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Role of SMOC2 in adenomyosis: implications for ECM remodeling and EMT pathogenesis

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Abstract

Background Adenomyosis is a common gynecological disorder characterized by the invasion of endometrial tissue into the myometrium, resulting in severe dysmenorrhea and menorrhagia. This study aimed to explore the role of SMOC2 (SPARC related modular calcium binding 2), an extracellular matrix (ECM) -associated protein, in the pathogenesis of adenomyosis and its potential as a therapeutic target.

Methods We conducted a clinical study involving 35 patients diagnosed with adenomyosis and 30 controls. Ectopic endometrial tissue samples were collected and analyzed using immunohistochemistry (IHC), Masson staining, and cell culture techniques. The proliferative effect of SMOC2 on cells was evaluated using CCK- 8 assay, while the expression of SMOC2 and epithelial-mesenchymal transition (EMT) was assessed using real-time PCR and western blot analysis.

Results SMOC2 expression was significantly higher in the ectopic endometrial tissue of adenomyosis patients compared to controls. SMOC2 could promote cell proliferation. Overexpression of SMOC2 significantly upregulated mesenchymal markers N-cadherin and α-SMA, and downregulated epithelial marker E-cadherin. Conversely, knocking down SMOC2 with siRNA reversed these effects. These findings indicate that SMOC2 promotes EMT in adenomyotic stromal cells. Additionally, SMOC2 also activated the MMP9 signaling pathway, which plays a crucial role in the extracellular matrix (ECM) remodeling.

Conclusions SMOC2 appears to be a key regulator in the pathogenesis of adenomyosis, promoting ECM remodeling and EMT, both of which are characteristic of the disease. Targeting SMOC2 may provide a novel therapeutic strategy for the treatment of adenomyosis.

Keywords Adenomyosis, SMOC2, Epithelial-Mesenchymal Transition, The Extracellular Matrix

Introduction

Adenomyosis (ADM) is characterized by the infiltration of endometrial glands and stroma into the myometrium, resulting in diffuse or localized lesions. Common clinical manifestations include increased menstrual volume (40%– 50%), prolonged menstruation, and exacerbated dysmenorrhea (25%). The underlying mechanisms of adenomyosis remain incompletely understood but are likely influenced by factors such as injury to the endometrial basal layer, genetic predisposition, and hormonal dysregulation.

Adenomyosis poses significant clinical challenges for gynecologists, particularly for women who have not

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completed their reproductive requirements. The ectopic adenomyotic lesions persistently respond to cyclic hormonal stimulation, leading to progressive inflammation and fibrosis. This pathology reduces uterine receptivity and elevates the risk of embryo implantation failure [1]. Even when embryos are obtained via assisted reproductive technology (ART), implantation and live birth rates are markedly diminished (20%– 30% reduction in IVF live birth rates), with increased miscarriage risks [2]. For women without children, disease management must prioritize uterine function preservation. However, current therapeutic strategies (medical or surgical) face inherent conflicts between efficacy and fertility protection. For instance, Long-term use of GnRH agonist (GnRH-a) may suppress ovarian function, inducing hypoestrogenic states (e.g., bone mineral loss), while exhibiting high recurrence rates post-cessation and poor long-term sustainability [3].Conservative surgery (adenomyotic lesion resection) carries risks of incomplete lesion removal due to ill-defined boundaries, residual disease, iatrogenic myometrial injury, postoperative adhesions, and uterine rupture [4]. Postoperative pregnancy rates remain suboptimal (30%– 50%) [5].

The multifaceted nature of adenomyosis, coupled with its unpredictable disease progression, high recurrence rates [6], and limited therapeutic efficacy, underscores the need for further research to develop more effective uterus-specific targeted drugs and optimize conservative surgical techniques.

The extracellular matrix (ECM) plays a pivotal role in the pathogenesis of adenomyosis. Excessive deposition of ECM components and abnormal ECM remodeling disrupt the myometrial architecture, facilitating the infiltration and growth of endometrial tissue into the myometrial layer [7]. Furthermore, ECM components influence the adhesion, migration, and proliferation of endometrial cells, creating a microenvironment conducive to their survival and expansion within the myometrium [8]. Dysregulation of ECM-related signaling pathways further exacerbates ECM synthesis and deposition, aggravating the pathological progression of adenomyosis. Investigating the relationship between ECM and adenomyosis not only enhances our understanding of its pathogenesis but also provides a foundation for developing novel therapeutic strategies.

