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ORIGINAL PAPER

Early growth response 2, a novel target of pelvic organ prolapse, is highly expressed in anterior vaginal wall tissues with pelvic organ prolapse

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Abstract

Pelvic organ prolapse (POP) is a common disorder among women that negatively affects women's quality of life. Early growth response 2 (EGR2) is a transcription factor that regulates cell growth. The present study aimed to explore the role of EGR2 in POP progression and provided a new target for the treatment and prevention of POP. Firstly, we extracted primary vaginal anterior wall fibroblasts from POP tissues and non-POP tissues and then constructed an EGR2-silencing lentivirus for further study. Immunoblotting, qPCR, TUNEL assay, CCK-8 assay, dual luciferase assay, and ELISA assay were carried out. EGR2 expression was much higher in POP tissues than in control tissues, and EGR2 expression positively correlated with *cytokine signaling 3 (SOCS3)* expression. Knockdown of EGR2 increased cell proliferation, upregulated PCNA expression, and reduced apoptosis in POP fibroblasts. Moreover, we found that the knockdown of EGR2 increased *COL1A1*, *COL3A1*, and *Elastin* expression and decreased MMP2 and MMP9 activities, and knockdown of EGR2 increased TGF-β/Smad pathway activity in POP fibroblasts. Interestingly, the results of dual luciferase assay demonstrated that EGR2 was able to increase SOCS3 transcriptional activity. EGR2 knockdown alleviated the apoptosis of POP fibroblasts by reducing SOCS3 expression and improving the proliferation and collagen synthesis of POP fibroblasts. Overall, our study illustrated that EGR2 was highly expressed in POP tissues, and knockdown of EGR2 alleviated apoptosis and reduced matrix degradation in POP fibroblasts. This study might provide a new insight into the pathogenesis of POP.

Keywords Pelvic organ prolapse · EGR2 · Fibroblasts · Collagen

Introduction

Pelvic organ prolapse (POP) is a common disorder of the female reproductive system. Pelvic organ prolapse is defined as the downward displacement of female pelvic organs such as the uterus, bladder, and rectum due to defects in the pelvic floor support structure (Barber 2016). A survey of 53,178 women from six provinces in mainland China showed that the prevalence of symptomatic POP was 9.56% (Li et al. 2020). POP is most prevalent in women aged 60–69 years (Barber and Maher 2013). One of the most prevalent types of POP is prolapse of the anterior vaginal wall (Barber 2016). The anterior vaginal wall fibroblasts have an important role in maintaining the anterior vaginal wall integrity and mechanical stretch (De Landsheere et al. 2013). It is now well recognized that POP is caused by reducing collagen fiber synthesis in the pelvic floor tissue or an accelerated breakdown of that tissue (Barber 2016; Kerkhof et al. 2009). Fibroblasts are the main functional cells that secrete collagen. The production of pelvic floor collagen is closely associated with the number and activity of fibroblasts in the pelvic floor tissues (Kisling et al. 2019). The demand for medical assistance in the treatment of pelvic floor defects is expanding twice as quickly as the growth of the population (Wu et al. 2014). Thus, POP prevention has become a critical issue in obstetrics and gynecology.

Early growth response 2 (EGR2), also known as KROX20, is a member of the zinc finger transcription factor family (Beckmann and Wilce 1997). As a transcription factor, EGR2 regulates cell growth and activation (Du et al. 2014; Reddy et al. 2016). Interestingly, EGR2 has shown

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different effects on different cell types. A study showed that low expression of EGR2 promoted collagen I and collagen III transcription in uterine smooth muscle tumor cells (Yin et al. 2011). Also, studies have demonstrated that EGR2 suppressed the proliferation of Schwann cells and fibroblasts (Parkinson et al. 2004) and reduced the collagen expression and proliferating cell nuclear antigen (PCNA) expression in uterine smooth muscle tumor cells (Yin et al. 2011). On the other hand, EGR2 inhibited serum starvation-induced fibroblast apoptosis (Parkinson et al. 2004) and promoted the synthesis of collagen in fibroblasts (Vollmann et al. 2017). The Gene Expression Omnibus (GEO) database shows that EGR2 had a high expression level in the anterior vaginal parietal tissue of patients with POP (GSE53868). Moreover, as a transcription factor, EGR2 was defined to bind the suppressors of cytokine signaling 3 (SOCS3) promoters to regulate the SOCS3 transcriptional activity (Li et al. 2012). Several studies have illustrated that SOCS3 inhibited fibroblast activation (Dees et al. 2020; Li et al. 2022). Tao et al. showed that the downregulation of SOCS3 expression increased the number of cardiac fibroblasts (Tao et al. 2021). Hence, we speculated that EGR2 may play a role in POP by regulating the expression of SOCS3.

In this study, we aimed to explore the expression level of EGR2 in POP, investigate the role of EGR2 on apoptosis and collagen production of vaginal fibroblasts, and provide a new strategy for the diagnosis and prevention of POP.

Materials and methods

Patients and specimen collection

The anterior vaginal wall tissues from patients who underwent total hysterectomy for uterine benign lesions with no sign of POP were used as the control samples. The anterior vaginal wall tissues from patients who received vaginal hysterectomy and prolapse surgery with POP-Q stage greater than II were used as the POP tissues. Written informed consent was obtained from all participants before specimen collection. This study was approved by the Shengjing Hospital of China Medical University Ethics Committee (2022PS040K) and complied with the principles of the Declaration of Helsinki.

Immunohistochemistry (IHC) assay

IHC was performed using paraffin-embedded tissue sections including 45 POP section and 22 without POP section. Tissues were fixed in 4% paraformaldehyde and embedded in paraffin, then cut into 5-μm-thick sections. Sections were deparaffinized, rehydrated, and then subjected to antigen retrieval. Then, sections were incubated with 3% H_2O_2 for 15 min to block endogenous peroxide activity. Sections were blocked with 1% bovine serum albumin (BSA) for 15 min, then incubated with antibody anti-EGR2 (13,491-1-AP, Proteintech, China) overnight at 4 ℃ and then incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (#31,460, ThermoFisher, USA) at 37 ℃ for 60 min. 3,3′-Diaminobenzidine DAB-1031 (DAB, MaiXin, China) solution was used for color rendering. The sections for blank control were incubated with PBS instead of primary antibody. Images were taken with an Olympus BX53 fluorescence microscope with a DP73 camera with 18 million pixels (Olympus, Japan). The objective lens was 20×. The images were collected using cellSensV0114 software (64 bit).

