Original Article

Sarsasapogenin blocks ox-LDL-stimulated vascular smooth muscle cell proliferation, migration, and invasion through suppressing STIM1 expression

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Background: Atherosclerosis (AS) is a pathological vascular disorder responsible for the majority of cardiovascular deaths. Sarsasapogenin (Sar) is a natural steroidal compound which has been extensively applied to multiple human diseases due to its pharmacological properties. In the present paper, the impacts of Sar on oxidized low-density lipoprotein (ox-LDL)-treated vascular smooth muscle cells (VSMCs) and its possible action mechanism were investigated.

Methods: Firstly, Cell Counting Kit-8 (CCK-8) estimated the viability of VSMCs following treatment with ascending doses of Sar. Then, VSMCs were treated by ox-LDL to stimulate an *in vitro* cell model of AS. CCK-8 and 5-Ethynyl-2'-deoxyuridine (EDU) assays were used to assess cell proliferation. Wound healing and transwell assays were applied to measure the migratory and invasive capacities, respectively. The expression of proliferation-, metastasis-, and stromal interaction molecule 1 (STIM1)/Orai signaling-associated proteins was measured by western blot.

Results: The experimental data illuminated that Sar treatment noticeably protected against ox-LDL-elicited VSMCs proliferation, migration, and invasion. Besides, Sar lowered the elevated STIM1 and Orai expression in ox-LDL-treated VSMCs. Further, STIM1 elevation partially abrogated the impacts of Sar on the proliferation, migration, and invasion of VSMCs challenged with ox-LDL.

Conclusions: In conclusion, Sar might reduce STIM1 expression to impede the aggressive phenotypes of ox-LDL-treated VSMCs.

Keywords: Atherosclerosis; Sar; STIM1/Orai signaling; vascular smooth muscle cells (VSMCs); oxidized low-density lipoprotein (ox-LDL)

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Introduction

Cardiovascular and cerebrovascular diseases remain the primary cause of death all over the world and are accompanied with severe complications (1,2). As a pathological basis of cardiovascular and cerebrovascular diseases, atherosclerosis (AS) is viewed as a chronic and progressive disease of the arterial wall (3). Emerging evidence has expounded that the prevalence of AS is escalating annually, and increasingly affecting younger people (4). At present, statins are widely applied to prevent the progression of AS and reduce cardiovascular events (5). However, the adverse effects that the long-term application of statins cause may pose another great threat to the health of AS patients (6). AS demonstrates an increased rate of vascular smooth muscle cells (VSMCs) plasticity characterized by switching from the differentiated contractile phenotype to a de-differentiated synthetic state (7). Imbalances in the phenotypic switching of VSMCs can result in a variety of cardiovascular diseases, including AS, aortic aneurysms, and vascular calcification (8). MMPs is a family of endopeptidases that value metal ions (Ca2+ and Zn2+) as cofactors that has been reported to mediate the progress of various cardiovascular diseases (9). Alternations in MMPs expression are also associated with the progression of AS (10). Thence, effective therapeutic remedies for alleviating VSMCs malignant transformation upon stimulation are in great demand.

Timosaponin AIII, a steroidal saponin, is an active ingredient of the traditional Chinese herb *Anemarrhena asphodeloide* (Zhi Mu), which has been reported to elicit protective activities on cancers, neuronal disorders, and inflammation (11). The pivotal role of sarsasapogenin (Sar) (*Figure 1A*) as a secondary metabolite from Timosaponin AIII has also been extensively covered in human diseases. For instance, Sar potentiates podocyte autophagy and modulates GSK3β signaling in diabetic nephropathy (12). Sar alleviates neurotoxicity in Alzheimer's disease (13). Lim *et al.* proposed that Sar inactivates NF-κB and MAPK and mediates Th17/Treg cell balance to ameliorate colitis (14). All these findings have highlighted the potential protective role of Sar. Moreover, previous literature has supported that *Anemarrhena asphodeloide* suppresses vascular smooth muscle cell (VSMC) growth (15). Nonetheless, whether Sar participates in the process of AS remains unclear.

Store-operated calcium channel (SOCC) has been revealed to be expressed in immune cells, VSMCs, and endothelial cells and participate in immunologic functions, vascular contraction, cell proliferation, and migration (16,17). A large number of studies have specified the pivotal role of SOCC in cardiovascular diseases (18), such as hypertension (19) and AS (20). Stromal interaction molecule 1 (STIM1) located in the endoplasmic reticulum membrane and Orai are pivotal components of canonical store-operated calcium entry (SOCE) (21). Emerging evidence has supported that STIM1 and Orai both play significant roles in vascular disorders (22,23). Emerging evidence has shown that STIM1 expression is raised in oxidized low-density lipoprotein (ox-LDL)-challenged VSMCs and accelerates the malignant alternations in VSMCs phenotypes (24-26).

This study was conducted with the aim of illuminating the role of Sar and identifying the regulatory relationship between Sar and STIM1 in AS. We present this article in accordance with the MDAR reporting checklist (available at https://cdt.amegroups.com/article/view/10.21037/cdt-23-111/rc).

Highlight box

Key findings

- Sarsasapogenin (Sar) suppresses stromal interaction molecule 1 (STIM1) expression to block oxidized low-density lipoprotein (ox-LDL)-triggered vascular smooth muscle cells (VSMCs) proliferation, migration, and invasion.

What is known and what is new?

- The potential protective role of the source of Sar, *Anemarrhena asphodeloide*, has been revealed in AS.
- The role and regulatory mechanism of Sar in AS have remained elusive.

What is the implication, and what should change now?

- Sar has potential in protecting against VSMCs proliferation, migration, and invasion in response to ox-LDL and may be used as a new therapeutic agent in AS.

