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Original Article

The loss of Spinster homolog 2 drives endothelial mesenchymal transition via SMS2-mediated disruption of sphingomyelin metabolism

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Abstract

Endothelial-mesenchymal transition (EndMT) is the process by which endothelial cells transform into mesenchymal cells, driving stromatogenesis and inflammatory responses, thereby contributing to the development of atherosclerotic plaques. Spinster homolog 2 (SPNS2), a protein responsible for S1P transport, regulates sphingolipid metabolism and signaling in endothelial cells to maintain vascular homeostasis. In the present work, we investigated the involvement of SPNS2 in endothelial mesenchymal transition. Knocking down SPNS2 in endothelial cells resulted in significant phenotypic changes, marked by a decrease in endothelial markers (CD31, VE-cadherin) and an increase in mesenchymal markers (Vimentin, α-SMA), confirming the occurrence of EndMT. Notably, SPNS2 knockdown leads to alterations in sphingolipid metabolism, most prominently marked by a significant increase in sphingomyelin (SM) levels. Similar cellular alterations were observed with the exogenous addition of SM, leading to the transition of endothelial cells from a cobblestone-like morphology to a dispersed, spindle-shaped form. In contrast, the exogenous addition of sphingomyelinase, which degrades SM, was able to reverse the endothelial-to-mesenchymal transition induced by SPNS2 knockdown. Mechanistically, our study suggests that SPNS2 knockdown promotes endothelial-to-mesenchymal transdifferentiation by upregulating SMS2 expression, which subsequently enhances sphingomyelin synthesis.

Keywords: SPNS2, Endothelial-mesenchymal transition, Sphingomyelin metabolism, SMS2, Atherosclerosis.

1. Introduction

Atherosclerosis (AS) is a leading cause of various cardiovascular and cerebrovascular diseases, associated with high incidence and mortality rates[1]. Endothelial-mesenchymal transition (EndMT) in vascular endothelial cells contributes to the development and progression of atherosclerosis by disrupting endothelial homeostasis and promoting plaque formation and instability. Dong et al. discovered that the P300/ASF1A complex promotes atherosclerosis development by inducing EndMT through the activation of SNAI1 transcription, mediated by the regulation of H3K18 acetylation histone modification[2]. Inhibiting histone deacetylases (HDACs) effectively blocks EndMT by preserving the expression of endothelial-associated proteins and reducing the levels of mesenchymal markers, which in turn decreases the formation of vascular lipid plaques[3]. Autopsy results revealed 'transitional' cells within human atherosclerotic plaques that express both endothelial and mesenchymal markers, confirming the occurrence of EndMT during atherosclerosis development[4]. Although recent studies have indicated that pathways such as TGF-β[5, 6], Notch[7, 8], and Wnt[9, 10] can alleviate AS by regulating EndMT, their effects have been relatively modest. However, these signaling pathways play a wide range of roles in multiple physiological and pathological processes, and drug selectivity and specificity are major challenges. Therefore, exploring new EndMT regulatory mechanisms and targets is of great importance for the treatment of diseases.

Spinster Homolog 2 (SPNS2), a member of the Major Facilitator Superfamily (MFS), is primarily expressed in endothelial cells, where it regulates the transport and secretion of Sphingosine-1-Phosphate (S1P)[11, 12]. Sphingomyelin (SM) serves as a crucial reservoir for S1P, which is generated through the Ceramide (Cer)-Sphingosine (Sph) pathway by the action of sphingosine kinases. The SM/Cer/Sph and S1P axis establishes a crucial metabolic equilibrium, with their dynamic balance closely linked to physiological and pathological processes such as cell migration, apoptosis, vascular homeostasis, stress response, and inflammation[13, 14]. Meanwhile, previous studies have confirmed the involvement of SPNS2-mediated sphingolipid metabolism in cardiovascular disease in the regulation of vascular tone and hypertension[15, 16] as

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well as cholesterol accumulation. Additionally, the crosstalks between S1P and TGF-βmay engage epithelial-mesenchymal transition (EMT)/EndMT in pro-fibrotic inflammatory and fibrogenic processes[17]. SPNS2 and its role in sphingolipid metabolism have been shown to play a crucial role in regulating EMT[18, 19], however, its specific function in EndMT remains unclear.