Masson staining

For Masson staining, sections were dewaxed to water and stained with Regaud's hematoxylin for 6 min, then stained with Ponceau-acid fuchsin solution for 1 min and 1% phosphomolybdic acid solution for 5 min. The sections were then stained with aniline blue for 5 min and examined under an Olympus BX53 fluorescence microscope with a DP73 camera with 18 million pixels (Olympus, Japan). The objective lens was 20×. The images were collected using cellSensV0114 software (64 bit).

Cell isolation and treatment

The primary anterior vaginal wall fibroblasts were isolated from the tissues of patients with POP and patients without POP. According to the previous study (Chen et al. 2005), tissues were washed with PBS, and fascia was removed using a scalpel. The tissues were cut into 1 mm^3 fragments, and ten fragments were placed in a 25 cm^2 culture flask. The culture medium was added slowly to prevent tissues from floating away from the bottom of the culture flask. The tissues were removed after fibroblasts were observed to crawl out of the tissue. The fibroblasts were then digested with 0.25% trypsin for 1 min and centrifugated. After centrifugation, fibroblasts were collected and seeded into new 25 cm^2 culture flasks. Then, 10 ml of Dulbecco's Modified Eagle's Medium (DMEM, Servicebio, China) containing 10% fetal bovine serum (Zhejiang Tianhang Biotechnology, China) was added to culture the cells. When cell fusion approximately reached 90% confluence, they were passaged. The supernatant was discarded and the PBS was added, then 0.25% trypsin was added to digest the cells. When cells became round, the serum-containing medium was added to terminate the digestion. The cells were passaged at a split ratio of 1:2.

Lentiviral infection

The lentiviral vectors were purchased from Fenghui Biotechnology Co., Ltd (Hunan, China). The shRNA and overexpression sequences were synthesized and inserted into the vectors by General Biological System Co., Ltd (Anhui, China). shRNA vector used in this study was pLVX-shRNA1 (BR004, Fenghui Biotechnology, China) and overexpression vector was pLVX-IRES-puro (BR025, Fenghui Biotechnology, China). The 293T cells were seeded in 10 cm dishes and transfected with the lentiviral vectors and the two package vectors psPAX2 and pMD2.G. After 6 h transfection, the transfection mixture was discarded, and fresh medium was added. The lentiviral particles were collected 48 h after transfection and filtered through a 0.45 filter. The target sequences of the EGR2 mRNA were: 5′-AGCTGTCTG ACAACATCTACC-3′. The target sequences of the nonspecific mRNA were: 5′-TTCTCCGAACGTGTCACGT-3′. For lentiviral infection, the culture medium was discarded, a fresh culture medium containing lentivirus (MOI of 100) was added to treat the cells, and cells were incubated at 37 ℃ with 5% CO_2. The transfection efficiency was assessed by western blot.

Cell proliferation/viability assay

Cell viability was measured using the Cell Counting Kit-8 (CCK-8) and cell proliferation was determined by a 5-ethynyl-2-deoxyuridine (EdU) assay kit according to the instruction of the manufacturer (KeyGEN Bio TECH, China).

Immunofluorescence (IF) assay

The dewaxed sections were boiled in antigen retrieval solution to perform antigen retrieval for 10 min. Subsequently, the sections were blocked with BSA for 15 min at room temperature. Then, the sections were labeled with primary antibodies anti-Vimentin (A19607, ABclonal, China), anti-Cytokeratin (A0247, ABclonal, China), and anti-Desmin (A3736, ABclonal, China) at 4 ℃ overnight followed by Cy3-labeled secondary antibody (ab6939, Abcam, UK) at room temperature for 60 min.

The cell slides were fixed in 4% paraformaldehyde for 15 min and permeabilized with 0.1% Triton X-100 for 30 min followed by blocking with 1% BSA for 15 min. The slides were incubated with primary antibodies anti-Vimentin (A19607, ABclonal, China), anti-Cytokeratin (A0247, ABclonal, China), and anti-Desmin (A3736, ABclonal, China) at 4 ℃ overnight followed by Cy3-labeled secondary antibody (A27039, Invitrogen, USA) at room temperature for 60 min. The 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI, D106471-5 mg, Aladdin, China) was used to counterstain nuclei. The sections for blank control were incubated with PBS instead of primary antibody. The images were collected with an Olympus BX53 fluorescence microscope with a DP73 camera with 18 million pixels (Olympus, Japan). The objective lens was 20×. The images were collected using cellSensV0114 software (64 bit).

TUNEL assay

The TUNEL assay was performed using an in situ cell death detection kit (Roche, Basel, Switzerland). In brief, samples were fixed and permeabilized with 0.1% Triton X-100 for 15 min. The TUNEL reaction solution was prepared in the ice according to the ratio of enzyme solution:label solution = 1:9. The TUNEL reaction solution was added dropwise until the cell slide was completely covered and incubated for 60 min at 37 ℃ in the dark. Then the slides were washed with PBS and the DAB staining solution was added for 5 min. The images were taken by an Olympus BX53 fluorescence microscope with a DP73 camera with 18 million pixels (Olympus, Japan). The objective lens was 20×. The images were collected using cellSensV0114 software (64 bit).

Dual-luciferase reporter assay

We performed a dual-luciferase reporter assay to examine the transcriptional regulation of SOCS3 by EGR2. The 293T cells (Zhong Qiao Xin Zhou Biotech, Shanghai, China) were cultured in DMEM, supplemented with 10% FBS, and used as tool cells. The pGL3 containing SOCS3 promoter, EGR2 overexpression plasmid, and corresponding empty vector plasmid were constructed. The 293T cells were co-transfected with EGR2 overexpression plasmids and SOCS3 promoter plasmids. Relative luciferase activity was determined using Dual Luciferase Reporter Gene Assay Kit (KeyGEN Bio TECH, Jiangsu, China).