Methods

Cell culture and treatment protocol

Dulbecco's modified Eagle medium (DMEM; KeyGEN Biotech, Nanjing, China) with 10% fetal bovine serum (FBS; KeyGEN Biotech) was placed in an incubator at 37 ℃ with 5% CO_2 to culture human VSMCs (iCell Bioscience Inc., Shanghai, China). The VSMCs of passage 4 were used in the following experiments. To construct an *in vitro* cell model of AS, VMSCs were treated with

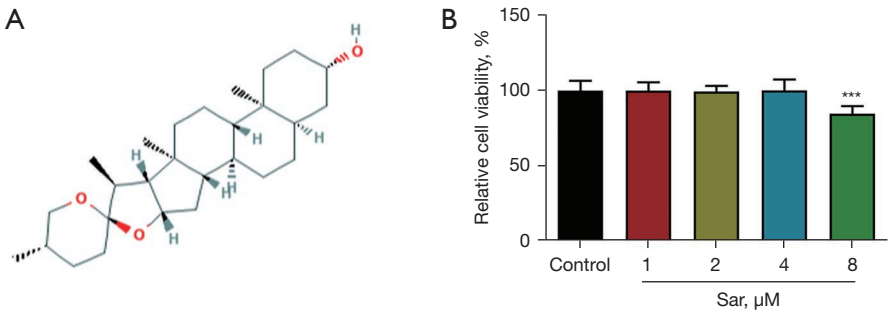

Figure 1 The impacts of Sar on VSMCs viability. (A) Molecular structure of Sar. (B) CCK-8 assay appraised VSMCs viability when treated by 1, 2, 4, and 8 μM of Sar (one-way ANOVA). Mean ± SD, n=3. ***, P<0.001 *vs.* Control. Sar, sarsasapogenin; CCK-8, Cell Counting Kit-8; VSMCs, vascular smooth muscle cells; ANOVA, one-way analysis of variance.

50 mg/L oxidized low-density lipoprotein (ox-LDL; Biosynthesis Biotechnology Company, Beijing, China) for 24 hours. To evaluate the impacts of Sar on ox-LDL-treated VSMCs, VSMCs were pretreated by Sar (1, 2, or 4 μM; Aladdin, Shanghai, China) for 24 hours prior to incubation with 50 mg/L ox-LDL.

Cell Counting Kit-8 assay

Human VSMCs inoculated in 96-well plates overnight with 5×10^3 cells/well received treatment with ascending concentrations of Sar (1, 2, 4, and 8 μM) alone for 48 hours. Prior to the capture of OD450 nm value using a microplate reader (Beijing Potenov Technology Co., Ltd., Beijing, China), 10 μL Cell Counting Kit-8 (CCK-8) solution (ABclonal, Woburn, MA, USA) was supplemented for extra 2 hours at 37 ℃ strictly referring to the manual from the manufacturer.

Western blot

The proteins collected from human VSMCs were prepared in radioimmunoprecipitation assay (RIPA) buffer (Bestbio, Shanghai, China), subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and bound to polyvinylidene fluoride (PVDF) membranes which were then immersed in 5% non-fat milk. The membranes were subsequently successively hybridized with primary antibodies overnight at 4 ℃ and goat anti-rabbit HRP antibody (cat. no. ab205718; 1/2000; Abcam, Cambridge, MA, USA) for 1 hour. The blots were prepared with the aid of the enhanced chemiluminescence (ECL) reagent (Bestbio) and the gray analysis was carried out with ImageLab4.0 software (Bio-Rad, Hercules, CA, USA). Proliferating cell nuclear antigen (PCNA; cat. No. ab92552; 1/1000; Abcam), Ki67 (cat. No. ab92742; 1/5000; Abcam), matrix metallopeptidase 9 (MMP9; cat. No. ab76003; 1/1000; Abcam), matrix metallopeptidase 2 (MMP2; cat. No. ab23981; 1/1000; Abcam), STIM1 (cat. No. ab108994; 1/1000; Abcam), Orai (cat. No. ab111960; 1/1000; Abcam), and GAPDH (cat. No. ab9485; 1/2500; Abcam) primary antibodies were utilized in this study.

5-Ethynyl-2'-deoxyuridine staining

The proliferation of VSMCs was measured via the employment of kFlour555 Click-iT EdU kit (KeyGEN Biotech, Nanjing, China). Briefly, VSMCs (1×10^4 cells/well) were resuspended in DMEM composed of 5-Ethynyl-2'-deoxyuridine staining (EDU; 50 μM per well; Ribobio, Guangzhou, China) for 2 hours at 37 ℃ as per the manufacturer's instructions. Subsequently, 4% paraformaldehyde and 1% Triton X-100 were successively added for immobilization for 30 minutes and permeabilization for 10 minutes, respectively, at room temperature. Cells were labeled with 0.1 μg/mL 4',6-diamidino-2-phenylindole (DAPI) for 20 minutes at room temperature. Images were prepared for observation under a fluorescence microscope (Shanghai Batuo Instrument Co., Ltd., Shanghai, China).

Wound healing assay

A single scratch was formed across the monolayer with the aid of a 200-μL pipette tip when VSMCs inoculated in 6-well plates (4×10^5 cells/well) reached 70–80% confluency.

The suspended cells were then rinsed with serum-free DMEM medium. A light microscope (Shanghai Batuo Instrument Co., Ltd.) was used to observe the wound area at 0 and 24 hours.