SMOC2 (SPARC-related modular calcium-binding 2), an ECM-associated protein, plays a crucial role in fibril assembly and co-localizes with fibronectin. Studies have shown that SMOC2 promotes fibroblast-to-myofibroblast transformation (FMT), enhancing myofiber formation, proliferation, migration, and the extracellular matrix(ECM) deposition [9]. Elevated expression of SMOC2 has been detected in patients with endometriosis, suggesting its involvement in processes such as cellular migration, angiogenesis, and tissue invasion, which may contribute to the pathogenesis of ectopic endometrial lesions [10]. However, the role of SMOC2 in adenomyosis remains poorly understood, warranting further investigation to elucidate its mechanisms and potential as a therapeutic target.

In this study, we validated the differential expression of SMOC2 in ectopic endometrial tissues from patients with adenomyosis using clinical specimens. We further explored the role of SMOC2 in promoting epithelial-mesenchymal transition (EMT) in primary cells derived from adenomyosis patients. These findings may provide a novel therapeutic approach for the clinical management of adenomyosis.

Methods

Patients and samples

From March 2020 to December 2021, 35 patients with adenomyosis confirmed by preoperative transvaginal ultrasound and postoperative histopathological examination were recruited from the Department of Obstetrics and Gynecology of the Third People's Hospital of Luoyang as the experimental group. Ectopic endometrial tissue samples were collected during hysterectomy and immediately fixed with 10% formalin buffer, followed by paraffin embedding. The average age of the patients was 44.39 years. At the same time, 30 cases of normal endometrial tissue from patients undergoing benign gynecological surgery (ovarian cysts, CIN II, infertility, etc.) were selected as the control group, with an average age of 40.76 years. The Inclusion Criteria are as follows:1) Postoperative pathological confirmation of adenomyosis by two independent pathologists.2) Women of reproductive age (typically 30–50 years), excluding pre-pubertal or postmenopausal patients. 3) Presence of typical clinical symptoms (e.g., progressive dysmenorrhea, menorrhagia, chronic pelvic pain, or infertility). 4)Voluntary participation with signed informed consent and willingness to comply with follow-up and sample collection (e.g., endometrial tissue). 5)For the healthy control group: No history of adenomyosis, endometriosis, or other gynecological organic diseases, and normal imaging findings. The Exclusion Criteria are as follows: 1) Comorbidities including uterine fibroids (≥ 3 cm), endometriosis (excluded via laparoscopy or pathology), or malignancies (e.g., endometrial/cervical cancer). 2)History of pelvic surgery (e.g., myomectomy, uterine artery embolization) within the past 6 months. 3)Use of hormonal medications (e.g., GnRH agonists/antagonists, oral contraceptives, or progesterone therapy) within the past 3 months. 4) Severe systemic diseases (e.g., autoimmune disorders, diabetes, hepatic/renal dysfunction) or

immunosuppressant use, to avoid confounding inflammatory/immune mechanisms. 5) Current pregnancy or lactation. 6) Insufficient clinical or imaging data (e.g., incomplete participant records).

IHC

Paraffin sections were deparaffinized, rehydrated, and subjected to antigen retrieval. Endogenous peroxidase activity was blocked. Sections were blocked with serum, then incubated overnight at 4 °C with primary antibodies: SMOC2 (1:200, #Ab198715, Abcam); CK1 (#16848–1-AP, Proteintech); Vimentin (#10366–1-AP, Proteintech); HRP-labeled secondary antibody (1:500, #BL003 A, Biosharp) was applied. DAB chromogen solution was used for signal detection, followed by hematoxylin counterstaining of nuclei. Sections were dehydration through graded ethanol (75%, 85%, 100% twice), butanol, and xylene (5 min each), then coverslipped and analyzed microscopically.

Masson staining

The Masson staining method was used to detect collagen fibers in tissue samples. Paraffin-embedded sections were deparaffinized and rehydrated through graded alcohols to distilled water. Sections were stained with Weigert's iron hematoxylin (5–10 min), differentiated with acid solution and rinsed. They were counterstained with Ponceau fuchsin (5–10 min), rinsed with weak acid solution (2:1 water-to-acid ratio), Briefly treated with phosphotungstic acid, and stained with Aniline blue (1–2 min) to highlight collagen. Sections were rapidly dehydrated in 95% ethanol, thoroughly dehydrated in absolute ethanol, cleared in xylene, and mounted with neutral resin.