For the first time, we demonstrated that SPNS2 knockdown leads to a significant decrease in endothelial markers such as CD31 and VE-cadherin, accompanied by a marked upregulation of mesenchymal markers like Vimentin and α-SMA, indicating that SPNS2 plays a crucial regulatory role in the EndMT process. Subsequent metabolic analysis revealed that SPNS2 knockdown induced substantial reprogramming of sphingolipid metabolism, characterized by a significant increase in SM levels. These findings suggest that SPNS2 may modulate endothelial cell phenotypic transitions through its regulatory effects on sphingomyelin metabolism. Exogenous supplementation with sphingomyelin replicated the phenotypic changes observed in endothelial cells following SPNS2 knockdown. Moreover, the addition of sphingomyelinase, which facilitates sphingomyelin degradation, effectively reversed EndMT in SPNS2-deficient cells. These results further validate the critical role of SPNS2 in regulating SM during the EndMT process. Mechanistically, analysis of sphingomyelin-related metabolic gene expression in SPNS2-deficient endothelial cells revealed a significant upregulation of SMS2. Notably, SMS2 knockdown effectively reversed the EndMT induced by SPNS2 deficiency. This finding offers new insights into the role of SPNS2-mediated sphingomyelin metabolism in endothelial cell phenotypic transition.

2. Materials and methods

2.1. Cell culture and generation of transfected cells

Human HUVECs (Chinese Academy of Sciences, Shanghai, China) were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin (100 U/mL). Cells were incubated in a humidified incubator with 5% CO2 at 37°C. All cell lines were verified by STR analysis and tested negative for mycoplasma contamination. For cell processing: cells were transduced with lentivirus using the pLCDH vector (GeneCopoeia) containing shNC or shSPNS2 for transfection, and Western blot (WB) was performed to verify the knockdown efficiency. In sphingomyelin inhibition assays, cells were treated with sphingomyelinase (Sigma, 567707) at concentrations of 0 μg/mL, 0.1 μg/mL, 0.25 μg/mL, 0.5 μg/mL, 0.75 μg/mL, and 1 μg/mL for 24 hours to screen for the optimal inhibitory concentration. In experiments involving the aggregation of exogenous SM, cells were treated with 10 μM C12:0 SM (Sigma, 860517P) for an additional 24 hours.

2.2. Western blot analysis

Protein lysates were prepared following standard protocols. After denaturation, the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a PVDF membrane. The membranes were incubated overnight at 4°C with the following primary antibodies: SPNS2 (Affinity Biosciences, DF15844), VIMENTIN (Proteintech, 22031-1-AP), VE-cadherin (Proteintech, 66804-1-Ig), αSMA (Proteintech, 14395-1-AP), CD31 (Proteintech, 11265-1-AP), GAPDH (Proteintech, 60004-1-AP), SMS1 (Proteintech, 19050-1-AP), and SMS2 (Proteintech, Ag6095). Afterward, the membranes were incubated at room temperature with goat anti-rabbit IgG H&L or goat anti-mouse IgG H&L conjugated with horseradish peroxidase (HRP) as secondary antibodies. Chemiluminescent signals were detected using an imaging system, and data were analyzed with ImageJ software.

2.3. RT-qPCR

Total RNA was extracted using TRIzol reagent and reverse transcribed into cDNA with StarScript II RT Mix and gDNA Remover (Tolobio, 22107). Real-time PCR analysis was performed using 2dRealStar Fast SYBR qPCR Mix (Tolobio, 22204-1). Relative mRNA expression was determined using the 2-ΔΔCT method and normalized to GAPDH expression for each gene. The qPCR primer pairs used were: Gapdh:Sense: 5′-CCACTCCTCCACCTTTGAC-3′, Antisense: 5′-ACCCTGTTGCTGTAGCCA-3′; Spns2: Sense: 5′-ACTTTGGGGTCAAGGACCGA-3′, Antisense: 5′-AATCACCTTCCTGTTGAAGCG-3′; Sms1: Sense: 5′-TGACTGCTGACCTGCCAAGAG-3′, Antisense: 5′-TGCTCCAGAGGCTCACAGTATTC-3′; Sms2: Sense: 5′-CTTAGCCCTCCACTCCC-3′, Antisense: 5′-CAGAATCTGCGTCCCAC-3′; CD31: Sense: 5′-GTGCTGCAATGTGCTGTGAA-3′, Antisense: 5′-TGCTAGCCTTCTGCTTGGTC-3′; VE-cadherin: Sense: 5′-GTTCACCTTCTGCGAGGATATG-3′, Antisense: 5′-GATGGTGAGGATGCAGAGTAAG-3′; αSMA: Sense: 5′-GAAGAAGAGGACAGCACTG-3′, Antisense: 5′-TCCCATTCCCACCATCAC-3′; Vimentin: Sense: 5′-AAAACACCCTGCAATCTTTCAGA-3′, Antisense: 5′-CACTTTGCGTTCAAGGTCAAGAC-3′; aSmase: Sense: 5′-AACTCTGAGCCGACCACTAGCT-3′, Antisense: 5′-GTCCAGGACCACATGAGAGCTT-3′; nSMase1: Sense: 5′-CTGCGGATCTTCAACCTCAACTG-3′, Antisense: 5′-GTCCTGCTCACTCCACACCTC-3′; nSMase2: Sense: 5′-GATAACTGCTCCTCTGACGACAAG-3′, Antisense: 5′-CCACGGCTTCTCCTCACCAG-3′.