Quantitative real-time polymerase chain reaction (qPCR)

Total RNA was isolated from tissues and cells by using TriPure according to the kit protocol (RP1001, BioTeke, China). For reverse transcription, the BeyoRT™ II M-MLV (D7160L, Beytotime, China) reverse transcriptase was used. qPCR was conducted using 2×Taq PCR Master Mix and SYBR green reagent (PC1150, Solarbio, China). The sequences of the primers were as follows: *EGR2*: forward: 5′-GTTCTCCCGCTCTGACG-3′, reverse: 5′-TTTCCG CTCTTTCTGTCTC-3′; *SOCS3*: forward: 5′-TCGCCACCT ACTGAACCCT-3′, reverse: 5′-GGTCCAGGAACTCCC GAAT-3′; *COL1A1*: forward: 5′-CGAAGACATCCCACC AATC-3′, reverse: 5′-ATCACGTCATCGCACAACA-3′;

COL3A1: forward: 5′-CCCGTATTATGGAGATGAAC-3′, reverse: 5′-ATCAGGACTAATGAGGCTTTC-3′; *Elastin*: forward: 5′-CCGCAGTTACCTTTCCG-3′, reverse: 5′-AGA CACTCCTAAGCCACCAA-3′.

Western blot (WB) assay

Proteins were extracted using RIPA protein lysis buffer (R0010, Solarbio, China), and the bicinchoninic acid (BCA) protein assay kit (PC0020, Solarbio, China) was used to detect the protein concentration. Proteins were separated by SDS−PAGE and then transferred to PVDF membranes (IPVH00010, Millipore, USA). In this study, the concentration of the separating gel was 10% and 15%, and that of the stacking gel was 5%. The membranes were blocked with 5% nonfat milk for 1 h followed by incubated with primary antibodies against EGR2 (13,491-1-AP, Proteintech, China), PCNA (A0264, Abclonal, China), TGF-β1 (AF1027, Affinity, China), Smad2/3 (A18674, Abclonal, China), p-Smad2/3 (AP0548, Abclonal, China), and SOCS3 (DF6133, Affinity, China) at 4 °C overnight. Then, the membranes were incubated with goat anti-rabbit IgG–HRP (SE134, Solarbio, China) or goat anti-mouse IgG-HRP secondary antibodies (SE131, Solarbio, China). Protein bands were visualized using an enhanced chemiluminescence (ECL) detection kit (PE0010, Solarbio, China) and measured using Gel-Pro-Analyzer. The manufacturer has provided primary antibody specificity in the technical specification.

Enzyme-linked immunosorbent assay (ELISA) and caspase-3 and caspase-9 activities detection

MMP-2 and MMP-9 activities were measured using ELISA kits according to the relevant kits. The ELISA kits were purchased form MultiScienceBiotech Co., Ltd. The caspase-3 and caspase-9 activities were determined by caspase-3 and caspase-9 Activity Assay Kit (Beyotime, China).

Statistical analysis

Statistical analyses were performed using unpaired *t*-test or one-way ANOVA where appropriate. Differences were considered statistically significant when $p < 0.05$.

Results

EGR2 is highly expressed in POP tissues and correlates with the SOCS3 expression

The qPCR and WB results showed that the expression of EGR2 and SOCS3 was higher in POP tissues than in control tissues, and the qPCR confirmed the positive correlation between *EGR2* and *SOCS3* (Fig. 1a–b). Moreover, in the control group, the EGR2 expression was rare, and the EGR2 was highly expressed in POP tissues compared with control tissues (Fig. 1c). We found that collagen was reduced, and the apoptotic cells were increased in the POP group compared with the control group (Fig. 1d–e).

Knockdown of EGR2 improves cell proliferation and reduced cell apoptosis in POP fibroblasts

We conducted IF staining in vaginal wall tissues, and the result showed that there were Vimentin-positive cells, Cytokeratin-positive cells, and Desmin-positive cells in vaginal wall tissues (Supplementary Fig. 1). The cell purity was confirmed by IF staining. The results showed that Vimentin expression was positive, and the Cytokeratin expression and Desmin expression were negative (Fig. 2a). The results of WB and qPCR demonstrated that EGR2 expression was significantly increased in POP fibroblasts than in control fibroblasts (Fig. 2b–c). Transfection efficiency was assessed by WB, the results showed EGR2 was successfully knocked down in POP fibroblasts (Fig. 3a). As shown in Fig. 3b, the knockdown of EGR2 increased the viability of fibroblasts. Moreover, EGR2 silencing increased the number of EdU-positive cells, indicating that knockdown of EGR2 improved cell proliferation (Fig. 3c). Interestingly, the expression level of PCNA in EGR2 silencing fibroblasts was 3.4 times higher than POP fibroblasts (Fig. 3d). Knockdown of EGR2 decreased the caspase-3 and caspase-9 activities and reduced apoptosis (Fig. 3e–f).

Knockdown of EGR2 inhibits extracellular matrix (ECM) degradation in POP fibroblasts

The gene expression levels of *COL1A1*, *COL3A1*, and *Elastin* were significantly upregulated in EGR2 knockdown fibroblasts compared with POP fibroblasts (Fig. 4a). Moreover, we found that EGR2 silencing significantly decreased the activity of MMP-2 and MMP-9 compared with Lv-NC cells (5.82 ± 0.71 ng/ml versus 3.65 ± 0.63 ng/ml, 3.88 ± 0.70 versus 1.82 ± 0.23 ng/ml, $p < 0.01$, respectively, Fig. 4b). The protein expression levels of TGF-β1 in EGR2 knockdown fibroblasts was 5.5 times higher than Lv-NC fibroblasts, and the phosphorylation level of Smad2/3 was increased significantly ($p < 0.01$, Fig. 4c).