Transwell assay

Matrigel [Becton, Dickinson, and Co. (BD) Biosciences, Franklin Lakes, NJ, USA] pre-coating was performed for 30 minutes at 37 ℃. In short, the upper sides of Matrigel-coated transwell inserts (Beijing Unique Biotech Co., Ltd., Beijing, China) were loaded with VSMCs (5×10^4) in serum-starved medium whereas the undersides were supplied with 500 µL DMEM medium decorated by 10% FBS as a chemoattractant. Then VSMCs might invade to the lower chamber with high nutritional content and the number of invaded cells might reflect the invasive ability of VSMCs. After 24 hours, non-invaded cells were discarded, and crystal violet staining of the invaded cells was conducted and observed under a fluorescence microscope (Shanghai Batuo Instrument Co., Ltd.).

Reverse transcription-quantitative polymerase chain reaction

Following the preparation of total RNA from human VSMCs using the Trizol reagent (Genenode, Beijing, China), complementary DNA (cDNA) was generated as per the user guide of First Strand cDNA Synthesis Kit (Genenode). Following the implementation of polymerase chain reaction (PCR) analysis with the employment of the SYBR Green I (Genenode), the alternations in messenger RNA (mRNA) levels were reflected in compliance with the $2^{-\Delta\Delta Ct}$ method (27), with GAPDH as the housekeeping gene. The primer sequences used were as follows: STIM1, forward, 5'-AGTCACAGTGAGAAGGCGAC-3' and reverse, 5'-CAATTCGGCAAAACTCTGCTG-3'; GAPDH, forward, 5'-AATGGGCAGCCGTTAGGAAA-3' and reverse, 5'-GCGCCCAATACGACCAAATC-3'.

Plasmid transfection

STIM1 overexpression vector (OV-STIM1) and the empty vector OV-NC were provided by EK-Bioscience (Shanghai, China). Human VSMCs were subjected to plasmid transduction adopting Lipofectamine®2000 (Hengfei Biotechnology, Shanghai, China) as per the manufacturer's recommendation. Subsequent experiments were carried out 24 hours post-transfection.

Statistical analyses

All experiments were independently repeated in triplicate and all experimental data were biologically repeated in triplicate. The values were given as mean ± standard deviation (SD) employing GraphPad Prism 8 software (GraphPad Software, Inc., San Diego, CA, USA). The differences were deemed statistically significant at $P<0.05$ adopting one-way analysis of variance (ANOVA) followed by Turkey's test.

Results

Sar concentration-dependently suppressed ox-LDL-stimulated VSMCs proliferation

To elucidate the effects of Sar on AS, VSMCs viability was appraised following treatment with ascending concentrations (1, 2, 4, or 8 µM) of Sar and the results of CCK-8 assay revealed no apparent alternations in VSMCs viability upon exposure to 1, 2, and 4 µM Sar. In particular, 8 µM Sar significantly reduced the viability of VSMCs (*Figure 1B*). Hence, 1, 2, and 4 µM of Sar that were nontoxic to VSMCs were chosen for the ensuing assays. Further, EdU staining also corroborated that the fortified VSMCs proliferation imposed by ox-LDL was notably impeded by Sar (*Figure 2A*). Also, western blot revealed that the augmented Ki67 and PCNA expression in the ox-LDL-treated VSMCs were both lessened by Sar (*Figure 2B*). Overall, Sar protected against ox-LDL-triggered VSMCs proliferation.

Sar prevented ox-LDL-induced VSMCs migration and invasion

Further, through transwell assay, it was noticed that ox-LDL challenging markedly potentiated the invasive abilities of VSMCs and Sar treatment concentration-dependently obstructed ox-LDL-provoked VSMCs invasion (*Figure 3A*). Similarly, wound healing assays presented that the exacerbated VSMCs migration in response to ox-LDL was inhibited following the administration with Sar (*Figure 3B*). To verify this finding, western blot was used to test the expression of metastasis-associated proteins MMP9 and MMP2 and the results showed that ox-LDL-elevated MMP9 and MMP2 expression were both diminished by Sar (*Figure 3C*). To sum up, Sar functioned as a suppressor in