2.4. Crystal violet staining

The culture medium was removed, and the cells were gently washed with PBS to eliminate any remaining floating cells. Next, 4% paraformaldehyde was added to the wells to fix the cells for 10-15 minutes. After fixation, the paraformaldehyde was discarded, and 0.1% crystal violet solution was applied to stain the cells for 10-20 minutes. Following staining, the cells were rinsed several times with PBS to remove any unbound dye, and the stained cells were then observed under a microscope.

2.5. Immunofluorescence staining

Cells were fixed with 4% paraformaldehyde to preserve cell morphology, followed by three washes with PBS. The cells were permeabilized with 0.05% Triton X-100 for 15 minutes and subsequently blocked with 5% bovine serum albumin (BSA) for 30 minutes at room temperature. The cells were then incubated overnight at 4°C with the primary antibody solution. After washing three times with PBS, the cells were incubated with Alexa Fluor 488-conjugated secondary antibody (Proteintech, SA00013-2) or Alexa Fluor 568-conjugated secondary antibody (Bioworld Technology, BS21696) for 2 hours at room temperature. Finally, the cells were counterstained with DAPI to visu-

alize nuclei. Fluorescent images were captured using an inverted fluorescence microscope, and quantitative immunofluorescence analysis was performed with ImageJ.

2.6. Wound healing assay

A scratch assay was performed to evaluate cell migration ability. Cells were seeded in 6-well plates and cultured until they reached approximately 90% confluence. A straight-line scratch was made in the cell monolayer using a sterile pipette tip. The cells were then washed with PBS to remove any detached cells. Serum-free medium was added to the wells to inhibit cell proliferation and encourage migration. The cells were incubated at 37°C in a 5% CO2 atmosphere. Microscopic images of the scratched area were taken at 0 h, 24 h, and 36 h to monitor cell migration. The scratch width was measured using image analysis software to quantify the migration rate.

2.7. Analysis of Sphingomyelin Content

Sphingomyelin content was measured using the Sphingomyelin Quantitative Colorimetric Analysis Kit (ab287856, Abcam). Cell suspensions were treated with sphingomyelinase as per the kit instructions. Following this, the enzyme mixture and probe were added to the samples. Sphingomyelin levels were quantitatively analyzed by measuring the absorbance at 570 nm using a colorimetric method.

2.8. Statistical analysis

The assumptions of normal distribution and equal variance were evaluated for all data sets using the Shapiro-Wilk test and Levene's test, respectively. Data are presented as mean ± standard deviation and were statistically analyzed using GraphPad Prism 7 software. Student's t-test was employed to compare differences between two groups, while one-way ANOVA followed by Tukey's multiple comparison post hoc test was used to assess differences among three or more groups. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Knockdown of SPNS2 promotes EndMT in endothelial cells

