Induction of SPARC on Oxidative Stress, Inflammatory Phenotype Transformation, and Apoptosis of Human Brain Smooth Muscle Cells Via TGF-β1-NOX4 Pathway

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Abstract
Secreted protein acidic and rich in cysteine (SPARC) has a close association with inflammatory response and oxidative stress in tissues and is widely expressed in intracranial aneurysms (IAs), especially in smooth muscle cells. Therefore, it is inferred that SPARC might be involved in the formation and development of IAs through the inflammatory response pathway or oxidative stress pathway. The aim of this study is to investigate the pathological mechanism of SPARC in oxidative stress, inflammation, and apoptosis during the formation of IAs, as well as the involvement of TGF-β1 and NOX4 molecules. Human brain vascular smooth muscle cells (HBVSMCs) were selected as experimental objects. After the cells were stimulated by recombinant human SPARC protein in vitro, the ROS level in the cells was measured using an ID/ROS fluorescence analysis kit combined with fluorescence microscope and flow cytometry. The related protein expression in HBVSMCs was measured using western blotting. The mitochondrial membrane potential change was detected using a mitochondrial membrane potential kit and laser confocal microscope. The mechanism was explored by intervention with reactive oxygen scavengers N-acetylcysteine (NAC), TGF-β1 inhibitor (SD-208), and siRNA knockout. The results showed that SPARC upregulated the expression of NOX4 through the TGF-β1-dependent signaling pathway, leading to oxidative stress and pro-inflammatory matrix behavior and apoptosis in HBVSMCs. These findings demonstrated that SPARC may promote the progression of IAs.

Keywords SPARC • Intracranial aneurysms • Oxidative stress • Phenotype transformation • TGF-β1-NOX4 pathway • Human brain smooth muscle cells

Introduction
Intracranial aneurysms (IAs) are relatively common lesions, leading to catastrophic subarachnoid hemorrhage with high mortality and morbidity rates. The pathological mechanism of formation of IAs is associated with inflammatory cell infiltration, smooth muscle cell apoptosis, and degradation of extracellular matrix. According to our and others studies, secreted protein acidic and rich in cysteine (SPARC) is widely expressed in human IAs tissues, especially in vascular smooth muscle cells (VSMCs) (Li et al. 2013), and can reduce the self-healing capacity and promote damage to the tunica media and internal elastic membrane (Ye et al. 2016), suggesting that SPARC might participate in the development and progression of IAs.

SPARC is a secretory cell matrix glycoprotein and is also known as BM-40 and the bone adhesion protein. Recent studies have outlined the relationship between SPARC and reactive oxygen species (ROS) in the pathogenesis of renal and epithelial injury, mature fat cells, and liver cells (Aseer et al. 2017; Shen et al. 2014; Shibata and Ishiyama 2013). ROS induces an important process leading to the formation of cerebral aneurysms, which include direct endothelial injury, inflammatory phenotypic transformation of smooth muscle cells, and apoptosis (Starke et al. 2013). However, the relationship between SPARC and ROS in VSMCs has not been reported to date.
Emerging evidence has reported that SPARC and TGF-β1 regulate each other in other cell lines (Shubham and Mishra 2012; Tumbarello et al. 2016). TGF-β1 up-regulates the expression of NOX4 in VSMCs (Liu et al. 2016), and the major sources of ROS in VSMCs are mainly from NOX1 and NOX4 (two kinds of NADPH-oxidases) during the pathological conditions (Lassegue and Clempus 2003). Therefore, we speculated that in human brain vascular smooth muscle cells (HBVSMCs), SPARC might also assist in up-regulating the expression of NOX4 through TGF-β1 to produce ROS. This, in turn, induces inflammatory phenotypic transformation and apoptosis of smooth muscle cells. In addition, the smooth muscle cells in the media of cerebral vessels and their extracellular matrix provide structural and functional support for the cerebral artery, and these are considered to play a key role in the formation and development of aneurysms. Therefore, HBVSMCs were used as a model of arterial media and directly supplemented the recombinant SPARC protein in vitro to explore the relationship between oxidative stress injury, pro-inflammatory behavior, and apoptosis between SPARC and HBVSMCs. The results might, in turn, provide us with the knowledge and method for prevention and treatment of IAs.