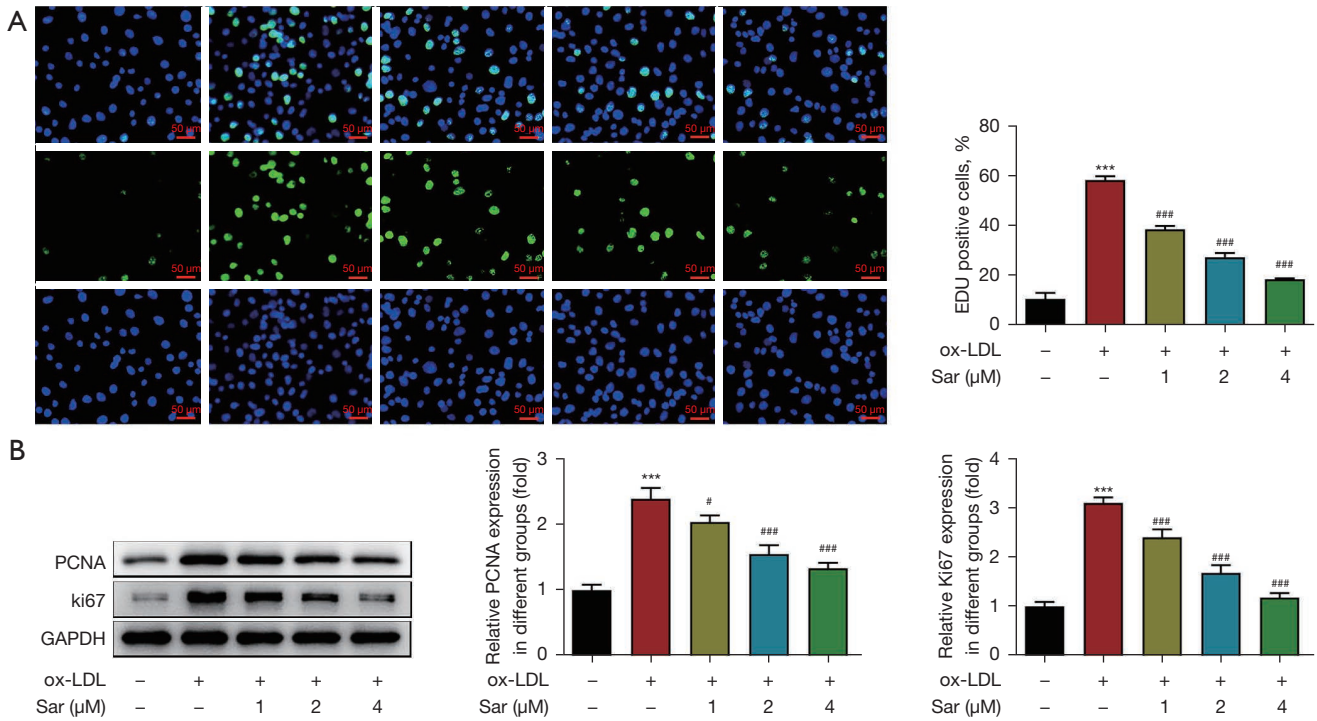

Figure 2 Sar concentration-dependently suppresses ox-LDL-stimulated VSMCs proliferation. VSMCs were pretreated by Sar (1, 2, or 4 μM) for 24 hours prior to incubation with 50 mg/L ox-LDL. (A) EDU assay appraised VSMCs proliferation (one-way ANOVA). EDU was used for staining. Magnification: 200×. (B) Western blot examined the expression of proliferation-associated proteins (one-way ANOVA). Mean ± SD, n=3. ***, P<0.001 *vs.* (ox-LDL-/Sar-). $^{\#}$, P<0.05 and $^{\#\#\#}$, P<0.001 *vs.* (ox-LDL+/Sar-). Sar, sarsasapogenin; VSMCs, vascular smooth muscle cells; ox-LDL, oxidized low-density lipoprotein; EDU, 5-Ethynyl-2'-deoxyuridine; PCNA, proliferating cell nuclear antigen; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ANOVA, one-way analysis of variance.

ox-LDL-evoked VSMCs migration and invasion.

Sar declined STIM1 and Orai expression in ox-LDL-challenged VSMCs

As predicted by the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database, STIM1 might serve as a potential target of Sar. Moreover, STIM1 activates the Orai channels. Interestingly, STIM1 and Orai mRNA and protein expression were discovered to be augmented by ox-LDL in VSMCs and be depleted by Sar in ox-LDL-exposed VSMCs. Due to its prominent effect, 4 μM Sar was chosen applied to the ensuing experiments (*Figure 4A*,*4B*).

STIM1 elevation reversed the impacts of Sar on the proliferation of ox-LDL-exposed VSMCs

To validate that Sar functioned in AS via STIM1-dependent mechanism, STIM1 was overexpressed by transduction of OV-STIM1 plasmids and the transfection efficacy was verified by reverse transcription-quantitative PCR (RT-qPCR) and western blot (*Figure 5A*,*5B*). As shown in *Figure 5C*, Sar overtly suppressed ox-LDL-stimulated VSMCs proliferation and STIM1 up-regulation partially exacerbated VSMCs proliferation again. Additionally, the ascending Ki67 and PCNA expression in ox-LDL-treated VSMCs were diminished by Sar, which were then further elevated by STIM1 (*Figure 5D*). Accordingly, Sar blocked the proliferation of VSMCs exposed to ox-LDL through cutting down STIM1 expression.

STIM1 overexpression reversed the impacts of Sar on the migration and invasion of ox-LDL-exposed VSMCs

Meanwhile, ox-LDL-induced VSMCs invasion was diminished by Sar and the impacts of Sar were partially abrogated by STIM1 elevation (*Figure 6A*). As expected,