EGR2 promotes SOCS3 transcriptional activity

Firstly, we detected the expression of SOCS3 in POP fibroblasts, and the results of WB and qPCR demonstrated that the knockdown of EGR2 decreased the protein and mRNA expression of SOCS3 (Fig. 5a–b). Then, we found that

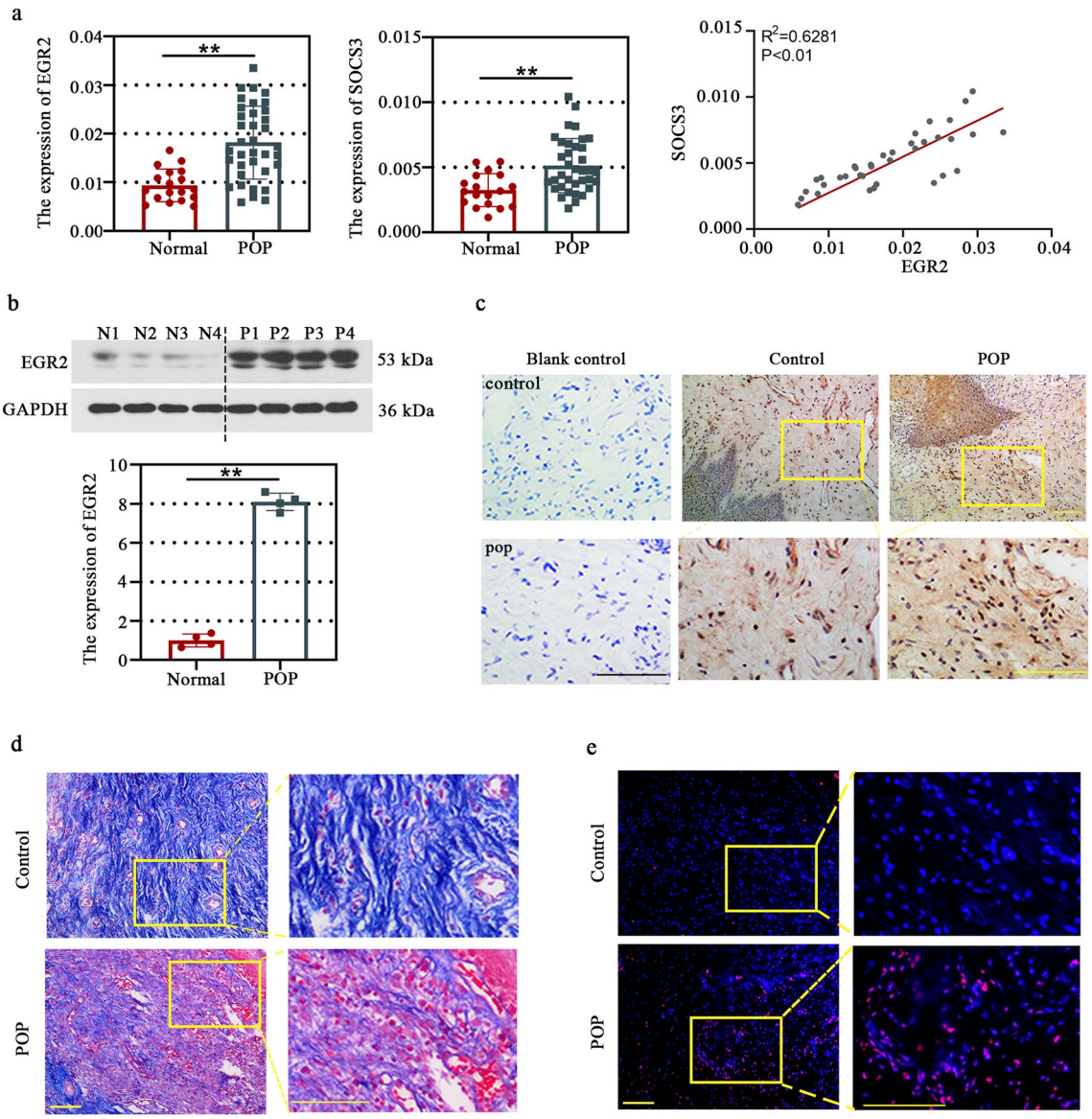

Fig. 1 EGR2 is highly expressed in POP tissues. **a** The mRNA expression of *EGR2* and *SOCS3* in POP ($n=36$) and non-POP ($n=18$) tissues was determined by qPCR; the correlation analysis was conducted to investigate the relationship between *EGR2* and *SOCS3*. **b** Western blot was used to measure the protein expression of EGR2 in tissues (POP: $n=36$; non-POP: $n=18$). **c** IHC staining of EGR2 in tissues and representative images were shown. **d** Representative images of Masson staining. **e** Representative image of TUNEL staining. Scale bar: 100 μm. Data are shown as mean ± SD

EGR2 overexpression enhanced the transcriptional activity of SOCS3 by binding to the SOCS3 promoter (Fig. 5c).

EGR2 inhibits cell proliferation and promotes apoptosis via activation of SOCS3

As shown in Fig. 6a, b, SOCS3 overexpression reduced the number of EdU-positive cells and decreased cell viability. Interestingly, co-transfection of POP fibroblasts with Lv-EGR2^{sh} and Lv-SOCS3^{oe} alleviated the reduction of cellular activity caused by SOCS3 overexpression (Fig. 6a, b). The results of qPCR showed that SOCS3 overexpression reduced the mRNA expression of *COL1A1*, *COL3A1*, and *Elastin*, while EGR2 knockdown reversed it (Fig. 6c). Moreover, TUNEL assay results showed EGR2 silencing alleviated apoptosis caused by SOCS3 overexpression in POP fibroblasts (Fig. 6d).