To investigate the impact of SPNS2 on endothelial cell function, we established an SPNS2 low-expression model in HUVECs using lentiviral transduction. The results showed a significant downregulation of both SPNS2 mRNA and protein levels (Figures 1A-B). Crystal violet staining revealed marked alterations in cell morphology following SPNS2 knockdown. These morphological changes underscore the significant impact of SPNS2 knockdown on HUVEC function (Figure 1C). To further elucidate the morphological and potential functional changes, we performed RNA sequencing analysis on endothelial cells with SPNS2 knockdown and control cells. The analysis identified 3,743 genes with downregulated expression and 3,934 genes with upregulated expression (Figure 1D). Pathway enrichment analysis of differentially expressed genes revealed that SPNS2 knockdown resulted in the enrichment of pathways associated with protein-containing complex scaffold activity, cell adhesion molecule binding, and cadherin binding (Figure 1E). These pathways are primarily involved in cytoskeleton dynamics, cell-cell junctions, cell adhesion, and signaling, all of which are critical for cellular phenotypic transitions and closely associated with EndMT. This suggests that EndMT may be a potential mechanism underlying the observed morphological changes in endothelial cells following SPNS2 knockdown. Therefore, we evaluated the mRNA and protein expression of endothelial and mesenchymal markers using RT-qPCR, Western blotting, and immunofluorescence staining. The results indicated that SPNS2 knockdown promoted the expression of mesenchymal markers α-SMA and Vimentin while reducing the levels of endothelial markers CD31 and VE-cadherin (Figures 1F-H). Meanwhile, the scratch assay observed enhanced cell migration after knockdown of SPNS2 (Figure 1I). This phenomenon aligns with the characteristics of EndMT. These findings suggest that SPNS2 plays a crucial role in maintaining endothelial function and that its expression is critical for regulating the process of EndMT.

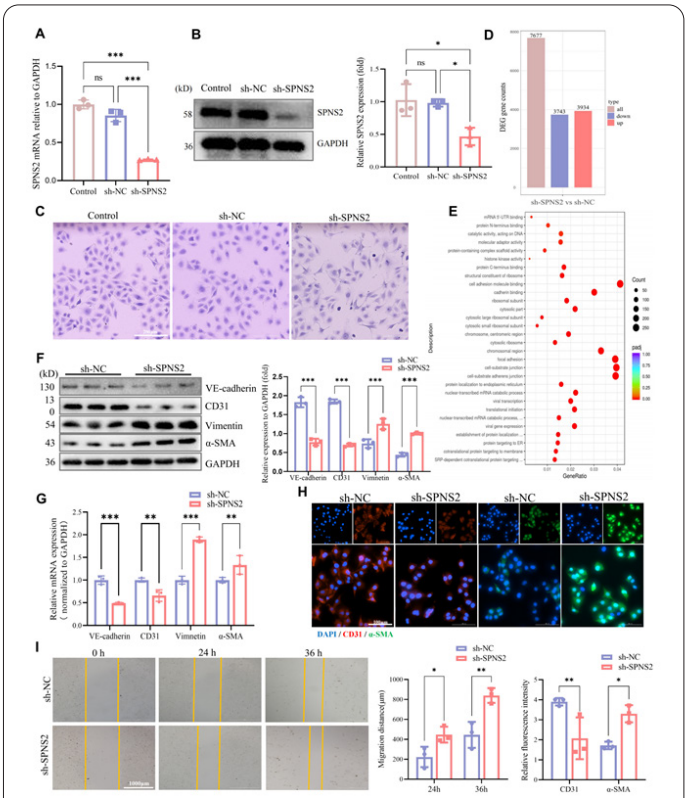

Fig. 1. Knockdown of SPNS2 promotes EndMT in endothelial cells. A. RT-qPCR detection of SPNS2 mRNA level in endothelial cells of control, sh-NC and sh-SPNS2 groups. B. Western blotting to detect SPNS2 protein expression in endothelial cells of control, sh-NC, and sh-SPNS2 groups. C. Morphological observation of endothelial cells in control, sh-NC, and sh-SPNS2 groups (Scale bar=200μm). D. histogram of differential genes in endothelial cells of sh-NC and sh-SPNS2 groups. E. First 30 biological processes of differential gene enrichment in endothelial cells of sh-NC vs. sh-SPNS2 groups. F. RT-qPCR analysis of CD31, VE-cadherin, α-SMA, Vimentin mRNA expression in endothelial cells of sh-NC vs. sh-SPNS2 group. G. Western blot analysis of CD31, VE-cadherin, α-SMA, Vimentin protein expression in endothelial cells of sh-NC and sh-SPNS2 groups. H. Immunofluorescence staining to analyze the relative quantification of CD31 and α-SMA in endothelial cells of sh-NC and sh-SPNS2 groups (Scale bar=100μm). I. Scratch assay to observe the migration ability of endothelial cells in sh-NC vs. sh-SPNS2 group (Scale bar=1000μm). The experimental samples in all groups satisfy n=3. Values are mean ± SEM. *:P<0.05, **:P<0.01,***:P<0.001