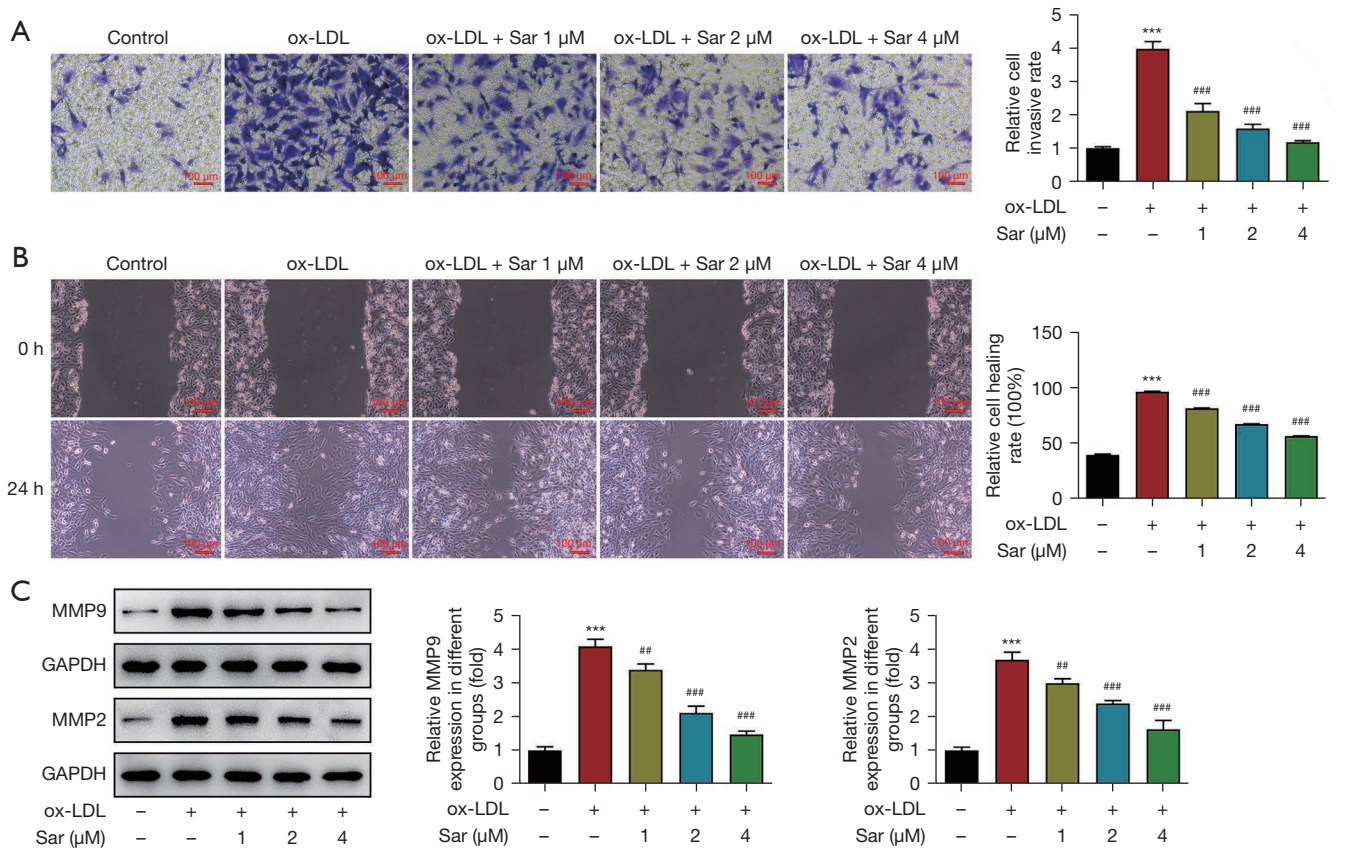

Figure 3 Sar prevents ox-LDL-elicited VSMCs migration and invasion. VSMCs were pretreated by Sar (1, 2, or 4 μM) for 24 hours prior to incubation with 50 mg/L ox-LDL. (A) Transwell assay estimated VSMCs invasion (one-way ANOVA). Magnification: 100×. (B) Wound healing assay estimated VSMCs migration (one-way ANOVA). Crystal violet was used for staining. Magnification: 100×. (C) Western blot tested the expression of metastasis-associated proteins (one-way ANOVA). Mean ± SD, n=3. ***, P<0.001 *vs.* Control (ox-LDL-/Sar-). ##, P<0.01 and ###, P<0.001 *vs.* ox-LDL (ox-LDL+/Sar-). Sar, sarsasapogenin; VSMCs, vascular smooth muscle cells; ox-LDL, oxidized low-density lipoprotein; MMP9, matrix metallopeptidase 9; MMP2, matrix metallopeptidase 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ANOVA, one-way analysis of variance.

STIM1 overexpression reversed the suppressive role of Sar in the migration of ox-LDL-challenged VSMCs (*Figure 6B*). This finding was accompanied with the results that the upregulated MMP9 and MMP2 expression in ox-LDL-challenged VSMCs were both reduced by Sar and further raised again after STIM1 was overexpressed (*Figure 6C*). Collectively, Sar obstructed ox-LDL-induced VSMCs migration and invasion via inactivating STIM1.

Discussion

AS represents an underlying driver of cardiovascular events associated with unstable plaques and poor prognosis (28). VSMCs are major constituents of the normal tissue structure of vascular walls and can maintain vascular tension (29). Under normal physiological conditions, VSMCs exist in a quiescent, non-proliferative and non-migratory state in healthy vessels (29). Phenotypic conversion of VSMCs including dedifferentiation, migration, and transdifferentiation into other cell types is responsible for structural remodeling and further leads to the occurrence of vascular remodeling diseases, such as hypertension, AS, vascular restenosis, and so on (30,31). The increased endothelium permeability to low density lipoprotein molecules, which accumulate in the intima and are oxidized by vascular cells, forming ox-LDL, is a dominant contributor to the formation of atherosclerotic plaques (32). Concurrently, ox-LDL accumulation has been identified as