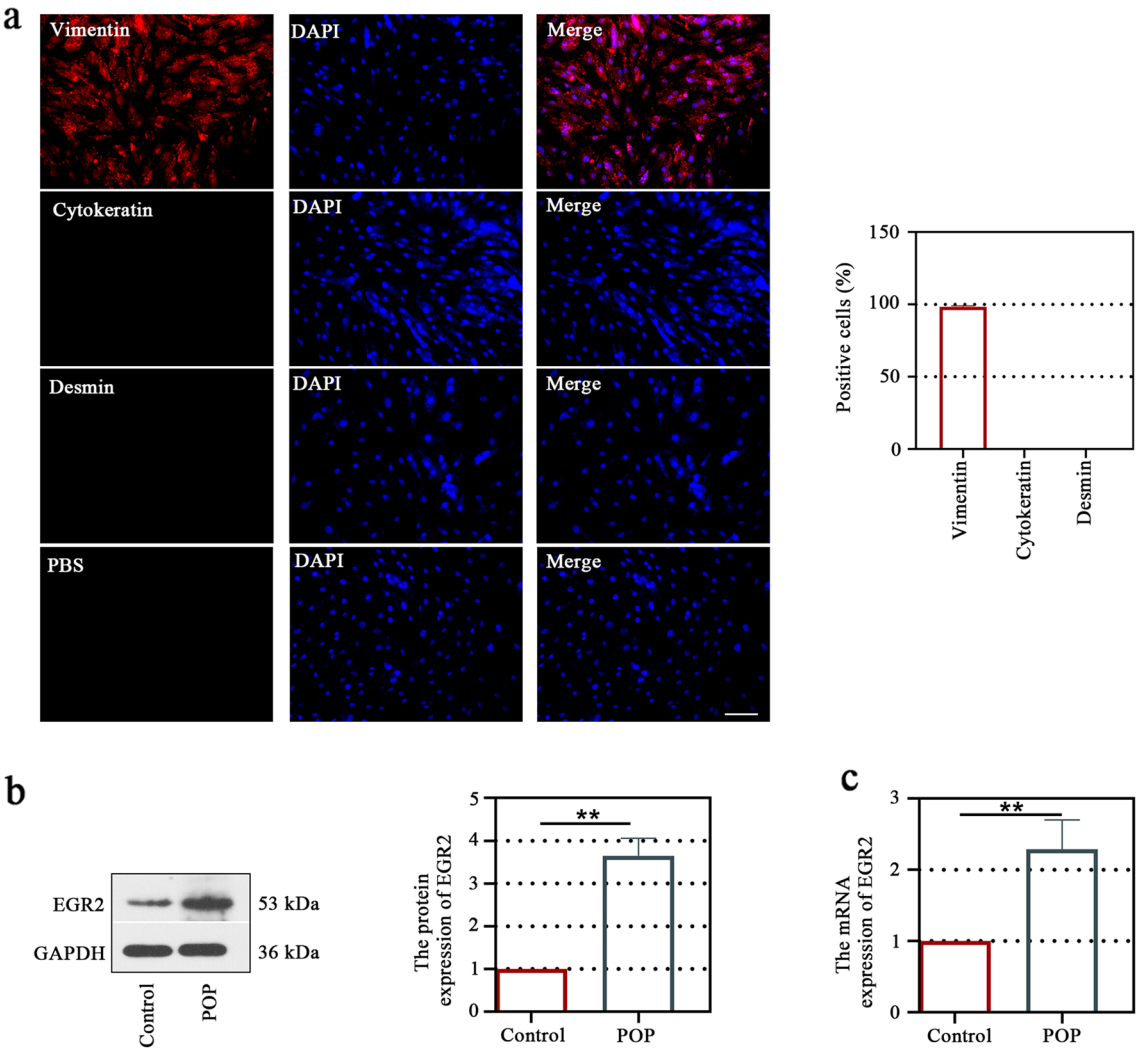

Fig. 2 Identification of the primary anterior vaginal wall fibroblasts. **a** IF staining of Vimentin, Cytokeratin, and Desmin. **b–c** EGR2 expression was measured by western blot (b, $n=3$) and qPCR (c, $n=3$). Scale bar: 100 μm. Data are shown as mean ± SD

Discussion

POP is a common reproductive disease among women, causing a major medical and financial burden and severely affecting women's quality of life (Zhang et al. 2020; Elneil 2009; Emmerson et al. 2019). EGR2 is a well-known transcription factor, and a recent study found that it is upregulated in multiple senescence models, indicating that EGR2 is a senescence marker (Tyler et al. 2021). In addition, studies have reported that prolonging the expression of EGR2 causes fibroblast senescence (Tyler et al. 2021; Vollmann et al. 2017).Yin et al. study revealed that EGR2 inhibited the proliferation of fibroblasts (Yin et al. 2011). The vaginal fibroblasts maintain the integrity of the vaginal wall tissues, which is essential for maintaining the position of the pelvic organs and avoiding POP. In the present study, the expression of EGR2 was significantly higher in the POP group than in the non-POP group. The knockdown of EGR2 expression improved the proliferation of POP fibroblasts. These results support our hypothesis that EGR2 is involved in the pathogenesis of POP.

PCNA is related to DNA synthesis and cell proliferation (Maga and Hubscher 2003). In cardiac fibroblast, the PCNA expression was inhibited under a hypoxia condition (Zhang et al. 2021). In this study, EGR2 silencing increased the protein expression of PCNA in POP fibroblasts. Caspases are crucial mediators of programmed cell death. Caspase-3 and caspase-9 are common apoptotic proteins in the caspase family, and their activation catalyzes specific cleavage of many key proteins (Porter and Janicke 1999). Kim et al. found that caspase-3 and caspase-9 were activated in patients with pelvic organ prolapse, and TUNEL-positive cells were increased in patients with pelvic organ prolapse (Kim et al. 2013). EGR2 plays a vital role in the PTEN-induced