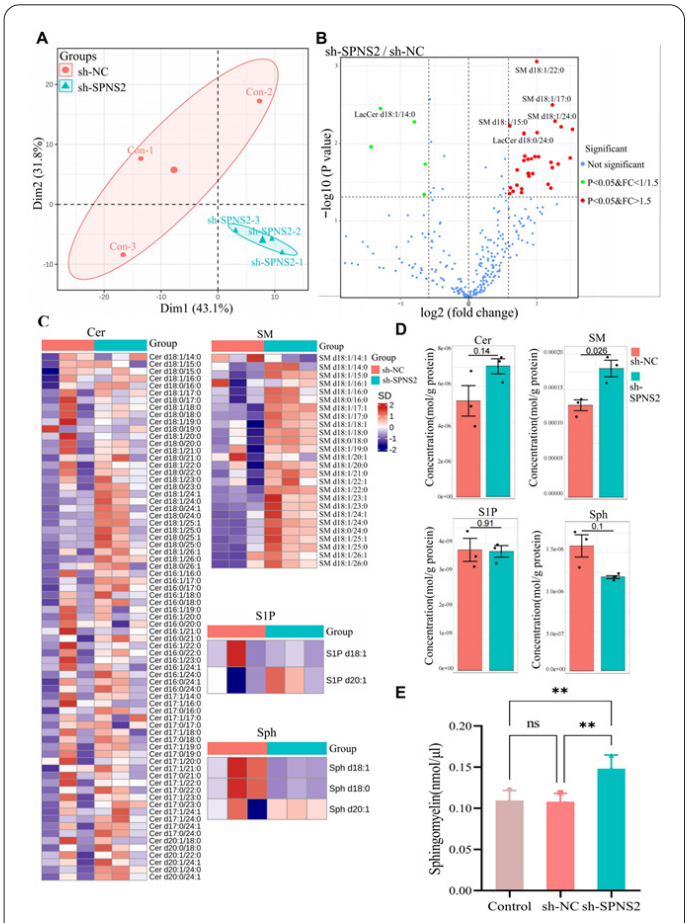

Fig. 2. SPNS2 lacking increases sphingomyelin levels in endothelial cells. A. Principal component analysis plot of endothelial cell sphingolipid composition assay in sh-NC vs. sh-SPNS2 groups. B. Volcano plot of endothelial cell sphingolipids in sh-NC vs. sh-SPNS2 groups. C/D. Heat map and quantitative statistics of Cer, SM, S1P, Sph expression content. E. Quantitative analysis of sphingomyelin levels in Control, sh-NC and sh-SPNS2 groups.

3.2. SPNS2 laching increases sphingomyelin levels in endothelial cells

SPNS2 is pivotal in the sphingolipid metabolic network, regulating the dynamic balance of SM, Sph, S1P, and Cer. This regulation is crucial for maintaining membrane stability[20], signal transduction, and cellular function[21, 22]. Sphingolipidomic analysis of endothelial cells before and after SPNS2 knockdown revealed significant biological differences. Principal component analysis (PCA) showed a clear separation of the samples in the principal component space (Figure 2A). Differential analysis of sphingolipid components revealed that SPNS2 knockdown led to the downregulation of 5 sphingolipid species and the upregulation of 30 sphingolipid species (Figure 2B). Notably, the most significant change was observed in SM levels (Figures 2C-D). The expression of SPNS2 significantly impacts SM levels in endothelial cells, as confirmed by subsequent SM quantification (Figure 2E). Specifically, SPNS2 knockdown resulted in a marked increase in SM levels in endothelial cells.

3.3. Low expression of SPNS2 upregulates SM levels to promote EndMT

Sphingomyelin plays a crucial role in maintaining and modulating cell morphology by regulating membrane stability[23], curvature[24], signal transduction, and cytoskeletal remodeling[25]. Studies have indicated that alterations in sphingomyelin metabolism can lead to EMT in renal collecting duct cells[19, 26]. To determine whether SPNS2 knockdown promotes EndMT by affecting SM levels, we first evaluated the impact of varying concentrations of sphingomyelinase (SMase) on sm levels in HUVECs (Figure 3A). Subsequently, we administered 10μM SM exogenously to HUVECs and applied 0.5μg/mL SMase to SPNS2 knockdown cells to modulate SM levels (Figure 3B). SM treatment increased sphingomyelin levels compared to the Control group. Additionally, SMase treatment effectively reduced the elevated SM levels induced by SPNS2 knockdown. Moreover, RT-qPCR and Western blot analyses showed that SM treatment significantly promoted EndMT compared to the control group.

