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SOCS3, Transcriptionally Activated by *NR4A1*, Induces Apoptosis and Extracellular Matrix Degradation of Vaginal Fibroblasts in Pelvic Organ Prolapse

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Background: Pelvic organ prolapse (POP) is a common gynecological chronic disorder. Human vaginal fibroblasts (HVFs) that maintain the integrity of vaginal wall tissues are essential for keeping pelvic organs in place. Apoptosis and the degradation of the extracellular matrix in HVFs contribute to the progression of POP. The cytokine signal transduction inhibitor 3 (*SOCS3*) exerts significant regulatory effects on cell signal transduction pathways, thereby affecting various pathological processes.

Aims: To explore the role and mechanism of *SOCS3* on HVFs in the context of POP.

Study Design: In vitro cell lines and human-sample study.

Methods: Anterior vaginal wall tissues were obtained from POP or non-POP patients for the analysis of *SOCS3* expression. HVFs were isolated from the vaginal tissues of POP patients, and *SOCS3* was either overexpressed or knocked down in HVFs via lentivirus infection. Subsequently, the biological function and mechanism of *SOCS3* in HVFs were investigated.

Results: *SOCS3* was highly expressed in the vaginal tissues of POP patients compared to non-POP patients. Functionally, the overexpression of *SOCS3* suppressed cell viability while promoting cell apoptosis in HVFs. The overexpression of *SOCS3* also accelerated extracellular matrix degradation (decreasing collagen I, collagen III, and elastin, and increasing *MMP2* and *MMP9*). In terms of mechanism, *NR4A1* transcriptionally activated *SOCS3* by binding to its promoter. Furthermore, rescue experiments revealed that *SOCS3* knockdown hindered *NR4A1* overexpression-induced cell apoptosis and extracellular matrix degradation in HVFs.

Conclusion: *SOCS3* mediated the apoptotic and extracellular matrix degradation effects of *NR4A1* on HVFs, underlining that the restraining of the *SOCS3* expression may be a promising strategy for POP treatment.

INTRODUCTION

Pelvic organ prolapse (POP), a common gynecological disease among females, is characterized by the protrusion of the pelvic organs (vagina, uterus, bladder, and/or rectum) into or out of the vaginal canal.[1] Treatment options for POP include pelvic floor muscle training, vaginal pessaries, and reconstructive pelvic surgery. However, the therapeutic outcomes are often unsatisfactory and relapse is a common occurrence.[2,3] Therefore, it is imperative to elucidate the pathogenesis of POP to develop effective treatments for this condition.

The most common form of POP is anterior vaginal wall prolapse, commonly known as cystocele. In the vaginal wall, connective tissues provide the supportive strength for the pelvic floor.[4] The connective tissue layer consists of an extracellular matrix (ECM), which obtains its strength from collagen I, collagen III, and elastin.[5] Vaginal fibroblasts control ECM production and remodeling, by generating anabolic proteins, like collagens, and activating catabolic enzymes, such as matrix metalloproteinases (*MMPs*), thus modifying their surrounding matrix.[6] In patients with POP, fibroblast dysfunction leads to damaged collagen metabolism and an unbalanced disturbed ECM production.[7,8] Additionally, some studies have confirmed increased fibroblast apoptosis in POP.[9] However, there is a limited number of studies that focus on the underlying mechanism. Hence, enhancing the functions of fibroblasts emerges as a viable approach to impede the progression of POP.

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The cytokine signal transduction inhibitor 3 (*SOCS3*), one of the SOCS family proteins, interacts with various activated proteins of the tyrosine kinase signaling pathway.[10] *SOCS3* suppresses cell proliferation and its deletion inhibits myocardial apoptosis and fibrosis.[11,12] Moreover, the downregulation of *SOCS3* promotes collagen release and aggravates fibrosis in murine models.[13] Database analysis showed an upregulation in *SOCS3* expression in the anterior vaginal wall tissue of patients with POP. Therefore, *SOCS3* may participate in the development of POP.

Human nuclear receptor 4A 1 (*NR4A1*) is an orphan nuclear receptor associated with fibrosis of multiple organs. *NR4A1* limits fibrotic diseases by regulating transforming growth factor-β (*TGF-*β) signaling.[14] Hiwatashi et al.[15] reported that *NR4A1* inhibits vocal fold fibrosis, while *NR4A1* knockdown promotes collagen I expression in fibroblasts. *NR4A1* also participates in the pathological progression of POP by promoting the apoptosis of fibroblasts.[16] Furthermore, 9-AA, an agonist of *NR4A1*, enhances the expression levels of *SOCS3*.[17] Based on these published results, we hypothesize that the *NR4A1*/*SOCS3* axis may play a role in the progression of POP.

In this study, we investigated the function and mechanism of *SOCS3* in POP. We found that *SOCS3* expression was higher in the vaginal tissues of POP patients than that in non-POP patients. *SOCS3* overexpression induced cell apoptosis and ECM degradation in human vaginal fibroblasts (HVFs) from patients with POP. Additionally, we detected that *SOCS3* was transcriptionally activated by *NR4A1*, and the effect of *SOCS3* on HVFs was regulated by *NR4A1*. This study provides evidence for the role of *SOCS3* in POP.