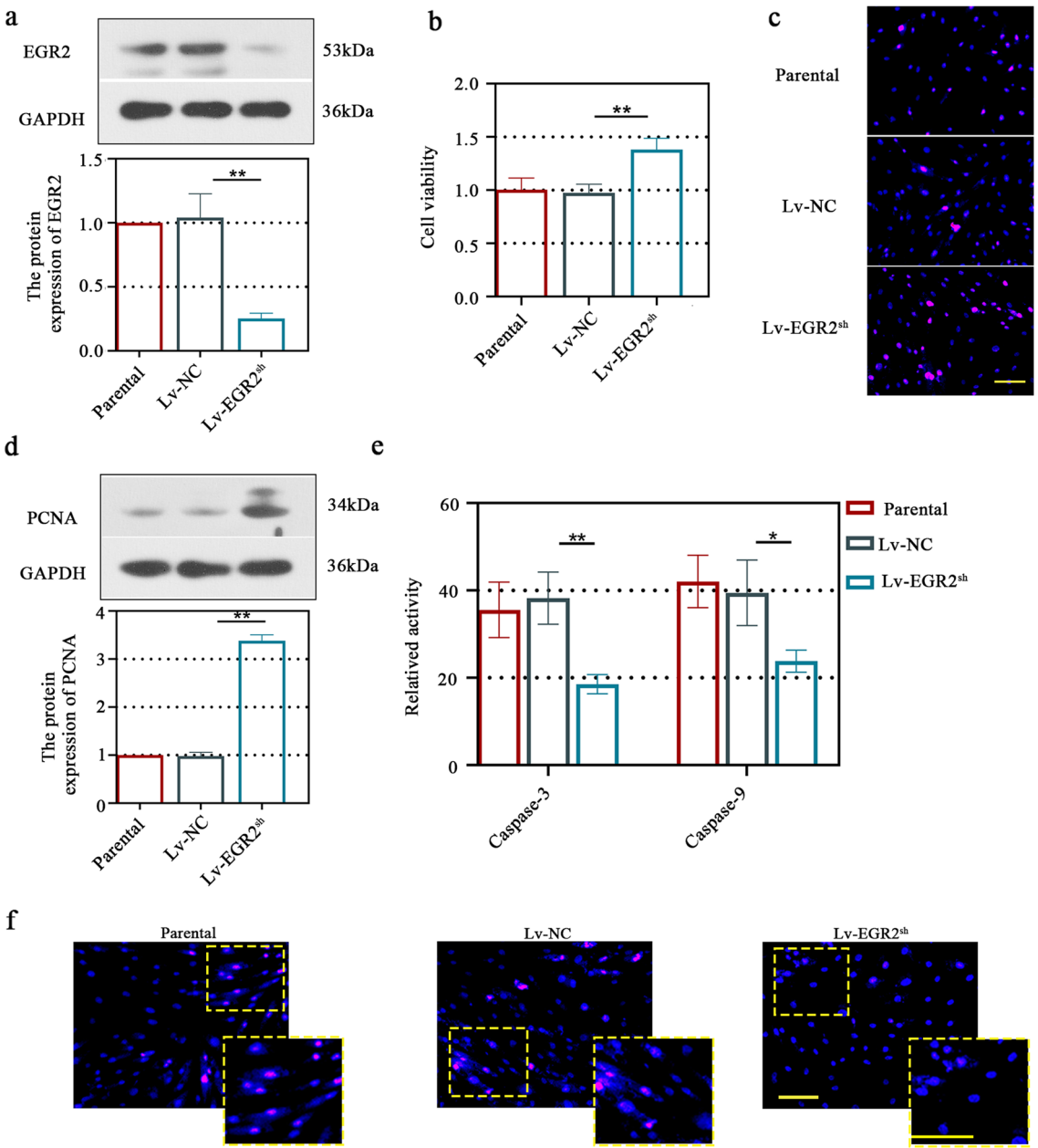

Fig. 3 EGR2 silencing improves cell survival of POP fibroblasts. EGR2 silencing lentivirus was constructed to infect the primary anterior vaginal wall fibroblasts of patients with POP. **a** The expression of EGR2 was analysis by western blot ($n=3$). **b** CCK8 assay was performed to determine cell viability ($n=3$). **c** EdU staining was conducted to determine cell proliferation. **d** PCNA expression was measured by western blot ($n=3$). **e** The activities of caspase-3 and caspase-9 were detected by detection kit ($n=3$). **f** TUNEL assay was conducted. Scale bar: 100 μm. Data are shown as mean ± SD

apoptotic pathway; EGR2 is able to induce apoptosis by activating caspase-3, caspase-8, and caspase-9. Herein, we illustrated that knockdown of EGR2 inhibited the activation of caspase-3 and caspase-9, and improved cell proliferation.

Collagen and elastin are the components of connective tissue and play an essential role in the resistance to deformation in tissues (Chen and Yeh 2011; Moon et al. 2011). Liu et al. found that the expression of COL1A1, COL3A1, and Elastin was decreased in POP tissues. However, the changing trend of COL1A1, COL3A1, and Elastin levels is controversial. Gabriel et al. illustrated that there was no significant difference in collagen I expression between POP and non-POP tissues (Gabriel et al. 2005). A study found that Collagen III expression was upregulated in women with POP (Yucel et al. 2013). The difference between results from the studies mentioned above may be due to experimental

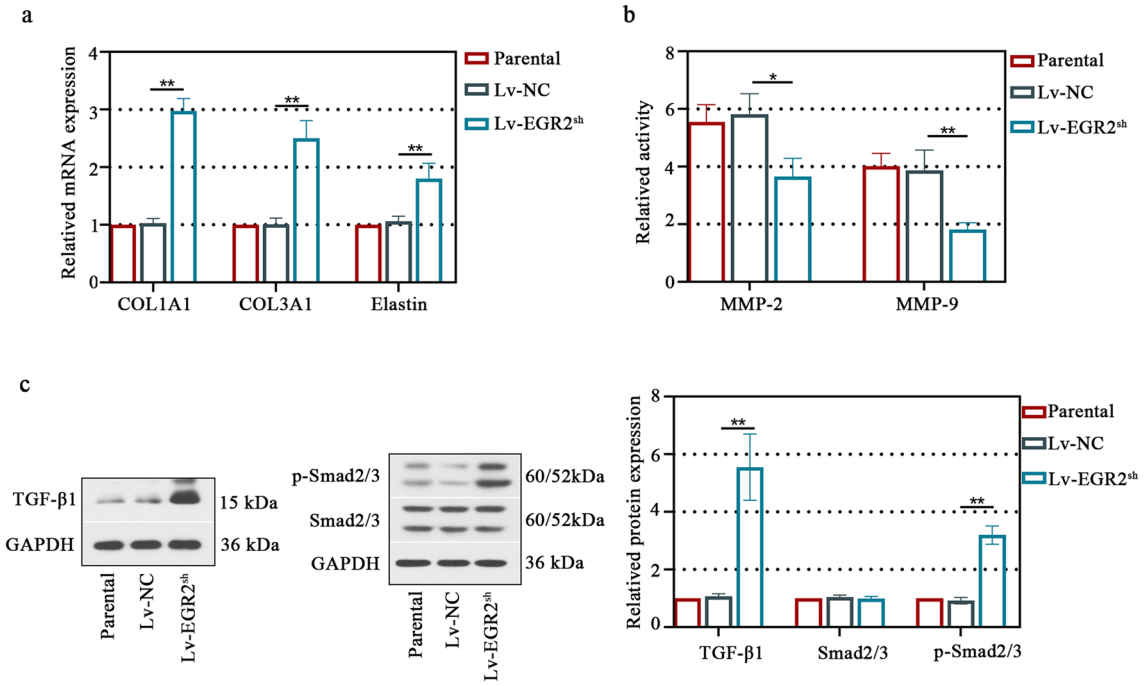

Fig. 4 Knockdown of EGR2 reduces the ECM degradation. **a** The expression of *COL1A1*, *COL3A1*, and *Elastin* was measured using qPCR ($n=3$). **b** MMP-2 and MMP-9 activities were detected by ELISA ($n=3$). **c** The expression of TGF-β1 and Smad2/3 and the phosphorylation level of Smad2/3 were measured by western blot ($n=3$). Data are shown as mean ± SD