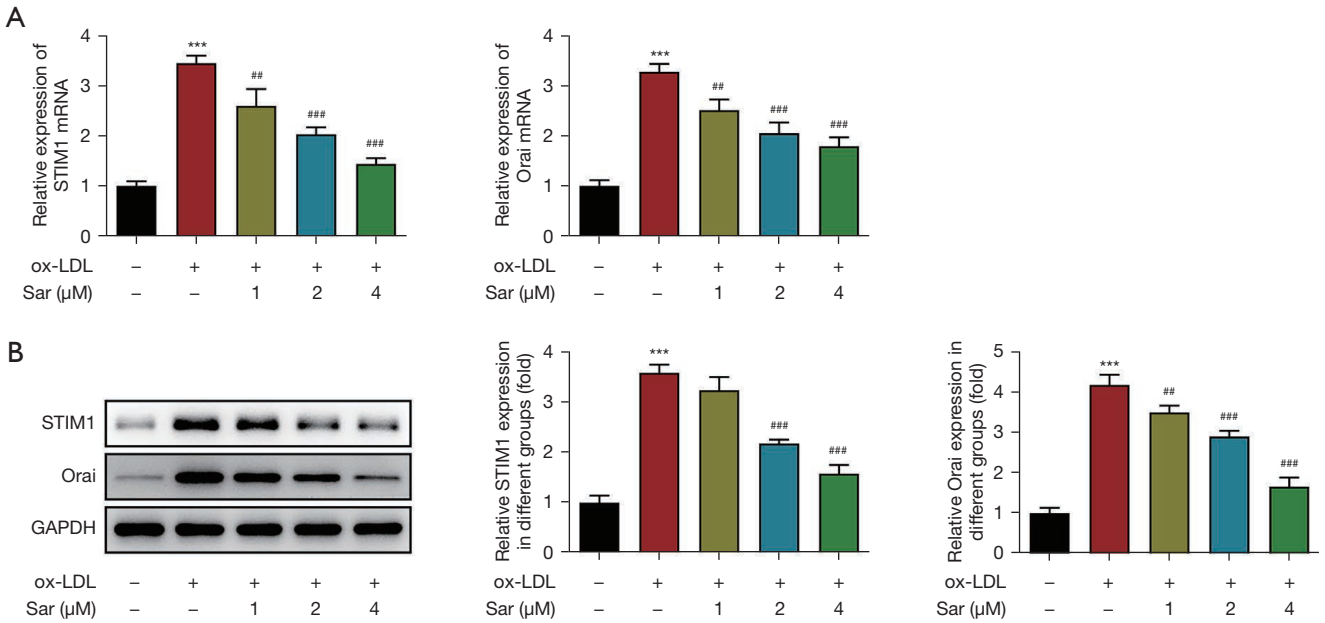

Figure 4 Sar declines STIM1 and Orai expression in ox-LDL-challenged VSMCs. VSMCs were pretreated by Sar (1, 2, or 4 μM) for 24 hours prior to incubation with 50 mg/L ox-LDL. (A) RT-qPCR and (B) western blot tested STIM1 and Orai expression (one-way ANOVA). The relative mRNA level of STIM1 was normalized to GAPDH mRNA. Mean ± SD, n=3. ***, P<0.001 *vs.* Control (ox-LDL-/Sar). ##, P<0.01 and ###, P<0.001 *vs.* (ox-LDL+/Sar-). Sar, sarsasapogenin; ox-LDL, oxidized low-density lipoprotein; VSMCs, vascular smooth muscle cells; STIM1, stromal interaction molecule 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ANOVA, one-way analysis of variance.

a regulatory factor involved in VSMCs calcification which is closely linked to multiple cardiovascular diseases (33). In addition to AS, chronic kidney disease, diabetes, hypertension and aging may be also induced (34). Therefore, this paper utilized ox-LDL to induce human VSMCs to stimulate an *in vitro* cell model of AS.

Anemarrhena asphodeloide, a traditional Chinese medical component, has been supported to suppress VSMCs growth (15). As a major effective component from *Anemarrhena asphodeloide*, Sar has been identified as a potential molecule for future drug development due to its properties in inflammatory response, cancers, diabetes, as well as neurological disorders (35). At the same time, Sar suppresses mesangial cell proliferation in diabetes (36) and Sar derivatives halt breast cancer cell MCF-7 proliferation (37). In this study, the experimental data elaborated that the ascending concentrations of Sar elicited no significant activities on human VSMCs viability. Following exposure to ox-LDL, the aberrant increase of VSMCs viability and proliferation occurred and administration with Sar remarkably impeded the viability and proliferation of ox-LDL-challenged VSMCs. In addition, the fortified expression of proliferation-associated proteins Ki67 and PCNA in ox-LDL-stimulated VSMCs were both downregulated by Sar in a concentration-dependent manner.

Dysregulation of VSMCs migration and invasion has been well documented as a pivotal event in the process of AS (38). Our investigations revealed that ox-LDL treatment markedly strengthened the migratory and invasive abilities of VSMCs, which were then further diminished by Sar. Active MMPs present in the atherosclerotic lesions may contribute to plaque destabilization by degrading ECM components (39). Atherosclerotic human vessels display increased MMPs expression as compared with healthy human vessels (40). MMP9 and MMP2 have been understood to be indispensable for VSMCs migration (41). Here, we discovered that the augmented MMP2 and MMP9 expression in ox-LDL-challenged VSMCs were both depleted by Sar.

STIM1 and Orai, the molecular basis for SOCC, has been reported to mediate calcium release-activated calcium signaling and regulate calcium homeostasis due to the initial binding between the SOAR domain of STIM1 and the C terminus of Orai docks STIM1 onto the N terminus of Orai (42). STIM1 is a multidomain protein that clusters