MATERIALS AND METHODS

Tissue samples collection

Anterior vaginal wall tissues were collected from POP patients (n = 36, age range: 47-74) and non-POP patients (n = 18, age range: 39-57). All patients participating in the study signed the informed consent. This study was conducted following the Declaration of Helsinki, and approved by the Shengjing Hospital of China Medical University Ethics Committee (2022PS040K).

Fibroblast culture and identification

Primary HVFs were isolated from anterior vaginal wall tissues and cultured as described previously.[18,19] The cells were cultured in Dulbecco's modified Eagle's medium (Servicebio, China) supplemented with 10% fetal bovine serum (Sijiqing, China) at 37 °C and 5% CO_2. Cells were passaged and expanded until reaching 60% confluence, and those up to the fourth passage were used for subsequent experiments. Immunofluorescence was performed to identify cells at the fourth passage using markers for vimentin (a marker for fibroblasts), cytokeratin (a marker for epithelial cells), and desmin (a marker for smooth muscle cells).

Lentivirus infection

The coding sequences of *SOCS3* or *NR4A1* were inserted into pLVX-IRES-puro (Fenghbio, China) to generate *SOCS3* overexpression lentivirus (LV-*SOCS3*) or *NR4A1* overexpression lentivirus (LV-*NR4A1*). The map of pLVX-IRES-puro is shown in Figure S2a. Additionally, *SOCS3*-targeted shRNA was inserted into pLVX-shRNA1 (Fenghbio, China) to establish sh*SOCS3* lentivirus (LV-sh*SOCS3*). The map of pLVX-shRNA1 is shown in Figure S2b. The *SOCS3* shRNA sequence is as follows: 5′-GCTTCGACTGCGTGCTCAAGC-3′, LV-SOCS3, LV-NR4A1, LV-shSOCS3, and their negative controls (NC) were subsequently packaged in HEK293T cells. The lentiviral-containing supernatant was harvested and filtered. HVFs were then infected with lentivirus-containing supernatants at a multiplicity of infection of 100 and incubated at 37 °C with 5% CO_2.

Statistical analysis

The data were analyzed using GraphPad Prism 8.0 (GraphPad Software, USA). All data were presented as the mean ± standard deviation. T-tests were employed to compare data between two groups, while one-way ANOVA with Bonferroni post-hoc test was used for comparing data involving more than two groups. $P < 0.05$ was deemed statistically significant.

For detailed methods, please see the Supplementary material.

RESULTS

SOCS3 is highly expressed in the vaginal tissues of POP patients

Built on the differential expression analysis of the gene expression omnibus database, we found 265 upregulated and 13 downregulated genes in patients with POP. Subsequently, GO enrichment analysis of differential expression genes (*DEGs*) revealed an association among POP, ECM, and cell apoptosis (Figure 1a). In these *DEGs*, *SOCS3* knockdown inhibits cell apoptosis and promotes collagen release,[12,13] suggesting that *SOCS3* may be involved in the progression of POP. In addition to the GSE12852 dataset, the high expression of *SOCS3* in POP tissues was observed in the GSE53868 dataset (Figure 1b). To verify our conjecture, we collected vaginal tissues from POP and non-POP patients. Then, Masson trichrome staining and TUNEL staining were performed to explore the histomorphological differences. The results of Masson trichrome staining showed decreased collagen (blue) in tissues of POP patients compared with non-POP patients (Figure 1c). TUNEL staining revealed a higher incidence of apoptosis in tissues from POP patients (Figure 1d). These results indicated more prominent collagen degradation and apoptosis in patients with POP, consistent with previous reports[7] and our bioinformatics analysis. To further investigate the role of *SOCS3* in POP, we detected *SOCS3* mRNA expression in vaginal tissues. The results showed that *SOCS3* was significantly upregulated in patients with POP (Figure 1e). Western blot analysis further verified the upregulation of *SOCS3* at the protein level (Figure 1f). Moreover, IHC and the histologic score (H-score) were performed to further verify the expression pattern of *SOCS3* in POP. The results showed higher *SOCS3* expression levels in the POP group compared with the non-POP group (Figure 1g-h). Furthermore, we analyzed the association between the expression levels of *SOCS3* and patient characteristics in Table S1;

however, no correlation was observed. These results suggested that *SOCS3* was upregulated in the vaginal tissues of patients with POP.

Morphological identification of HVFs

Primary HVFs obtained from the vaginal tissues of POP patients were cultured and collected for microscopic observation (Figure S1a). Then, the fourth passage of HVFs was subjected to immunofluorescence staining to identify the phenotype using specific biomarkers (Figure S1b). The results revealed high cytoplasmic expression of vimentin (a marker of fibroblasts) in the fourth passage of HVFs. In contrast, no significant increase in the expression of cytokeratin (a marker of epithelial cells) and desmin (a marker of smooth muscle cells) was observed. The above results illustrated that cells isolated from vaginal tissues were mainly fibroblasts without the contamination of epithelial and smooth muscle cells.