Cell culture and transfection

Ectopic endometrial tissues from adenomyosis patients (Third People's Hospital of Luoyang, December 2021) were minced into 1 mm^3 fragments and enzymatically digested with 0.2% collagenase II at 37 °C for 1.5 h. Digested tissue was sequentially filtered through 70 μm and 100 μm nylon meshes to remove debris. Stromal cells in the filtrate were pelleted by centrifugation, resuspended in DMEM/F12, and plated on 10 cm dishes. Non-adherent blood cells were removed by PBS rinsing after 30-min incubation at 37 °C. Cells were cultured in DMEM/F12 supplemented with 10% FBS and 1% antibiotics. These cells were identified as ectopic endometrial stromal cells and cryopreserved. Thawed cells were cultured in DMEM/F12 with 20% FBS under 5% CO_2 at 37 °C.Three groups were established for the experiments: control group (blank), Over/si-NC group (negative control), Over/si-SMOC2 group (experimental).

For transfection, over/si-NC and over/si-SMOC2(0.1 μg) were diluted in 5 μl serum-free medium. Lipofectamine 2000 (0.3 μl) was diluted in 5 μl serum-free medium, gently mixed, and incubated at room temperature (RT) for 5 min. The diluted plasmid and Lipofectamine solutions were combined, mixed, and incubated at RT for 20 min. The Supernatant was aspirated from cells cultured in 6-well plates. Cells were washed with PBS, and 10 μl transfection mixture + 90 μl complete medium were added to each well. Cells were incubated in a 37 °C, 5% CO_2 incubator for 24 h. The Supernatant was aspirated, cells were washed twice with PBS, and 100 μl fresh complete medium was added per well. The biological replicates were conducted three times.

CCK- 8 test

After transfection, cells were incubated for 24, 48, 72, and 96 h. CCK- 8 solution(15 μl)was added to each well and incubated at 37 °C, in a 5% CO2 for 2 h. The OD value was measured at 450 nm using a microplate reader. The measured OD value were used to analyze cell proliferation. The biological replicates were conducted three times.

Real-time PCR analysis

Total RNA was isolated from the over/si-NC and over/si-SMOC2 transfected cells using Trizol reagent (Bio sharp, shanghai, China), and cDNA synthesis was performed using the reverse transcription kit (Ye SEN, Shanghai, China). EMT-related factor gene expression was evaluated by real-time PCR using SYBR Green Master Mix (Targetmol). Oligonucleotide primers were synthesized by Sheng-gong Corporation (Shanghai, China). Expression values were normalized to the geometric mean of β-actin measurements and the quantification was done with the 2-ΔΔCt method. The biological replicates were conducted three times.

Western blot analysis

Cells from the over/si-NC and over/si-SMOC2 groups were collected 48 h post-transfection. Total protein was extracted using RIPA lysis buffer and quantified.

60 μg of protein per sample was separated by SDS-PAGE and transferred to a PVDF membrane. Membranes were blocked with 5% skim milk for 2 h, incubated with primary antibody at 4 °C for 24 h, followed by HRP-conjugated secondary antibody at room temperature for 1.5 h. Band intensity was quantified using Quantity One software. The loading control is β-actin. Target protein expression was normalized to β-actin (target band gray value/β-actin band gray value). The biological replicates were conducted three times.

Statistical analysis

Data were analyzed using R 4.3.1. Count data were described using cases, rates, or percentages. we compared the distributions of continuous variables between two groups using the Wilcoxon test and among more than two groups using the Kruskal–Wallis test. The Chi-square test was used for comparisons among categorical data. For descriptive statistics, boxplots were used to graphically depict groups of SMOC2 immunoreactivity data, analyzed by T-test. In cell studies, data were statistically analyzed using GraphPad Prism 8.0 software, with one-way ANOVA followed by Tukey's post hoc test, $p < 0.05$ was considered statistically significant.

Results

Clinicopathological data

The characteristics of the patients and the control group are shown in Table 1. The control group was comparable to the adenomyosis patients in terms of menstrual period and parity, but the control group was significantly younger than the adenomyosis patients, with less dysmenorrhea, normal menstrual flow, and no manifestations of endometriosis (Table 1). Menstrual flow was categorized into three classes: light, moderate, and heavy, based on the frequency of sanitary pad changes (< 3 times/day, 3–6 times/day, or >6 times/day, respectively) [11].