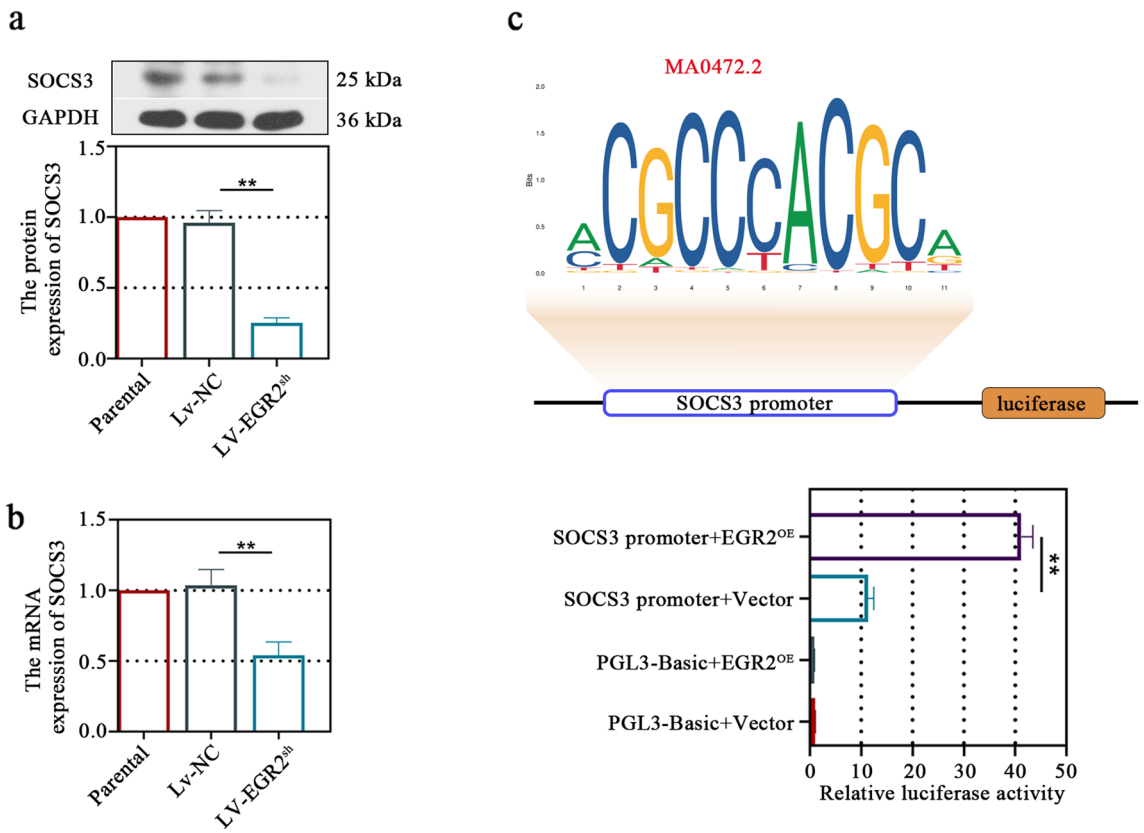

Fig. 5 EGR2 promotes SOCS3 transcriptional activity. **a**–**b** The expression of SOCS3 was verified by western blot (**a**, $n=3$) and qPCR (**b**, $n=3$). **c** Dual luciferase assay was performed to determine the relationship between EGR2 and SOCS3 ($n=3$). Data are shown as mean ± SD