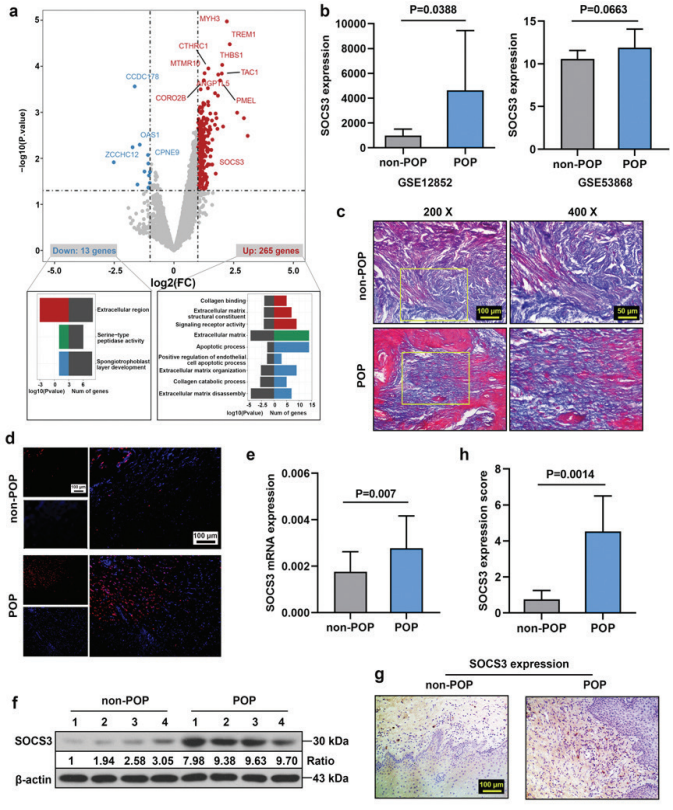

FIG. 1. *SOCS3* is upregulated in the anterior vaginal wall tissues of patients with pelvic organ prolapse (POP). (a) A volcano plot of differentially expressed genes (*DEGs*) and GO enrichment analysis of *DEGs* based on the GSE12852 dataset. (b) The expression level of *SOCS3* in anterior vaginal wall tissues and uterosacral ligament tissues of patients with POP from gene expression omnibus (GEO). (c) Collagen deposition in anterior vaginal wall tissues of POP and non-POP patients was detected by Masson trichrome stain. (d) Apoptotic protein expression was detected by TUNEL assay. (e) The mRNA expression of *SOCS3* was detected by RT-qPCR. (f) The protein expression of *SOCS3* was detected by western blot. (g) *SOCS3* expression was detected by immunohistochemistry (IHC), and *SOCS3* expression scores were shown in (h). The results were presented as the mean ± SD.

SD, Standard deviation

SOCS3 overexpression inhibits viability and induces apoptosis in HVFs

Considering the increased expression of *SOCS3* in POP, the function of *SOCS3* in POP was further investigated. *SOCS3* was overexpressed in the fourth passage of HVFs through lentivirus infection, and the gene overexpression was confirmed with RT-qPCR and western blot (Figure 2a, b). Subsequently, the results from the CCK-8 assay showed that *SOCS3* overexpression inhibited the cell viability of HVFs (Figure 2c). Additionally, a significantly higher apoptosis rate was observed in *SOCS3* overexpression groups compared to the control groups (Figure 2d). The TUNEL assay further verified the above results, with more TUNEL-positive cells observed in *SOCS3* overexpression HVFs (Figure 2e). In addition, pro-apoptotic protein Bax and cleaved-caspase-3, as well as anti-apoptotic protein Bcl-2, were detected by western blot. The expression levels of Bax and