Evidence for fibrosis in adenomyotic lesions

Masson staining was performed on adenomyosis and control endometrium. In the control uterus, fibrotic tissue staining was negative, while the adenomyosis lesions contained extensive collagen fibers (Fig. 1). The Masson staining of adenomyotic lesions showed that most of the interstitial compartment was stained red, indicating myofibers or epithelial cells, while blue fibers indicated collagen fibers. These results suggested extensive fibrosis in the tissues of patients with adenomyosis.

Evidence of positive expression of SMOC2 in ectopic endometrial tissue

Protein expression of SMOC2 in the ectopic endometrium of ADM and normal patients was analyzed by immunohistochemistry (Fig. 2A). The percentage of positive area and H-SCORE ($\sum$ (pi $\times$i) =(percentage of weak intensity $\times 1$) +(percentage of moderate intensity $\times 2$) +(percentage of strong intensity $\times 3$) were analyzed (Fig. 2B). SMOC2 expression was higher in ADM patients than in normal patients, with statistically significant differences. These results suggested that SMOC2 expression in the ectopic endometrium of ADM patients was higher than in normal patients in terms of staining intensity and area.

Evidence of the correlation between SMOC2 and EMT-related factors in Ectopic Endometrial Cells

Cultured ADM ectopic endometrial cells were isolated by differential adherence for immunocytochemical identification. ADM ectopic endometrial cells were identified using specific markers CK1 (cytokeratin 1) and Vim (Vimentin). CK1 is an epithelial cell marker, typically expressed in normal endometrial cells, while Vim is a mesenchymal cell marker, typically expressed in ectopic

Table 1 Characteristics of patients recruited with adenomyosis and controls

	Control ($n=30$)	Adenomyosis ($n=35$)	χ^2	*P*
Age (year)			10.519	0.001
≤ 40	13 (43.3)	3 (8.3)		
> 40	17 (56.7)	32 (91.7)		
Maternity (times)			0.229	0.632
≤ 2	7 (23.3)	11 (30.6)		
> 3	23 (76.7)	24 (69.4)		
The degree of menstrual cramps			65.000	< 0.001
None	30 (100.0)	0 (0.0)		
Mild/moderate	0 (0.0)	20 (61.1)		
Seriousness	0 (0.0)	15 (38.9)		
Menstrual flow			23.638	< 0.001
Normal	25 (83.3)	9 (25.0)		
More than normal	5 (16.7)	26 (75.0)		
Menstrual cycle			0.015	0.901
< 28	9 (30.0)	10 (30.6)		
≥ 28	21 (70.0)	25 (69.4)		

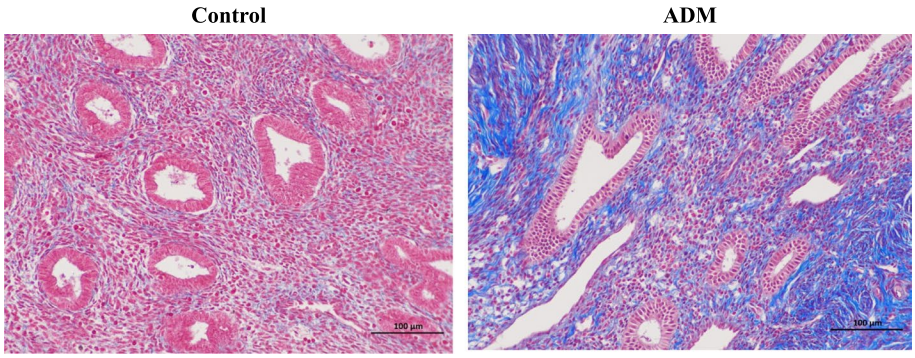

Fig. 1 Evidence for increased fibrotic content in adenomyosis. In Masson staining, collagens are stained blue, while smooth muscle and epithelial cells are stained purple. No blue collagen fibers are seen in control endometrium, while collagen fibers are prominent in the stromal component of ectopic endometrium from women with adenomyosis. Magnification: ×200.Scale bar = 100 μm