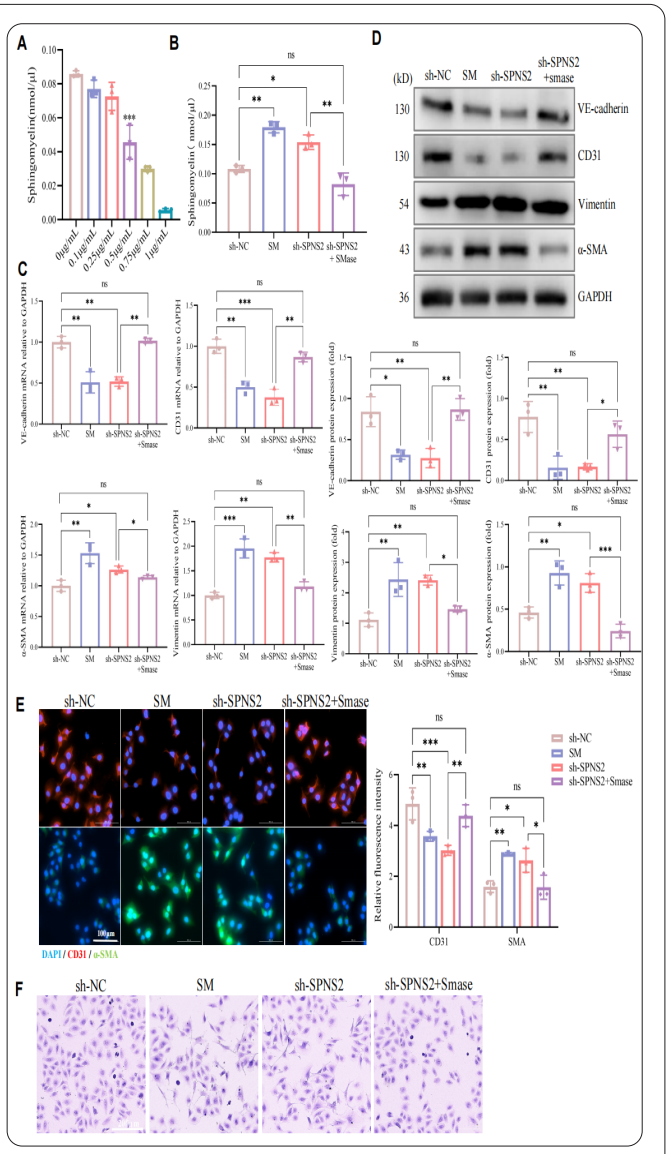

Fig. 3. Low expression of SPNS2 upregulates SM levels to promote EndMT. A. Detection of sphingomyelin levels in endothelial cells treated with different concentrations of smase. B. Quantitative analysis of sphingomyelin levels in each subgroup. C. RT-qPCR analysis of CD31, VE-cadherin, α-SMA, Vimentin mRNA expression. D. Western blot analysis of CD31, VE-cadherin, α-SMA, Vimentin mRNA expression. E. Morphological observation of endothelial cells in control group, SM group, sh-SPNS2 group, and sh-SPNS2+smase group (Scale bar=200μm). F. Immunofluorescence staining analysis of CD31, α-SMA relative quantification (Scale bar=100μm). (The experimental samples in all groups satisfy n=3. Values are mean ± SEM. *:P<0.05, **:P<0.01,***:P<0.001.)

Furthermore, treatment with SMase, which reduced SM levels, partially reversed the EndMT induced by SPNS2 knockdown (Figures 3C-D). Immunofluorescence staining of endothelial cells corroborated these findings (Figure 3E). Microscopic morphological analysis showed that SM treatment induced a spindle-shaped morphology in endothelial cells, resembling the changes observed after SPNS2 knockdown. Compared to the deformation observed in the sh-SPNS2 group, the addition of SMase led to a restoration of cell morphology to a cobblestone-like appearance, resembling that of the Control group (Figure 3F). These results indicate that the sphingosine, SM, is involved in the EndMT process, suggesting that SPNS2 regulates endothelial cell mesenchymal transition by upregulating sphingomyelin levels.