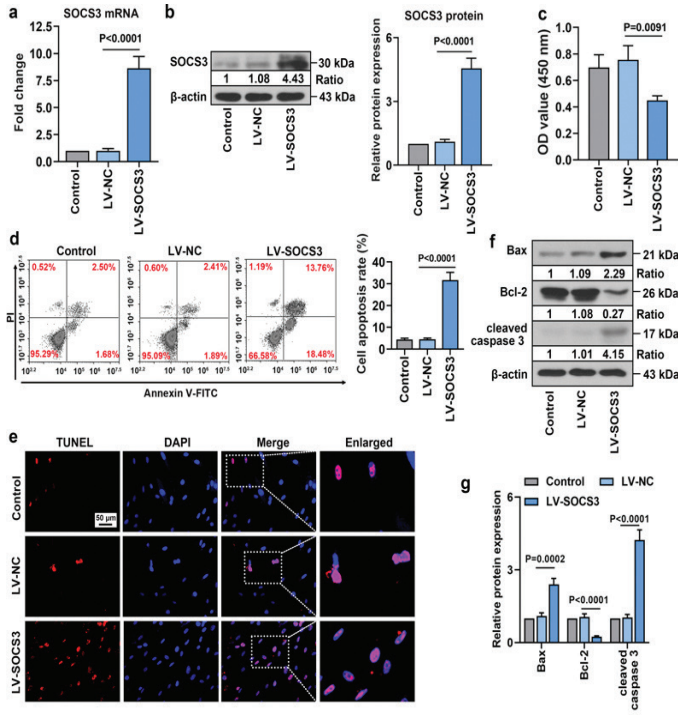

FIG. 2. *SOCS3* overexpression inhibits viability and induces apoptosis in human vaginal fibroblasts (HVFs). HVFs isolated from patients with POP were infected with *SOCS3* overexpression lentivirus (LV-SOCS3) or the negative control (LV-NC), 48 h postinfection, (a) the mRNA level was detected by RT-qPCR. (b) The protein expression level was detected by western blot. (c) Cell viability was detected by CCK-8 assay. (d) Cell apoptosis was detected by flow cytometry. (e) Cell apoptosis was detected by TUNEL staining. (f) The protein expression levels of Bax, Bcl-2, and cleaved-caspase 3. (g) Quantification of the protein levels of Bax, Bcl-2, and cleaved-caspase 3. The results were presented as the mean ± SD.

SD, Standard deviation

cleaved-caspase 3 increased, whereas that of Bcl-2 decreased, as observed after *SOCS3* overexpression (Figure 2f, g). These results suggested that *SOCS3* overexpression inhibited viability and induced apoptosis of HVFs.

SOCS3 overexpression induces ECM degradation in HVFs

Previous experiments validated the significance of collagen degradation as a prominent characteristic in patients with POP.[20] Thus, to explore whether *SOCS3* participates in the regulation of ECM components, the protein expression of collagen I, collagen III, and elastin was analyzed. The results revealed that *SOCS3* overexpression reduced the expression of collagen I, collagen III, and elastin (Figure 3a). Besides, we detected the protein expression of *MMP2* and *MMP9*. Collagen-degrading *MMPs* are involved in ECM remodeling processes, as shown in Figure 3b, increased expression of *MMP2* and *MMP9* was observed after *SOCS3* overexpression. Moreover, the expression of components related to collagen synthesis pathways, including *TGF-β*, Smad2/3, and phosphorylated Smad2/3 (p-Smad2/3) was detected.[21] We found that *TGF-β1* and p-Smad2/3 showed low expression in the *SOCS3* overexpression HVFs, and the expression of Smad2/3 was almost unchanged (Figure 3c, d). Together, all the signs indicated that the upregulation of *SOCS3* caused ECM degradation in HVFs.

SOCS3 is transcriptionally activated by *NR4A1* via binding the *SOCS3* promoter

NR4A1 accelerates the pathological process of POP by inducing fibroblast apoptosis.[16] To investigate the relationship between *SOCS3* and *NR4A1*, we constructed *NR4A1*-overexpressing lentivirus (LV-*NR4A1*) to infect HVFs. A clear rise of *NR4A1* mRNA and protein expression was shown in HVFs (Figure 4a, b). *NR4A1* overexpression was accompanied by increased *SOCS3* mRNA and protein levels

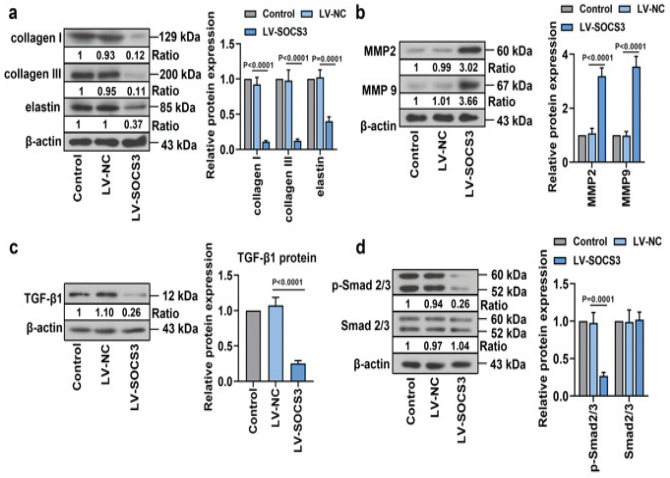

FIG. 3. *SOCS3* overexpression induces ECM degradation in HVFs. (a) The protein expression of collagen I, collagen III, and elastin. (b) The protein expression of *MMP2* and *MMP9*. (c) The protein expression of *TGF-β1*. (d) phosphorylated Smad2/3 (p-Smad2/3) and Smad2/3. The results were presented as the mean ± SD.

SD, Standard deviation

(Figure 4c, d), suggesting that *SOCS3* expression might be regulated by *NR4A1*. Based on the above results, we predicted *NR4A1*-binding sites on the *SOCS3* promoter (Figure 4e), and the interaction between *NR4A1* and *SOCS3* was further confirmed by the dual luciferase reporter assay. As shown in Figure 4f, when fragment A (-1948, -1585 bp, containing four sites) or fragment B (-1585, -643 bp, containing two sites) was truncated, the luciferase activity was decreased. Moreover, when there was only fragment C (-643 to +36, containing one site), the promoter activity was increased compared with the control vector. These results indicated that the regions from -1948 to -1585 bp, -1585, -643 bp, and -643 to +36 bp contained binding sites of *NR4A1*. The above results suggest that *NR4A1* transcriptionally activates *SOCS3* by binding to its promoter.