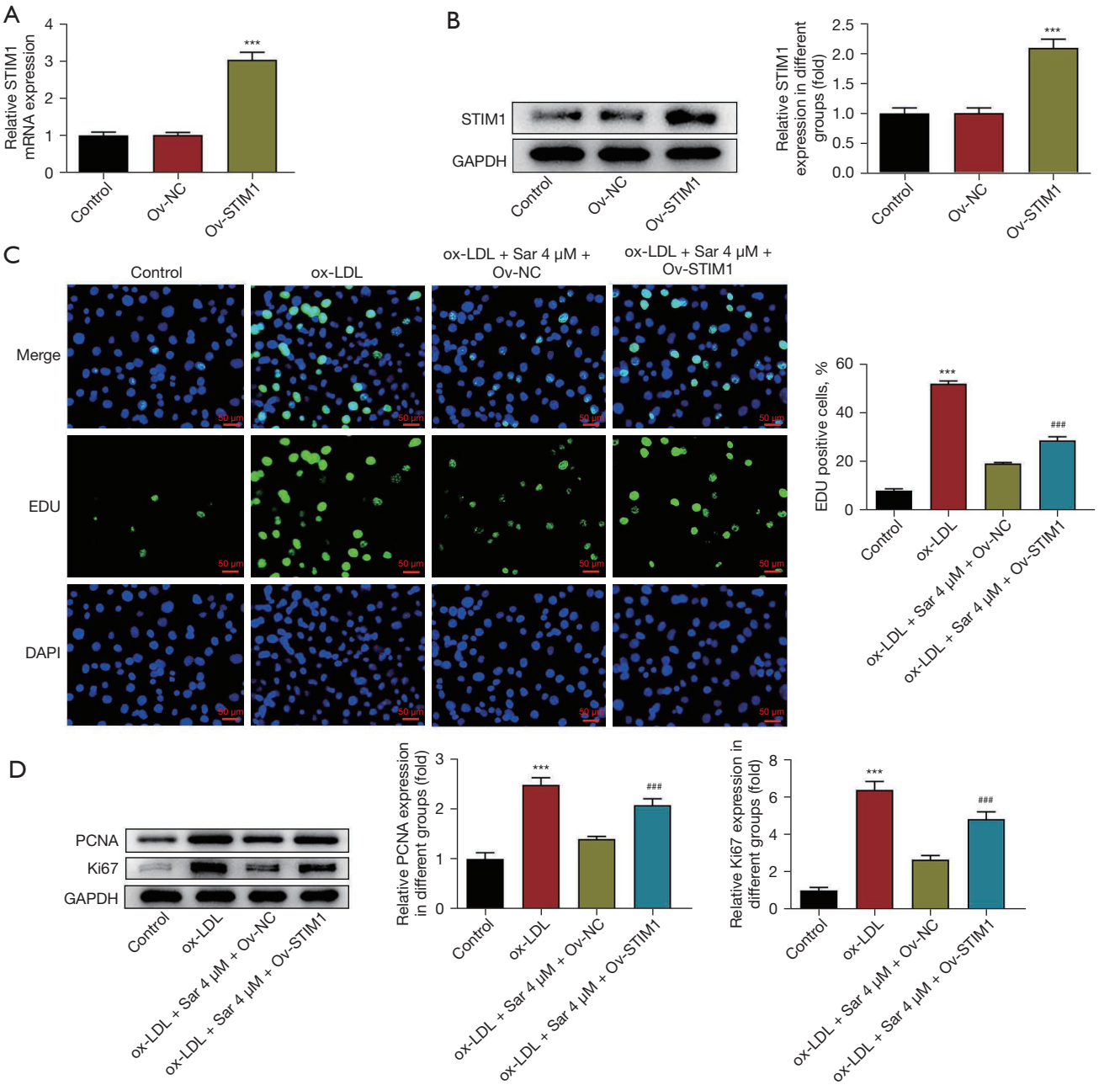

Figure 5 STIM1 elevation reverses the impacts of Sar on the proliferation of ox-LDL-exposed VSMCs. (A) RT-qPCR and (B) western blot tested the transduction efficacy of Ov-STIM1 plasmid (one-way ANOVA). The relative mRNA level of STIM1 was normalized to GAPDH mRNA. ***, P<0.001 *vs.* Ov-NC. VSMCs transfected with Ov-STIM1 and Ov-NC were pretreated by Sar (1, 2, or 4 μM) for 24 hours prior to incubation with 50 mg/L ox-LDL. (C) EDU assay appraised VSMCs proliferation (one-way ANOVA). EDU was used for staining. Magnification: 200×. (D) Western blot examined the expression of proliferation-associated proteins (one-way ANOVA). Mean ± SD, n=3. ***, P<0.001 *vs.* Control. ###, P<0.001 *vs.* ox-LDL + Sar 4 μM + Ov-NC. Sar, sarsasapogenin; VSMCs, vascular smooth muscle cells; ox-LDL, oxidized low-density lipoprotein; STIM1, stromal interaction molecule 1; EDU, 5-Ethynyl-2'-deoxyuridine; RT-qPCR, reverse transcription quantitative polymerase chain reaction; PCNA, proliferating cell nuclear antigen; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ANOVA, one-way analysis of variance.