Materials and Methods

Cell Culture and Stimulation with SPARC

Primary HBVSMCs were purchased from ScienCell Research Laboratories (Cat No.1100, Carlsbad, CA), and were cultured in the recommended smooth muscle cell culture medium (SMCM, Cat.No.1101, ScienCell Research Laboratories, Carlsbad, CA) at 37 °C under 95% air and 5% CO₂. After the cells reached the fusion stage, they were digested by trypsin for subculturing, and 3rd to 6th generation HBVSMCs were used for the experiments. The SPARC in plasma was 1.5–10 μg/ml (Serebruany et al. 1999) under pathological conditions. Thus, 6 μg/ml SPARC (Genscript, Jiangsu, China) was used to simulate overexpression in cells, and the effect at different time periods (0, 3 h, 6 h, 12 h, 18 h, and 24 h) was detected. When needed, the cells were pretreated with NAC 10 μM for 1 h or SD-208 1 μM for 2 h.

Western Blot

The cells were seeded in 6-well plates, and then treated in specific experiments. The total protein was then extracted from VSMCs using an extraction kit (BestBio, Shanghai, China) according to the manufacturer’s instructions. The total proteins were separated by 8–12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then were transferred onto a polyvinylidene fluoride membrane. The membrane was sealed with 5% skim milk and incubated with a specific primary antibody at 4 °C overnight. This was followed by washing and further incubation with a horseradish peroxidase-labeled secondary antibody (CST, 1:500) at room temperature for 2 h. Immunoassay bands were developed using Millipore’s enhanced chemiluminescence ECL, and detected using ChemiDoc XRS+ (Bio-Rad; Hercules, CA, USA) chemiluminescence. Protein expression was quantitatively analyzed using ImageJ software. The main antibodies were as follows: NOX4 (1:500, 20,536-1-AP, Proteintech), NOX1 (1:500, 17,772-1-AP, Proteintech), TGF-β1 (1:500, 21,898-1-AP, Proteintech), matrix metalloproteinase-9 (MMP-9, 1:500, 10,375-2-AP, Proteintech), MCP-1 (1:1000, ab73680, ABCAM), SPARC (1:500, 15,274-1-AP, Proteintech), Bax (1:1000, 50,599-2-Ig, Proteintech), Bcl-2 (1:1000, 12,789-1-AP, Proteintech), Caspase3 (1:500, 19,677-1-AP, Proteintech), cleaved caspase 3 (1:1000, #9664, CST), GAPDH (1:5000, 10,494-1-AP, Proteintech), and β-actin (1:5000, No.20536-1-AP, Proteintech). The protein level was used as protein loading control. All experiments were conducted thrice.

Detection for Intracellular ROS

The superoxide levels in the living cells were measured by a superoxide fluorescence kit (enz-51,012, Enzo LifeScience, Farmingdale, NY), and intracellular hydrogen peroxide levels were measured by a hydrogen peroxide detection kit (MAK164, sigma-aldrich) (green fluorescence). In short, the cells were treated, and incubated with Hoechst 33342 2 μg/ml at 37 °C for 15 min according to the manufacturer’s instructions for staining the nucleus. Fluorescence intensity was measured by a fluorescence microscope, and photos were taken by a DP71 charge coupled device (CCD) digital camera (Olympus, Waltham, MA, USA). The images were analyzed by flow cytometry (BD Accuri™ C6 PLUS, BD Biosciences). All experiments were thrice-repeated.

Detection for Mitochondrial Membrane Potential (ΔΨm)

The cells were then inoculated in 8-well chamber slides(μ-Slide 8) at a density of 5×10³ cells/chamber and cultured for 18 h after specific treatment. The changes in mitochondrial membrane potential were measured using a JC-1 mitochondrial membrane potential assay kit (Beyotime, Shanghai, China) according to the instructions. In healthy cells with high mitochondrial membrane potential, JC-1 spontaneously formed J-aggregation in the mitochondria, emitting red fluorescence. While in unhealthy cells, the mitochondrial membrane potential...
decreases, and JC-1 was released from the mitochondria into cytoplasm as a monomer, generating green fluorescence. Therefore, mitochondrial membrane potential was indicated by the ratio of red to green fluorescence intensity. Staining was performed with JC-1, and the red and green fluorescence changes were detected using a laser confocal microscope (Leica TCS SP8) to obtain images. All experiments were conducted in triplicates.