NR4A1 overexpression induces cell apoptosis and ECM degradation in HVFs

To further verify the effects of *NR4A1* on POP, we assessed *NR4A1* expression in vaginal tissues from POP and non-POP patients. Higher *NR4A1* expression at the mRNA level was observed in patients with POP (Figure 5a). IHC staining and H-score further confirmed that *NR4A1* expression was higher in patients with POP (Figure 5b, c). However, the expression level of *NR4A1* had no association with the characteristics of POP (Table S2). Subsequently, *NR4A1* was overexpressed in the HVFs via lentivirus infection. *NR4A1* overexpression suppressed cell viability (Figure 5d) and

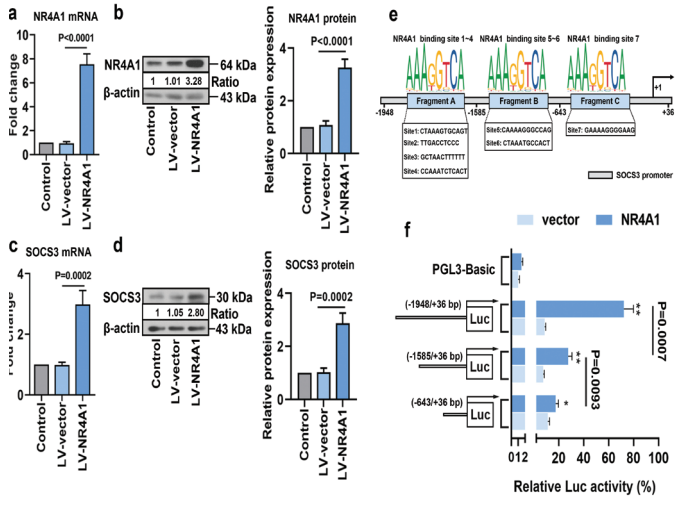

FIG. 4. *SOCS3* is transcriptionally activated by *NR4A1* via binding to the *SOCS3* promoter. HVFs were infected with *NR4A1* overexpression lentivirus (LV-*NR4A1*) or the negative control (LV-vector), 48 h postinfection, (a) the mRNA and (b) protein expression levels of *NR4A1* were detected by RT-qPCR and western blot. *SOCS3* expression both at (c) mRNA and (d) protein levels was also detected. (e) The putative binding sites of *NR4A1* on the *SOCS3* promoter. (f) The *SOCS3* promoter and truncated promoter with different lengths were inserted into PGL3-Basic vectors. *SOCS3* promoter vectors were co-transfected into cells with *NR4A1* overexpression plasmids, 48 h posttransfection, the luciferase activity was detected by a dual luciferase reporter assay. The results were presented as the mean ± SD. **p* < 0.05, ***p* < 0.01.

SD, Standard deviation

promoted cell apoptosis in HVFs (Figure 5e, f). In addition, the protein expression levels of collagen I, collagen III, and elastin were attenuated in *NR4A1*-overexpression HVFs (Figure 5g), indicating that *NR4A1* promoted ECM degradation.

SOCS3 mediates the effect of NR4A1 on HVFs

To determine whether *SOCS3* mediates the effect of *NR4A1* on HVFs, HVFs were co-infected with *NR4A1* overexpression lentivirus and sh*SOCS3* lentivirus. The results showed that *SOCS3* knockdown increased cell viability and decreased cell apoptosis in *NR4A1* overexpression HVFs (Figure 6a, b). Furthermore, upregulated protein expression of collagen I, collagen III, and elastin was observed when *SOCS3* was knocked down (Figure 6c). Together, these results revealed that the function of *NR4A1* on HVFs was blocked via *SOCS3* knockdown, indicating that *NR4A1* affected HVFs by *SOCS3*-mediated apoptosis and ECM degradation in HVFs.

The screening of potential genes regulated by SOCS3

RNA-seq analysis was further performed to detect the possible mechanism of *SOCS3* on HVFs. Initially, principal component analysis exhibited significantly different component profiles between *SOCS3* overexpression (LV-*SOCS3*) and control (LV-NC) groups (Figure 7a). *DEGs* were identified by volcano plot filtering (Figure 7b). We identified 203 downregulated and 914 upregulated *DEGs*. Then, *DEGs* were significantly enriched for POP-related GO or KEGG terms, such as ECM organization, programmed cell death, and collagen catabolic process (Figure 7c). Further analysis identified *DEGs* involved in these processes, presented in a heatmap (Figure 7d). Gene Set Enrichment Analysis (GSEA) confirmed significant enrichment of *DEGs* involved in the regulation of ECM organization and collagen catabolic process (Figure 7e). These findings suggested that *SOCS3* may regulate ECM and collagen-related downstream signaling.