3.4. Knockdown of SMS2 inhibits SPNS2 deficiency-induced SM elevation and EndMT

Sphingomyelin is regulated by various factors, including their synthesis, degradation, and transport pathways, which influence the rate and levels of sphingolipid production[27, 28]. Analysis of key genes involved in sphingomyelin synthesis and degradation revealed that SPNS2 knockdown resulted in an upregulation of sphingomyelin synthases SMS1 and SMS2, with SMS2 showing a more pronounced increase (Figures 3A-B). This suggests that SPNS2 may regulate sphingomyelin levels by modulating the expression of sphingomyelin synthases. To explore this further, we knocked down SMS2 in endothelial cells with knockdown of SPNS2 to assess the effect on SM levels. Inhibition of SMS2 was found to decrease SM levels in sh-SPNS2 endothelial cells (Figure 3C). After knocking down SGMS2, the morphology of sh-SPNS2 endothelial cells was restored from spindle-like change to cobblestone-like morphology (Figure 3D). Analysis of mRNA and protein expression of endothelial and mesenchymal markers revealed that SMS2 knockdown increased the levels of CD31 and VE-cadherin while decreasing α-SMA and Vimentin expression. This observation suggests that SMS2 suppression effectively reversed the endothelial-mesenchymal transition induced by SPNS2 knockdown (Figure 3E-G). Meanwhile, knockdown of SMS2 reversed the enhanced cell migration ability after knockdown of SPNS2 (Figure 1H). Collectively, these results demonstrate that SPNS2 knockdown increases SMS2 expression, which subsequently raises SM levels and promotes EndMT.

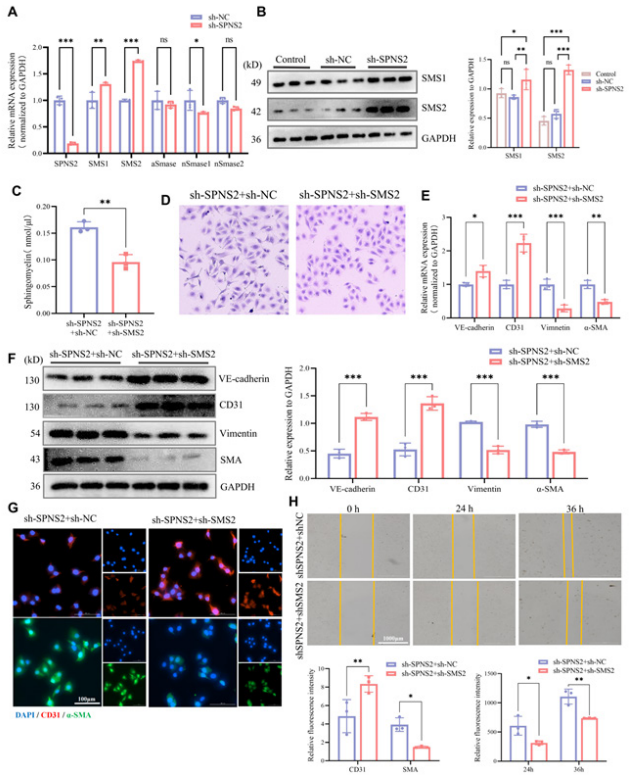

Fig. 4. Knockdown of SMS2 inhibits SPNS2 deficiency-induced SM elevation and EndMT. A. RT-qPCR detection of sphingomyelin metabolism-related gene expression. B. Western blotting to analyze the expression level of SMS protein in endothelial cells of sh-NC and sh-SPNS2 groups. C. Quantitative analysis of sphingomyelin level in sh-SPNS2+shNC group and sh-SPNS2+sh-SMS2 group. D. Morphological observation of endothelial cells in sh-SPNS2+sh-NC and sh-SPNS2+sh-SMS2 groups. E.RT-qPCR analysis of CD31, VE-cadherin, α-SMA, Vimentin mRNA expression. F. Western blot analysis of CD31, VE-cadherin, α-SMA, Vimentin mRNA expression. G. Immunofluorescence staining to analyze the relative quantification of CD31, α-SMA (Scale bar=200μm) . H. Scratch test was performed to observe the migration ability of endothelial cells in the sh-SPNS2+shNC group versus the sh-SPNS2+sh-SMS2 group (Scale bar=1000μm). (The experimental samples in all groups satisfy n=3. Values are mean ± SEM. *:P<0.05, **:P<0.01,***:P<0.001.)