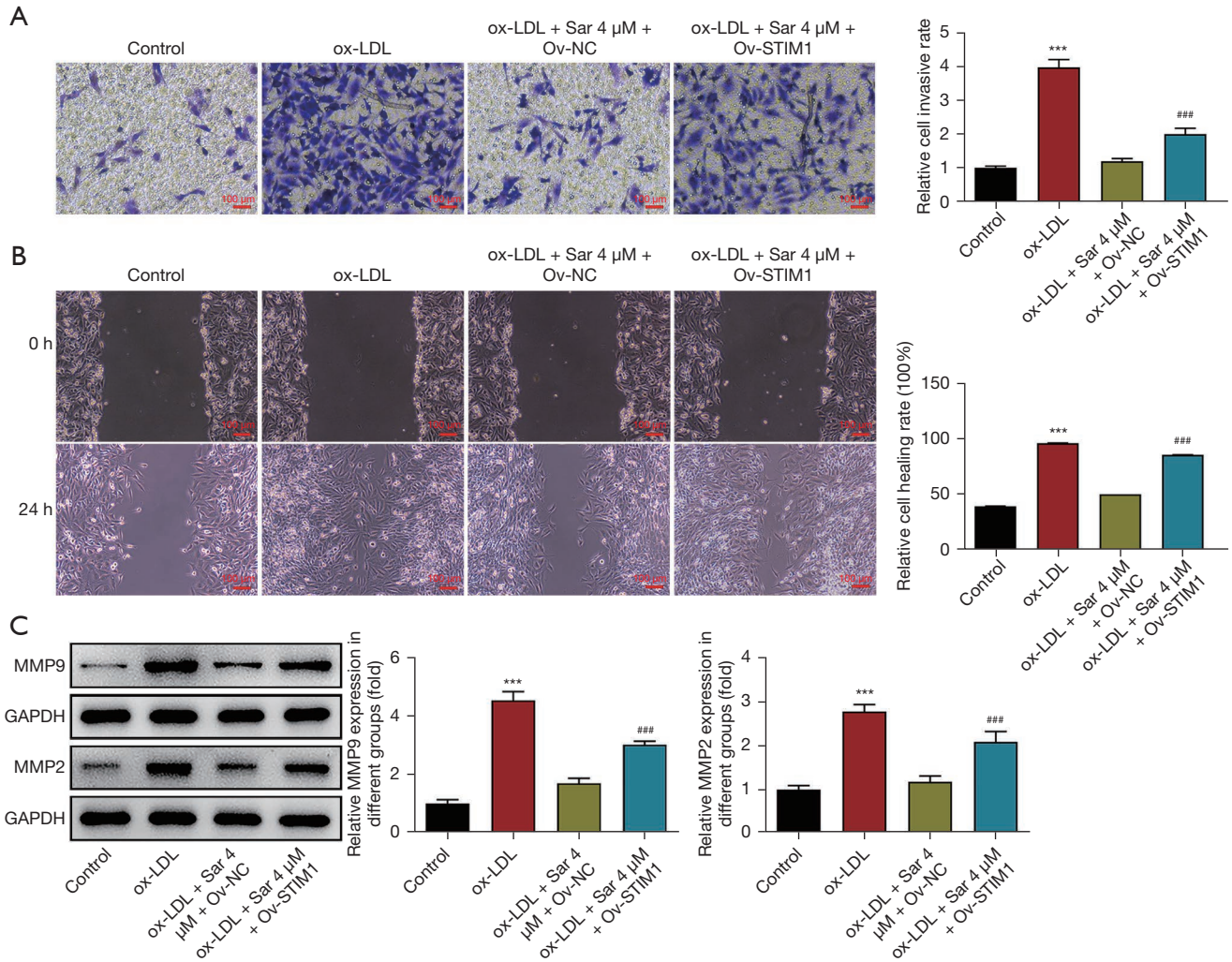

Figure 6 STIM1 overexpression reverses the impacts of Sar on the migration and invasion of ox-LDL-exposed VSMCs. VSMCs transfected with Ov-STIM1 and Ov-NC were pretreated by Sar (4 μM) for 24 hours prior to incubation with 50 mg/L ox-LDL. (A) Transwell estimated VSMCs invasion (one-way ANOVA). Crystal violet was used for staining. Magnification: 100×. (B) Wound healing assay estimated VSMCs migration (one-way ANOVA). Crystal violet was used for staining. Magnification: 100×. (C) Western blot tested the expression of metastasis-associated proteins (one-way ANOVA). Mean ± SD, n=3. ***, P<0.001 *vs.* Control. ###, P<0.001 *vs.* ox-LDL + Sar 4 μM + Ov-NC. Sar, sarsasapogenin; ox-LDL, oxidized low-density lipoprotein; VSMCs, vascular smooth muscle cells; STIM1, stromal interaction molecule 1; MMP9, matrix metallopeptidase 9; MMP2, matrix metallopeptidase 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ANOVA, one-way analysis of variance.

and dimerizes in response to Ca (2+) store depletion leading to activation of Orai (43). Through the STRING database, it has been predicted that Sar might target STIM1 and STIM2. Abundant evidence has corroborated that STIM1 participates in AS via regulating the malignant transformation including proliferation and migration of VSMCs in response to ox-LDL (24,26,44,45). Also, STIM1 participates in stable peripheral coupling in contractile VSMCs which is responsible for modulating blood flow and pressure in cardiovascular diseases (46). The existing experimental results expounded that ox-LDL elevated STIM1 and Orai expression in VSMCs, which were then further abolished by Sar. Besides, after STIM1 was overexpressed, the suppressed proliferation, migration, and

invasion of VSMCs challenged with ox-LDL on account of Sar treatment were all counteracted.

Conclusions

To sum up, Sar might concentration-dependently prevent ox-LDL-triggered VSMCs proliferation, migration, and invasion to protect against AS via the inhibition of STIM1 expression. This study hinted at the protective role of Sar in AS and further figured out the potential regulatory mechanism involving STIM1/Orai signaling, which introduced the potential application of Sar to the therapy for AS. Nevertheless, the impacts of Sar on AS need to be further verified in animal experiments in the future. In addition, STIM1 needs to be silenced in further experiments to assess the relative effects of Sar on ox-LDL-induced VSMCs migration/proliferation that are independent of STIM1. Orai also needs to be overexpressed to verify the involvement of STIM1/Orai signaling in the effects of Sar on the proliferation, migration, invasion of ox-LDL-treated VSMCs. As predicted by STRING, STIM2 was also a target of Sar. Hence, STIM2 also needs to be investigated in the future. Concurrently, in diabetic nephropathy, Sar has been mentioned to modulate GSK3β signaling which is an important regulator of VSMCs phenotype. Thereafter, whether Sar also protects against ox-LDL-induced VSMCs injury via GSK3β signalling also needs to be explored in the future.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://cdt.amegroups.com/article/view/10.21037/cdt-23-111/rc

Data Sharing Statement: Available at https://cdt.amegroups.com/article/view/10.21037/cdt-23-111/dss

Peer Review File: Available at https://cdt.amegroups.com/article/view/10.21037/cdt-23-111/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://cdt.amegroups.com/article/view/10.21037/cdt-23-111/coif). All authors report that the study was supported by National Health Commission Key Laboratory of Pulmonary Immune-related Diseases (No. 2019PT320003). The authors have no other conflicts of interest to declare

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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