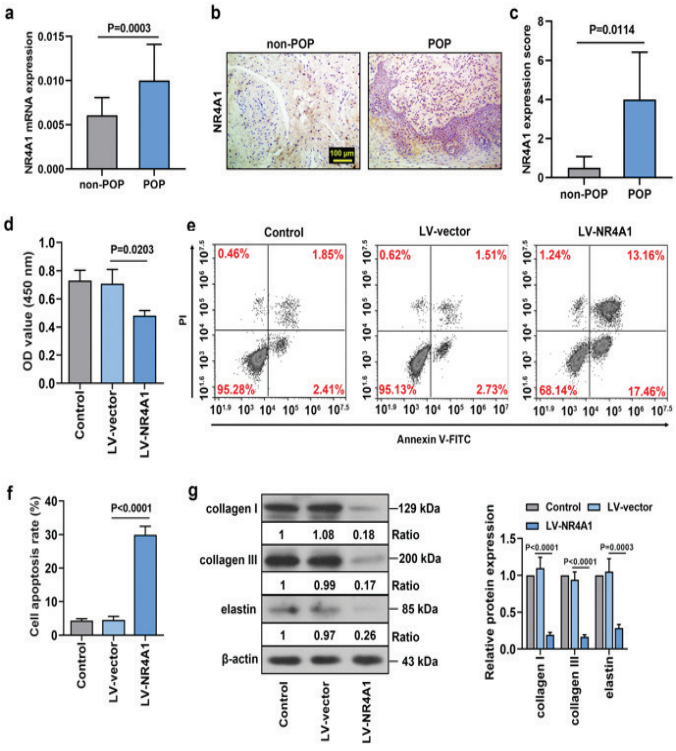

FIG. 5. *NR4A1* overexpression induces cell apoptosis and ECM degradation in HVFs. The expression of *NR4A1* in the POP or non-POP vaginal tissues was detected by (a) RT-qPCR or (b) immunohistochemistry. *NR4A1* expression scores were shown in (c). (d) HVFs were infected with LV-*NR4A1* or LV-vector, 48 h post-infection, and cell viability was detected by CCK-8 assay. (e) Cell apoptosis was detected by flow cytometry. (f) Cell apoptosis rate in each group. (g) The protein expression of collagen I, collagen III, and elastin. The results are presented as the mean ± SD.

SD, Standard deviation

DISCUSSION

POP, characterized by a descent of pelvic organs into the vaginal cavity, significantly impairs the quality of life for many women.[1,22] However, the etiopathogenesis of POP remains poorly understood. Anterior vaginal wall prolapse is the most common form of POP. Vaginal fibroblasts maintain the integrity of vaginal wall tissues, which is important for supporting pelvic organs and preventing POP.[23] Numerous studies have demonstrated that fibroblast apoptosis and ECM loss play critical roles in the progression of POP.[7,8] In our study, we selected vaginal tissues as experimental subjects to explore the pathogenesis of POP. As expected, the vaginal tissues showed decreased collagen deposition and enhanced apoptosis in patients with POP. Subsequently, through bioinformatics analysis, we observed an elevated expression of *SOCS3* in patients with

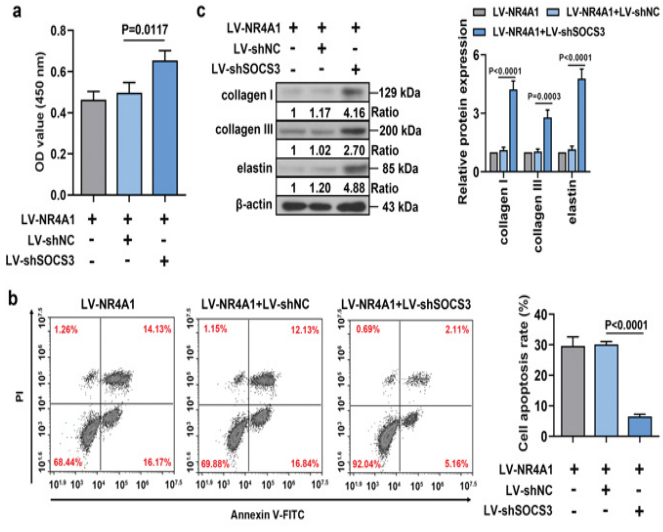

FIG. 6. SOCS3 mediates the effect of *NR4A1* on HVFs. HVFs were co-infected with LV-*NR4A1* or sh*SOCS3* lentivirus (LV-sh*SOCS3*), 48 h postinfection, (a) cell viability was detected by CCK-8. (b) Cell apoptosis was detected by flow cytometry. (c) The protein expression of collagen I, collagen III, and elastin. The results were presented as the mean ± SD.