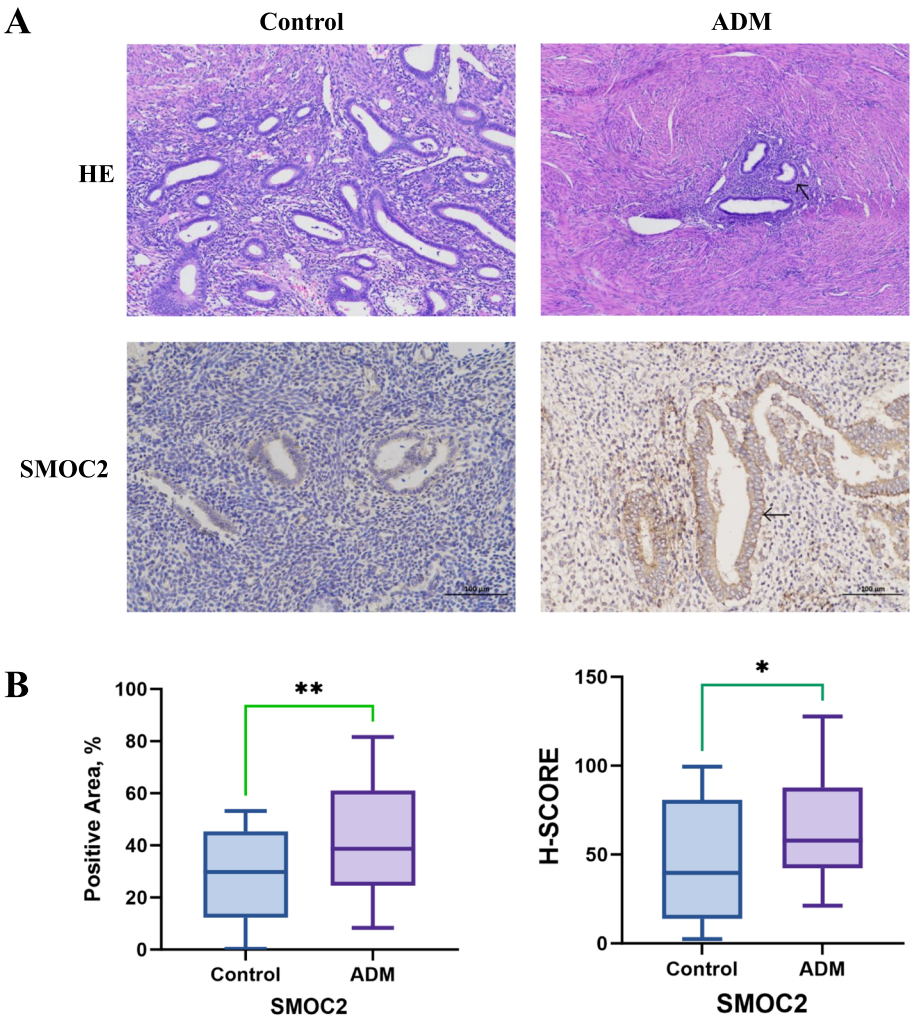

Fig. 2 Evidence of positive expression of SMOC2 in ectopic endometrial tissue. **A** HE staining and SMOC2 immunoreactivity staining in ADM patients (ectopic endometrial tissue indicated by arrows) and normal endometrial tissues; SMOC2 expression on glandular epithelial cell membranes (indicated by arrows). Magnification: ×200.Scale bar = 100 μm. **B** Boxplots of SMOC2 Positive Area (** $p < 0.05$) and H-SCORE (percentage of weak intensity × 1 + percentage of moderate intensity × 2 + percentage of strong intensity × 3) in adenomyotic lesions and control endometrium

endometrial cell. Microscope observation showed that CK1 antibody identified ADM ectopic endometrial cells as negative (colorless), while Vim antibody identified them as positive (brown), with a nuclear staining positivity rate of 94.47% (Fig. 3A). These results confirmed the successful isolation and culture of ADM ectopic endometrial cells, which were used for subsequent experiments.

In the CCK- 8 cell proliferation assay (Fig. 3B), the over-SMOC2 group demonstrated a significant increase in cell viability at 24, 48, 72, and 96 h compared to the over-NC group (*** $p < 0.01$), indicating a time-dependent effect. These results suggested that SMOC2 promotes cell proliferation.