**SiRNA Transfection**

The interference sequences of targeted genes designed by Shanghai Gema Biological Co., LTD. were used to knockout the expression of SPARC, Nox4 or TGF-β1 in HBVSMCs. Three interference sequences were designed for each target gene. The siRNA target sequences are listed in Table 1. According to the instructions, siRNA was instantaneously transfected into fused cells by Lipofectamine using random sequence siRNA (Scr siRNA) as a negative control. Briefly, the corresponding siRNA was mixed with premixed lipid complex (Lipofectamine 2000, Invitrogen, Carlsbad, CA, USA) and incubated at room temperature for another 20 min. The cells and sequence transfection medium were incubated for 6 h and then cultured with normal medium. After transfection for 48 h, the knockout efficiency was analyzed using western blotting, and the siRNA with the highest interference efficiency was selected from the three candidates.

**Statistical Analysis**

The data were expressed as mean ± SE and analyzed using SPSS19.0 software. Unpaired t-test or ANOVA was used to analyze the data between two groups and multiple groups. *p* < 0.05 was considered to be statistically significant.

**Table 1**  Target sequences of siRNA

| Genes  | Serial number | Target sequences Sense (5′-3′) | Target sequences Antisense (5′-3′) |
|--------|---------------|--------------------------------|----------------------------------|
| NOX4   | 540           | CCGGCUGCAUACGUCUUAATT          | UUAAGAUGCUGCAGCGGGTT             |
|        | 990           | GGAAGAGCGCAAGAGAGGAATT         | UUGGAAUGCGGCUUUCCTT              |
|        | 1132          | CCAAUGUGCCAACACUUUATT          | AAGAGGUGUUCGCCACAUUGTT           |
| TGF-β1 | 1168          | CCACCAUAAUUGCCAGAAATT          | UCAAUGGCAUUAGGUGGGTT             |
|        | 1410          | GACUCGCGAAGUGGGUUAATT          | AUAACCAUCUGCGAGCUTT              |
|        | 1582          | GCGGCUGCAUUGGGGAACUUATT        | GUCUCCCAUCAGCGACGGTT             |
| SPARC  | 306           | GCAUCGGACAGAAGGUAATT           | AUAAUCUUCUGUGAGUUGCTT            |
|        | 448           | GCAG                           | AUAACCAUCUGCGACGGTT              |
|        | 1073          | AGGUAGCACUGGGUGUAATT           | UUGUUCAUACACUCUGCCTT             |
| GAPDH  | control       | UGACCUCAACUACUGGUUTT           | AUAACCAUACUGGUUGGUAGGCATT        |
and the expression trend was consistent with that of fluorescence intensity of $H_2O_2$, indicating that the generation of $H_2O_2$ was positively correlated with the expression of NOX4. To further confirm that $H_2O_2$ was derived from NOX4, the cells were transfected with siRNA to reduce the expression of NOX4. As shown in Fig. 3a, b, compared with the control group, the fluorescence intensity of $H_2O_2$ was significantly decreased after re-stimulating SPARC after NOX4 knockout. This showed that NOX4 was the main contributor for the production of $H_2O_2$ induced by SPARC in HBVSMCs.

Fig. 1 SPARC induced accumulation of ROS in HBVSMCs. HBVSMCs were treated with SPARC (6 $\mu$g) at different time points (0, 3 h, 6 h, 12 h, 18 h, and 24 h). Fluorescence microscopy showed (a) $H_2O_2$ fluorescence intensity with MAK164 and (b) superoxide fluorescence intensity with enz-51,012. Flow cytometry analysis of (c) intracellular $H_2O_2$ levels with MAK164 and (d) intracellular superoxide levels with enz-51,012. Nuclei were stained with Hoechst 33342, and the original magnification was $100\times$ ($n=3$ per group)

Fig. 2 Effect of SPARC on HBVSMCs. a The expression levels of NOX1 and MCP-1 and (b) the expression levels of TGF-$\beta_1$, NOX4, MMP9 were detected using western blotting, HBVSMCs were treated using SPARC (6 $\mu$g) at different time points. $^*p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$ vs control, $n=3$ per group
(a) Scr siRNA  NOX4 siRNA

NOX4

GAPDH

(b) Hoechst  \( \text{H}_2\text{O}_2 \)  Merge

Scr siRNA

NOX4 siRNA

Scr siRNA + SPARC

NOX4 siRNA + SPARC
SPARC Induced NOX4 Expression and Regulated NOX4 Activity through TGF-β1 in HBVSMCs