SD, Standard deviation

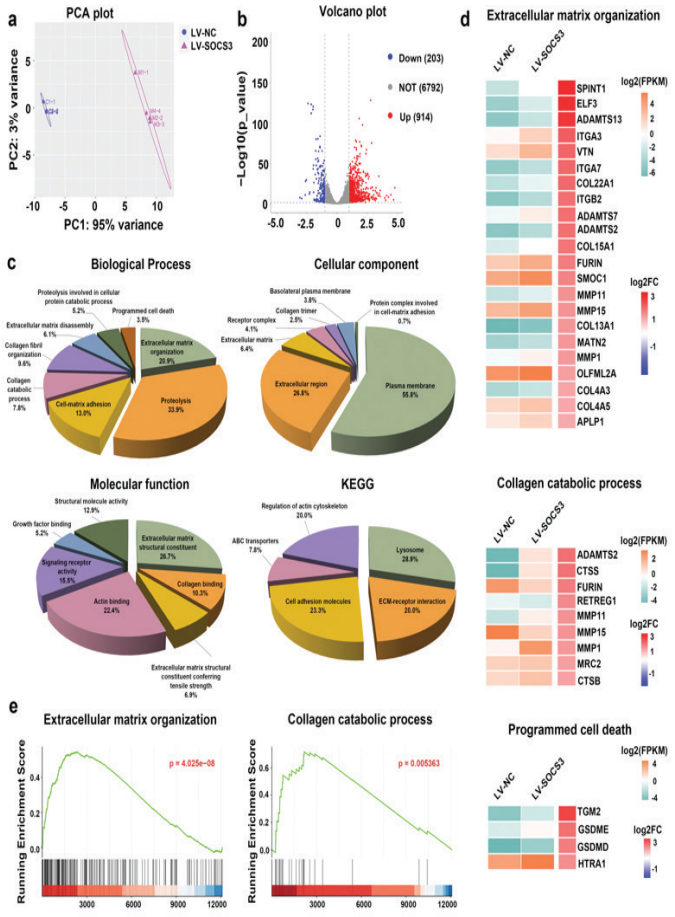

FIG. 7. RNA-seq analysis for differentially expressed genes (*DEGs*) between *SOCS3* overexpression and control HVFs. (a) Principal Component Analysis (PCA) plot. (b) Volcano plot showed the distribution of *DEGs* between *SOCS3* overexpression (LV-*SOCS3*) and control groups (LV-NC). (c) GO and KEGG analysis for the upregulated *DEGs*. (d) Heatmap of all the genes annotated to the ontologies related to extracellular matrix organization, collagen catabolic process, and programmed cell death. (e) GSEA for extracellular matrix organization and collagen catabolic process gene sets enriched in LV-*SOCS3* versus LV-NC.

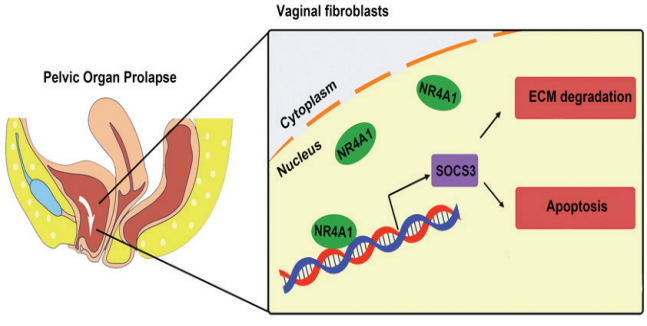

FIG. 8. Schematic diagram of *SOCS3*-mediated pathogenesis in HVFs in the context of POP.

POP, suggesting a potential association between *SOCS3* and the progression of POP.

SOCS3, as a member of the suppressor of the cytokine signaling family, is a potent inhibitor of Janus kinase/signal transducer and activator of the transcription (Jak/STAT) pathway.[10] *SOCS3* is implicated in various human diseases, including cancers, inflammatory diseases, cardiovascular diseases, diabetes, asthma, and neuronal diseases.[24] However, thus far, these studies provide limited information on the relationship between *SOCS3* and POP, and the mechanism of action of POP remains unclear. In our study, we confirmed that *SOCS3* expression was higher in the vaginal tissues of patients with POP, suggesting that increased *SOCS3* expression may participate in the progression of POP. Recent studies have reported that *SOCS3* inhibits cell proliferation and promotes cell apoptosis.[11,12] In our study, we also observed that *SOCS3* overexpression inhibited viability and induced apoptosis in HVFs. Additionally, collagen I, collagen III, and elastin are the main ingredients of ECM. The strength and resilience of vaginal tissues are determined by collagens and elastin, respectively.[5] Dees et al.[13] reported that the deletion of *SOCS3* promotes collagen release. In light of this finding, we sought to investigate whether *SOCS3* regulates the ECM in HVFs. Our results demonstrated that *SOCS3* overexpression led to decreased expression levels of collagen I, collagen III, and elastin. Moreover, *MMPs* are involved in the degradation of ECM and suppression of collagen deposition in POP.[6] Some studies have reported that *SOCS3* knockdown results in the downregulation of *MMP2* and *MMP9*.[25] Consistent with previous research, our study showed that upregulation of *SOCS3* elevated the expression of *MMP2* and *MMP9*. Besides, our studies further found that *SOCS3* also downregulated *TGF-β1* and p-Smad2/3 protein expression. *TGF-β1* is a crucial regulator in fibrosis-related diseases, which induces myofibroblast transformation and regulates ECM production.[26] It promotes ECM deposition by enhancing collagen synthesis and suppressing *MMPs*.[27] *TGF-β*1 exerts its diverse biological activities mainly through activating transcriptional regulators of the Smad pathway. The p-Smad2/3 produced upon *TGF-β1* activation is responsible for regulating collagen and fibronectin gene expression.[28] A previous study has reported that increased *SOCS3* expression inhibits *TGF-β1* expression.[29] *TGF-β*1 blocks the loss of ECM by suppressing *MMP2* and *MMP9* expression via the *TGF-β*1/Smad3 signaling pathway in the uterosacral ligament of patients with POP.[30] Our findings revealed that *SOCS3* decreased the expression of p-Smad3 and p-Smad2. Therefore, we hypothesized that *SOCS3* may enhance the expression of *MMP2* and *MMP9* by inhibiting *TGF-β1*/Smad2/3, thereby promoting ECM degradation. Nevertheless, these possible mechanisms and detailed interactions among them require further exploration. In summary, *SOCS3* overexpression induced apoptosis and ECM degradation of HVFs.