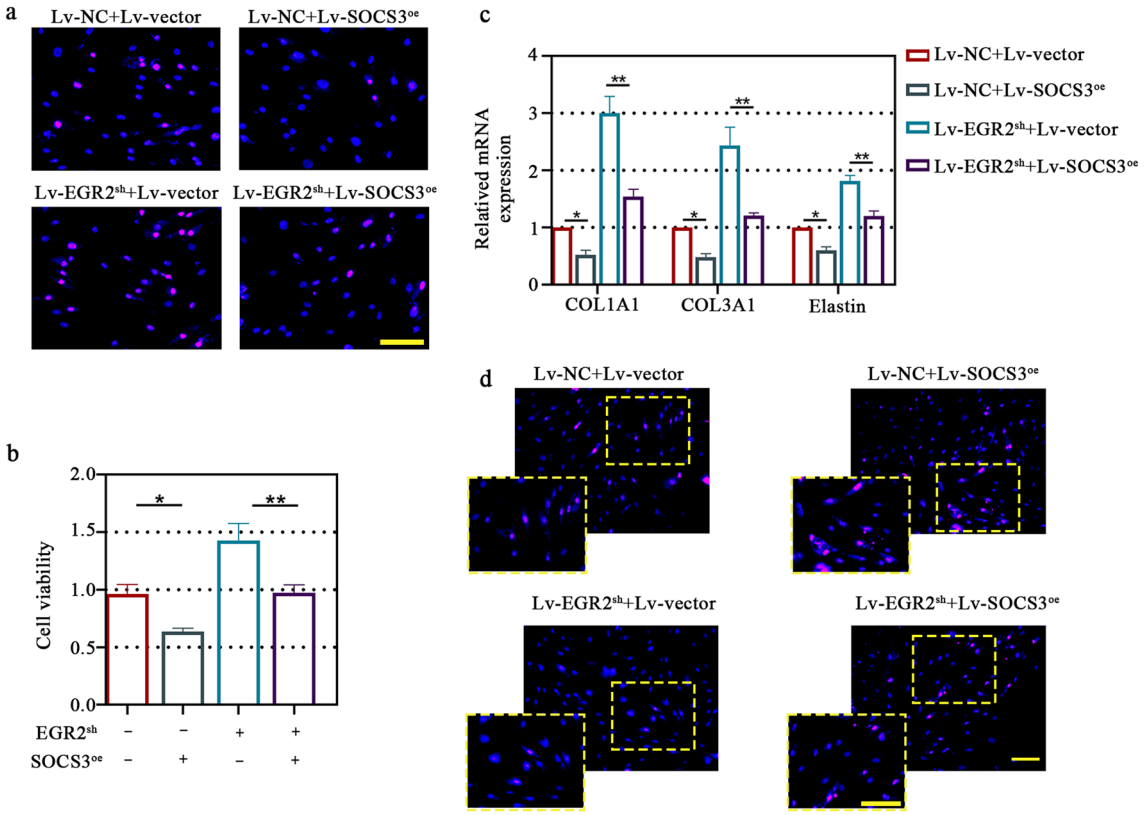

Fig. 6 EGR2 interaction with SOCS3 and inhibits cell proliferation and promotes apoptosis of POP fibroblast. **a** Cell proliferation analysis was conducted by EdU assay. **b** Cell viability was determined by CCK-8 ($n=3$). **c** The expression of *COL1A1*, *COL3A1*, and *Elastin* was measured by qPCR ($n=3$). **d** TUNEL assay was performed. $N=3$. Scale bar: 100 μm. Data are shown as mean ± SD

procedures, the data from the above studies were obtained from IHC assay, which might be limited by the biopsy site. In our study, we used qPCR to detect the mRNA levels of *collagen* and *Elastin*. The results showed that EGR2 knockdown promoted the expression of COL1A1, COL3A1, and Elastin in POP fibroblasts, confirming that EGR2 is involved in the regulation of collagen synthesis in fibroblasts.

The ECM provides a framework for pelvic support and plays a critical role in POP occurrence (Zhang et al. 2017). The dysregulation of ECM metabolism brought on by an imbalance of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) affects the structural remodeling of the vaginal lamina propria (Vetuschi et al. 2016). MMP-2 and MMP-9 are the main gelatinases that degrade gelatin and break down the basement membrane (Creemers et al. 1998; Min et al. 2017). A study showed that levels of MMP-2 and MMP-9 were higher in POP patients (Moon et al. 2011). Min et al. detected the expression of ECM components in parametrial ligament fibroblasts under mechanical strain loading and found that the protein expression levels of MMP-2 and MMP-9 significantly increased under increased mechanical strain (Min et al. 2017). The role of EGR2 in ECM deposition is unclear. Our results demonstrated that the knockdown of EGR2 decreased the activities of MMP-2 and MMP-9 in POP fibroblasts, suggesting that the downregulation of EGR2 may alleviate ECM degradation and prevent the occurrence of POP. TGF-β1 is a crucial fibrogenic cytokine and plays an important role in the promotion of fibroblast transdifferentiation. TGF-β promotes collagen synthesis and reduces degradation by inhibiting MMP and upregulating TIMP (Sampson et al. 2014). Moreover, TGF-β1 is capable of activating SMAD-2 and SMAD-3 to regulate fibrosis. Li et al. study showed that the TGF-β1/Smad pathway promotes the proliferation, migration, and activation of lung fibroblasts (Li et al. 2021). Our previous study also showed that the MMP-9 expression was upregulated and TGF-β1 expression was decreased in patients with POP (Zhao et al. 2021). In the present study, we found that the knockdown of EGR2 activated the TGB/SMAD pathway in POP fibroblasts.

Among the SOCS protein family, SOCS3 is an important member and a cytokine-induced negative regulator of cytokine signaling pathways (Carow and Rottenberg 2014). Moreover, Li et al. found that binding of EGR2 to the proximal region of the SOCS3 promoter led to increased promoter activity of SOCS3, and EGR2 could

directly regulate the expression of SOCS3 (Li et al. 2012). In the present study, the knockdown of EGR2 decreased SOCS3 expression, and EGR2 overexpression increased SOCS3 promoter activation. Moreover, we found that EGR2 expression was positively correlated with SOCS3 expression in anterior vaginal parietal tissues. Numerous studies have revealed SOCS3 plays a regulatory role in fibroblast activation (Dees et al. 2020; Hirakata et al. 2020; Chen et al. 2020). Hirakata et al. demonstrated that SOCS3 deletion increased fibroblasts in the aortic wall (Hirakata et al. 2020), Dees et al. demonstrated that downregulation of SOCS3 activated STAT3 to improve collagen release (Dees et al. 2020). Also, we found that overexpression of SOCS3 inhibited cell proliferation, decreased collagen expression, and increased apoptosis in POP fibroblasts. These results indicated that EGR2 may regulate fibroblast proliferation and apoptosis by promoting SOCS3 expression.

Overall, our study showed that EGR2 was highly expressed in patients with POP, and *EGR2* expression was positively correlated with *SOCS3* expression. The knockdown of EGR2 promoted cell proliferation, increased collagen and elastin expression, and reduced extracellular matrix degradation through the TGF/Smad pathway. Moreover, we found that EGR2 promoted the transcriptional activity of SOCS3. These results provide new insight into the treatment and diagnosis of POP, and EGR2 may be a novel biomarker of POP.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00418-023-02240-2.

Author contributions X.J.: writing—original draft, writing—review and editing, methodology, software, validation, formal analysis, and investigation. H.X.: writing—original draft, methodology, software, investigation, visualization, and data curation. Q.H.: methodology, validation, formal analysis, and visualization. Y.Y.: software, data curation, and visualization. M.Q.: formal analysis and visualization. Z.X.: conceptualization, writing—review and editing, and supervision.

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Data availability The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that the publication of this paper has no conflicts of interest.

Ethical approval and consent to participate The written informed consent was obtained from all participants before specimen collection. This study was approved by the Shengjing Hospital of China Medical University Ethics Committee (2022PS040K) and complied with the principles of the Declaration of Helsinki.

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