The expression of TGF-β1 in HBVSMCs was tested after SPARC stimulation. As shown in Fig. 2b, the expression of TGF-β1 was also increased in a time-dependent manner, and the time to increase was earlier than that of NOX4, suggesting that SPARC might first induce the production of TGF-β1 and then up-regulate the expression of NOX4 through TGF-β1. To further verify this, a TGF-β1 inhibitor SD-208 (HY13227, MCE, China) 1 μm/L was used to pre-treat the cells for 2 h, and then they were stimulated with SPARC for 18 h. The

![Fig. 3](image1.png)

**Fig. 3** The effect of SPARC on H$_2$O$_2$ level of HBVSMCs after NOX4 knock-down. a The expression level of NOX4 after knock-down. b The fluorescence images of H$_2$O$_2$ in different groups were observed by MAK164 under an inverted fluorescence microscope (original magnification 100×), and nuclei were stained with Hoechst 33342. The cells with or without SPARC 6 μg treatment for 18 h after NOX4 knockout and without knockout. **p < 0.01 vs the indicated groups, n = 3 per group

![Fig. 4](image2.png)

**Fig. 4** The effect of SPARC on HBVSMCs after TGF-β1 was inhibited. a The relative protein expression levels of NOX4, MMP9, and MCP-1 were detected using western blotting in different groups. b quantification of NOX4, MMP9, and MCP-1 proteins from panel (a). c fluorescence images of H$_2$O$_2$ in both groups of SPARC and SPARC+SD-208. Cells were pretreated for 2 h with or without SD-208 1 μm/L and then with or without SPARC 6 μg/ml treatment for 18 h. *p < 0.05 and **p < 0.01 vs the indicated groups, n = 3 per group
results showed that the expression of NOX4 was decreased significantly after TGF-β1 was suppressed (Fig. 4a, b), and this was accompanied by a decrease in H₂O₂ products (Fig. 4c). In addition, after significant knockout of the TGF-β1 gene (Fig. 5a), the expression of NOX4 was also decreased (Fig. 5b). To verify that the up-regulation of NOX4 expression is the direct effect of TGF-β1, HBVSMCs were directly stimulated by recombinant TGF-β1 at 1 ng (AmyJet Scientific, Wuhan, China) for 18 h (Sun et al. 2014). The significantly up-regulated expression of NOX4 was accompanied by a significant increase in H₂O₂ (Fig. 6a), while NOX1 expression and superoxide products showed no significant changes (Fig. 6b, c). These results suggested a positive correlation between NOX4 and TGF-β1 and that SPARC induced intracellular oxidative stress by up-regulating NOX4 by TGF-β1 to produce ROS (H₂O₂).

Next, we also found that TGF-β1 directly stimulated HBVSMCs and increased the expression up-regulation of SPARC (Fig. 6d). We suspected that SPARC might play a regulatory role in the up-regulation of NOX4 expression induced by TGF-β1. As a result, the expression of TGF-β1 and NOX4 was observed after the expression of SPARC was decreased due to knockdown of the SPARC gene. As shown in Fig. 7a, b, the expression of TGF-β1 and NOX4 was also decreased after SPARC knockout. After that, following the stimulation of TGF-β1 (1 ng) for 18 h, the decreased expression of NOX4 was almost reversed. However, the fluorescence intensity of H₂O₂ products in the SPARC interference group was significantly inhibited (Fig. 7c), indicating that the H₂O₂ products were not reversed. This indicated that the decrease in NOX4 expression was not the direct effect of SPARC knockdown but occurred due to the result of the corresponding decrease in TGF-β1 expression. However, a lack of SPARC affected the bioactivity of NOX4 induced by TGF-β1 (release of H₂O₂).

The above experiments proved that SPARC could induce the expression of TGF-β1, and TGF-β1, in turn, induces the expression of SPARC. In addition, SPARC
expression was decreased correspondingly after TGF-β1 knockdown (Fig. 5b), and TGF-β1 expression was decreased correspondingly after SPARC knockdown. This also indicated that SPARC and TGF-β1 interacted with each other in HBSVMCs in a positive feedback regulation.