The abnormal expression of *SOCS3* is often attributed to the transcriptional activation of its promoter. For example, the transcriptional activation of the farnesoid X receptor enhances *SOCS3* expression.[31] In this study, we confirmed that *NR4A1* overexpression enhanced the levels of *SOCS3*, and we identified the *NR4A1* binding sites on the *SOCS3* promoter. Thus, *SOCS3* might be transcriptionally

regulated by *NR4A1*. *NR4A1*, belonging to the orphan nuclear hormone receptors of the Nur77 family, is a transcription factor involved in the cell cycle, apoptosis, and tumorigenesis.[32] Similarly, Zeng et al.[16] demonstrated that *NR4A1* induces apoptosis by linking the actin cytoskeleton in parametrial ligament fibroblasts of POP. Here, we found that *NR4A1* was highly expressed in the vaginal tissues of POP patients. *NR4A1* overexpression promoted cell apoptosis and ECM degradation in HVFs. More importantly, *SOCS3* knockdown reversed the negative effects of *NR4A1* on HVFs, suggesting that *NR4A1* affected the function of HVFs by transcriptionally activating *SOCS3* (Figure 8).

Furthermore, the RNA-seq analysis was performed to investigate the potential downstream genes that may be regulated by *SOCS3*. When *SOCS3* was overexpressed, *DEGs* were significantly enriched in ECM organization, programmed cell death, and collagen catabolic process, which was consistent with our findings. *SOCS3* overexpression upregulated the expression of various genes, such as *HTRA1* and *CTSS*. The upregulated expression of *HTRA1* has been reported to inhibit cell proliferation and induce cell apoptosis.[33] Additionally, reducing the expression of *CTSS* increases ECM deposition.[34] These downstream factors may play a role in the POP process. However, follow-up experiments are needed to further study the functions of these factors.

The dysfunction of *SOCS3* may contribute to several disorders. The activation of *SOCS3* leads to insulin resistance and retinal endothelial cell apoptosis in the diabetic retina.[35] *SOCS3* deletion suppresses myocardial apoptosis, which results in the block of left ventricular remodeling after myocardial infarction.[36] Moreover, hepatocyte steatosis was accelerated by promoting *SOCS3* expression.[37] Selective inhibition of *SOCS3* may attenuate some of these consequences, suggesting the potential role of *SOCS3* inhibitors as a promising therapy for disease treatment. For example, PARγ agonist ROZ alleviated steatosis by decreasing *SOCS3* expression.[37] Similarly, the inhibition of *SOCS3* expression by ruxolitinib attenuates atherosclerosis.[38] Besides, microRNAs have been identified as regulators of *SOCS3* expression. Repression of microRNA-122 reduces *SOCS3* expression by promoter methylation.[39] These methods of inhibiting *SOCS3* expression may offer new clinical avenues for the treatment of POP.

In addition, we found no significant association between the expression of *SOCS3* or *NR4A1* and the characteristics of patients with POP. We speculated that this may be due to the insufficient number of samples. In the future, we will continue to collect clinical samples to clear the relationship between the expression levels of *SOCS3* or *NR4A1* and the characteristics of patients with POP.

In conclusion, this study provides evidence that *SOCS3*, transcriptionally activated by *NR4A1*, promotes the progression of POP by inducing HVF apoptosis and ECM degradation. Thus, the *NR4A1*/*SOCS3* axis emerges as a promising therapeutic target for the treatment of POP.

Ethics Committee Approval: This study was conducted following the Declaration of Helsinki, and approved by the Shengjing Hospital of China Medical University Ethics Committee (2022PS040K).

Data Sharing Statement: The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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Supplementary: http://balkanmedicaljournal.org/uploads/pdf/suplement_2023-10-60.pdf

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