4. Discussion

This study elucidates the significant effects of SPNS2 knockdown on HUVECs, particularly in promoting EndMT and altering cell migration and invasion capabilities. This discovery provides novel insights into the molecular mechanisms regulating endothelial cell function. Our research demonstrates that SPNS2 knockdown leads to increased sphingomyelin levels, which not only alters cell morphology but also induces the expression of markers associated with EndMT. Further experiments show that SPNS2 knockdown significantly upregulates SMS2 expression and activity, resulting in increased sphingomyelin synthesis. This change is closely linked to the process of EndMT in HUVECs. These findings highlight the critical role of SPNS2 in maintaining endothelial cell function. These results clearly indicate that SPNS2 plays an important role in the maintenance of endothelial cell function, and its knockdown leads to dysregulation of sphingomyelin metabolism and promotes EndMT.

Existing literature indicates that sphingomyelin and its metabolic products play a critical regulatory role in EMT[19, 26], particularly in the context of cell signaling and gene expression regulation. However, the specific mechanisms underlying EndMT, a subtype of EMT, have not yet been fully elucidated. The findings of this study are consistent with existing research and further support the critical role of sphingomyelin in regulating EndMT. Through sphingolipidomics and cellular biology experiments, this study systematically analyzed the impact of SPNS2 knockdown on HUVECs, providing new evidence on the relationship between sphingomyelin metabolism and EndMT.

Sphingomyelin, an active metabolite in sphingolipid metabolism, affects related metabolites and multiple intracellular signaling pathways through changes in its levels[29, 30]. Among the enriched differential pathways, the 'Protein-containing complex scaffold activity' pathway

regulates cytoskeleton stability and intercellular structural scaffolding, while the 'Cell adhesion molecule binding' pathway maintains the integrity of intercellular tight junctions and tissue structure by modulating cell adhesion molecule interactions. Cytoskeletal remodeling and changes in intercellular junctions are crucial steps in the transition of endothelial cells to mesenchymal cells[31]. During this process, cadherins, particularly VE-cadherin, play a key role in cell adhesion and signaling. The downregulation of VE-cadherin facilitates the phenotypic transition and migration of endothelial cells, thereby promoting the occurrence of EndMT. While these findings provide novel insights into the regulatory mechanisms of EndMT, further research is needed to elucidate the specific role of sphingomyelin in this process and its interactions with other molecular pathways.

Additionally, this study has certain limitations. Our investigation primarily focused on changes in sphingomyelin metabolism following SPNS2 knockdown, without thoroughly exploring other factors that may influence EndMT. Furthermore, while our experiments reveal the role of SMS2 in the EndMT process induced by SPNS2 knockdown, its interactions with other signaling pathways require further validation. The findings of this study align with existing research, particularly regarding the role of sphingomyelin and its metabolites in EMT and EndMT. Previous studies have shown that SMS2 facilitates the initiation of epithelial-mesenchymal transition through the TGF-β/Smad signaling pathway[32]. Additionally, as a key component of the cell membrane, increased sphingomyelin content can alter membrane fluidity, potentially leading to changes in cell morphology[33, 34]. This alteration may regulate the EndMT process by influencing the reorganization of membrane microdomains[35], the distribution of cell adhesion molecules, and related signaling pathways. However, the precise regulatory mechanisms remain unclear. Specifically, further investigation is needed to determine whether the effects of membrane fluidity on EndMT are consistent across different cell types and pathological conditions. Furthermore, the impact of sphingomyelin on membrane fluidity and the roles of other metabolic products in EndMT are areas that require further exploration. Our findings provide novel insights and offer new directions for deepening the understanding of the molecular mechanisms governing EndMT.

In conclusion, our data demonstrate that SPNS2 plays a significant role in EndMT in endothelial cells. SPNS2 promotes sphingomyelin synthesis by upregulating SMS2 expression, thereby inducing changes in EndMT-related pathways and facilitating cellular transition. Our findings provide novel insights into the molecular mechanisms underlying EndMT and may contribute to the development of new therapeutic strategies for diseases associated with EndMT, such as atherosclerosis, fibrosis, and cancer progression.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships.

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Credit author statement

Jin-feng Qin: contributed to experimental design, performed experiments, analyzed and interpreted the data, and wrote the manuscript. Yuan Li: participated in experimental design and execution. Xiao-dan Wang: performed data analysis. Shuang-xi Tu: conducted experiments. Xiao Zhu: assisted in data interpretation and manuscript writing. Kai Yin: conceived the study, provided project oversight, and designed the experiments. All authors have read and approved the final manuscript for publication.

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