**ROS Induced by SPARC Promoted Phenotypic Transformation of HBVSMCs**

During the early stage of aneurysm (Maradni et al. 2013), VSMCs showed inflammatory phenotypic transformation with increased synthesis of MMP9 and MCP-1.
Therefore, the expression of MMP9 and MCP-1 in HBVSMCs after stimulating exogenous SPARC protein (6 μg/ml) was detected. The results showed that the expression of MMP9 and MCP-1 was also increased in a time-dependent manner and reached the peak within 18 h. This was consistent with the expression trend of NOX4 (Fig. 1a, c, d). This suggested that SPARC induced the transformation into the inflammatory phenotype. In addition, after treatment with NOX4 knockout (Fig. 8) and TGF-β1 inhibition (Fig. 4a, b), the high expression of MMP9 and MCP-1 induced by SPARC was reduced, indicating the involvement of TGF-β1/NOX4. To verify whether ROS was involved in the SPARC-induced expression of MMP9 and MCP-1, the cells were pretreated with 1 mM NAC (ab143032, Abcam) for 1 h, followed by incubation with SPARC for 18 h. As shown in Fig. 9a, b, NAC significantly eliminated the fluorescence intensity of H2O2 and significantly inhibited the increase of MMP9 and MCP-1 expression. This means that the H2O2 is involved in the process of SPARC inducing the expression of MMP9 and MCP-1. These results suggested that ROS produced by SPARC through the TGF-β1/NOX4 pathway induces inflammatory phenotypic transformation of HBVSMCs.

To observe the effect of SPARC on the apoptosis of HBVSMCs, the expression of apoptotic proteins was detected using western blotting after stimulating the HBVSMCs by exogenous SPARC protein (6 μg/ml) for 18 h. As shown in Fig. 9c, the expression of anti-apoptotic protein (Bcl-2) was significantly down-regulated, while the pro-apoptotic protein (Bax), caspase3, and cleaved caspase3 were significantly up-regulated. The change in ΔΨm in the mitochondria induced by SPARC was detected using JC-1 as a fluorescent probe. These results indicated that SPARC significantly improved the ratio of the intracellular JC-1 monomer (Fig. 9d), suggesting the inhibitory effect of SPARC on ΔΨm. Because Bcl-2 family proteins (such as Bax, Bcl2, etc.) regulate apoptosis by controlling the permeability of the mitochondrial membrane and by releasing the cytochrome, the decreased mitochondrial membrane potential is also considered as a landmark event in the early apoptotic stage. Therefore, these results suggested that SPARC induced the apoptosis of HBVSMCs in the mitochondrial pathway. To verify whether the ROS participates in the apoptosis of HBVSMCs induced by SPARC, the cells were pretreated with NAC 1 mM for 1 h and then incubated with SPARC for 18 h to observe the expression of related apoptotic proteins and mitochondrial Δm. It was found that the up-regulation of the pro-apoptotic protein induced by SPARC and the down-regulation of anti-apoptotic protein (Bcl2) were reversed after ROS clearance (Fig. 9c), and the inhibitory effect on Δm (Fig. 9d) was also weakened. These results showed that SPARC induced apoptosis by inducing ROS accumulation.
Discussion

SPARC is highly expressed in intracranial aneurysmal tissues, and thus we explored its role in detail. In vitro, it was found that SPARC induced up-regulation of NOX4 expression through TGF-β1, resulting in the accumulation of H$_2$O$_2$ and induction of inflammatory phenotypic transformation and apoptosis in HBVSMCs.

