in downregulated expressions of contractile proteins, attenuated contraction and stimulated proliferative ability [18]. Proliferated VSMCs exert a certain compensatory effect on the damaged blood vessels by impairing them. Nevertheless, an abundant release of ECM results in vascular remodeling, increased peripheral resistance and blood pressure [19].

VSMCs are of two typical types: the contractile type and the proliferate type. α-SMA is an indicator for contractile VSMCs, and OPN is the hallmark of the latter [20]. In this study, AngII treatment markedly downregulated α-SMA and upregulated OPN in HAVSMCs, but this trend was antagonized by Losartan treatment.

TRPM7 contains 6 transmembrane α-helical domains (S1-S6). In S5 and S6, there is a

hydrophobic pole [21-23]. A functional ion channel is formed by hydrophobic pores of four TRPM7 channel subunits S5-S6, which non-selectively penetrates divalent cations (i.e. Zn^{2+}, Ni^{2+}, Co^{2+}, Mg^{2+} and Ca^{2+}) and monovalent cations (i.e. K^+ and Na^+). Schmitz *et al*. [24] pointed out that TRPM7 is of significance in regulating Mg^{2+} homeostasis through the functional coupling of its ion channel and kinase domain. Besides, TRPM7-mediated calcium influx participates in cell proliferation and migration. Zhang *et al* [11] demonstrated that TRPM7 is involved in AngII-induced phenotype changes in the vascular smooth muscle of the ascending aorta. Findings in this study have consistently illustrated that the overexpression of TRPM7 inhibited the stimulated migratory and proliferative potential in AngII-induced HAVSMCs. In addition, the plasma level of TRPM7 was positively correlated with DBP and SBP in hypertension patients.

CONCLUSION

Overexpression of TRPM7 blocks AngOO induced phenotype changes in HAVSMCs under the pathological condition of hypertension.

DECLARATIONS

Acknowledgement

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Yangrong Tan and Wang Zhao designed the study and performed the experiments, Ling Liu collected the data, Shuiping Zhao analyzed the data, Yangrong Tan and Wang Zhao prepared the manuscript. All authors read and approved the final manuscript.

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