Unlike other smooth muscle cells, VSMCs showed plasticity. In response to various stimuli of environmental factors, VSMCs perform phenotypic regulations, showing a proinflammatory, matrix remodeling, and dedifferentiation phenotype. This is characterized by increased expression of inflammatory factors and MMPs. In this study, it was found that SPARC induced over-expression of MMP9 and MCP-1 in HBVSMCs by NOX4-derived H$_2$O$_2$ for phenotypic transformation and apoptosis of HBVSMCs induced by SPARC. a The levels of H$_2$O$_2$ in different groups were observed under a fluorescence microscope (original magnification 100×). b, c The relative protein expression levels of apoptosis and inflammation were detected using western blotting. d The photomicrographs of mitochondrial membrane potential were observed with JC-1 using a confocal microscope. The cells were pretreated for 1 h with or without NAC 1 mM and then with or without SPARC 6 μg/ml treatment for 18 h. *p < 0.05 and **p < 0.01 vs the indicated groups, n = 3 per group.
transformation of inflammation and matrix remodeling. Excessive MMP-9 has been confirmed in IAs (Kim et al. 1997). MMP-9 forms aneurysms by degrading type 4 collagen, proteoglycan core protein, and elastin, and the incidence of aneurysms in MMP-9 knockout mice showed a significant reduction (Thompson et al. 1995). MCP-1 causes inflammation by recruiting monocytes/macrophages into the vessel wall. In an animal aneurysmal model (Aoki et al. 2009), the expression of MCP-1 in the arterial wall was increased with the progression of aneurysm and is accompanied by the accumulation of macrophages. MCP-1β−/− mice showed a significant decrease in macrophage recruitment to the arterial wall. From the above literature, it is observed that the inflammatory phenotypic changes of cerebrovascular smooth muscles play an important role in the formation and development of IAs. Therefore, SPARC promotes the development of IAs by inducing phenotypic transformation of cerebrovascular smooth muscle cells.

Sakaki et al. (Sakaki et al. 1997) first observed the characteristics of apoptosis in human IAs. Subsequent studies have also confirmed the apoptosis of VSMCs in the intima of the arterial wall (Guo et al. 2007). The loss of VSMCs reduced the strength of blood vessel walls, and reduced the synthesis of collagen, which thus loses the self-repair function. This is the main characteristic of growth and final rupture in IAs (Kassam et al. 2004). Our results showed that SPARC induced ROS by promoting apoptosis of the mitochondrial pathway through NOX4 in cerebrovascular smooth muscle cells. The relationships between NOX4 expression and vascular disease progression, ROS level and apoptosis have also been reported. For example, under normal growth conditions, NOX4 overexpression and H2O2 generation in aortic VSMCs increased the apoptosis (Xu et al. 2014). The 7-acetone cholesterol pre-treated aortic VSMCs also induced apoptosis of VCMCs by increasing NOX4 expression (Peduzzi et al. 2004). These data suggested that increased NOX4 expression in VSMCs increased the susceptibility to apoptosis, which was consistent with our current study results.

TGF-β is the main positive stimulator of Nox4 (Liu et al. 2016; Martin-Garrido et al. 2011; Xu et al. 2014), and stimulation intensity is parallel to the increased ROS production. The results showed that TGF-β1 also induced the expression and activation of NOX4 in cerebrovascular smooth muscle cells. In addition, it is clarified that SPARC is essential in the activation of NOX4 by TGF-β. However, the exact mechanism of SPARC enhancing NOX4 activity should be further studied.

The autocrine positive feedback loop between SPARC and TGF-β1 was proved for the first time in HBSMCs. Stabilization of the positive feedback autocrine loop is beneficial for maintaining cell function. When a few factors disrupt the local autocrine function balance, it was speculated that TGF-β1 and SPARC amplify their own effects continuously due to self-perpetuating cyclic behavior. As a result, the phenotypic transformation of cerebrovascular smooth muscle cells was induced continuously, the degradation of vascular wall was caused by secreting degradation of MMPs, and the inflammation was caused by the secretion of MCP-1 to recruit macrophages into the vascular wall. Besides the secretion of various pro-inflammatory cytokines by macrophages and other inflammatory cells, they also release MMP to amplify the inflammatory response. Accumulation of ROS products leads to oxidative stress injury and cell apoptosis. The above processes lead to aneurysm formation. This suggested that the disturbance of the MMP-9 level leads to matrix destruction and IAs formation, and it is local rather than systemic (Sakaki et al. 1997), which, in turn, might be related to a local autocrine disorder of blood vessels.

In conclusion, this study demonstrated that SPARC induces phenotypic transformation and apoptosis in HBSMCs through the TGF-β1-Nox4-ROS pathway, promoting the development of IAs. Controlling the SPARC signaling pathway might block the progression of aneurysm. The next step involves establishing an animal model based on the inhibitory characteristics of SPARC knockout to further verify the pathogenesis of SPARC in aneurysm development. Therefore, SPARC may assist in studying IAs, or it may become a potential target for the prevention and treatment of IAs.

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Author Contributions XT conducted experiments and wrote the manuscript. TL and WZ were responsible for the experimental design. SZ analyzed the data and revised the file. FL provided technical support. YW strictly reviewed the manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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