In RT-PCR experiments, overexpression of SMOC2 significantly increased mRNA levels of SMOC2, N-cadherin, and α-SMA, while decreasing E-cadherin mRNA levels compared to the over-NC (****$p < 0.001$, Fig. 4A). Western blot analysis confirmed that overexpression of SMOC2 significantly increased protein levels of α-SMA and N-cadherin, while decreasing E-cadherin protein levels compared to the over-NC (****$p < 0.001$, Fig. 4B). These results indicated that SMOC2 overexpression promotes EMT by upregulated mesenchymal markers (N-cadherin and α-SMA) and downregulating the epithelial marker (E-cadherin).

For SMOC2 knockdown, si-SMOC2 - 1 was the most efficient among the tested siRNAs and it was used in subsequent experiments (Fig. 5A).

Primary-cultured ADM ectopic endometrial cells were transfected with si-SMOC2 - 1, and RT-PCR analysis showed that si-SMOC2 - 1 group significantly decreased the mRNA levels of α-SMA, N-cadherin, while increasing E-cadherin mRNA levels compared to the si-NC group (****$p < 0.001$, Fig. 5B). Western blot analysis confirmed that si-SMOC2 - 1 significantly decreased protein levels of α-SMA, N-cadherin, while increasing E-cadherin protein level compared to the si-NC group (*** $p < 0.01$, Fig. 5C). These results indicated that silencing SMOC2 reversed the EMT process by downregulating mesenchymal markers (N-cadherin and α-SMA), while upregulating the epithelial marker (E-cadherin).

Evidence for activation of the MMP9 signaling pathway

We evaluated the mRNA and the protein expression levels of MMP9 in over-SMOC2/si-SMOC2 ectopic endometrial cell compared to controls. Overexpression of SMOC2 significantly increased MMP9 mRNA and

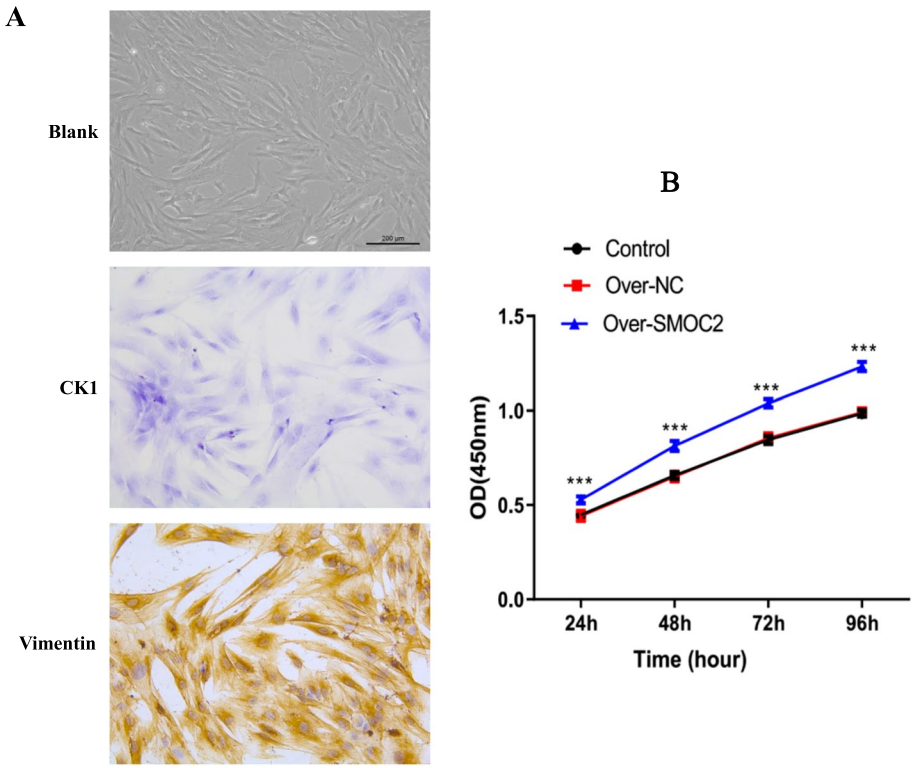

Fig. 3 Evidence of the identification of ADM ectopic endometrial cell and the effect of SMOC2 overexpression on cell proliferation. **A** Identification of ADM ectopic endometrial cells in primary culture; CK1 immunostaining was negative, while Vim staining was positive. **B** CCK-8 assay evaluate cell viability in over-SMOC2 cells. *** $p < 0.01$. The biological replicates were conducted three times

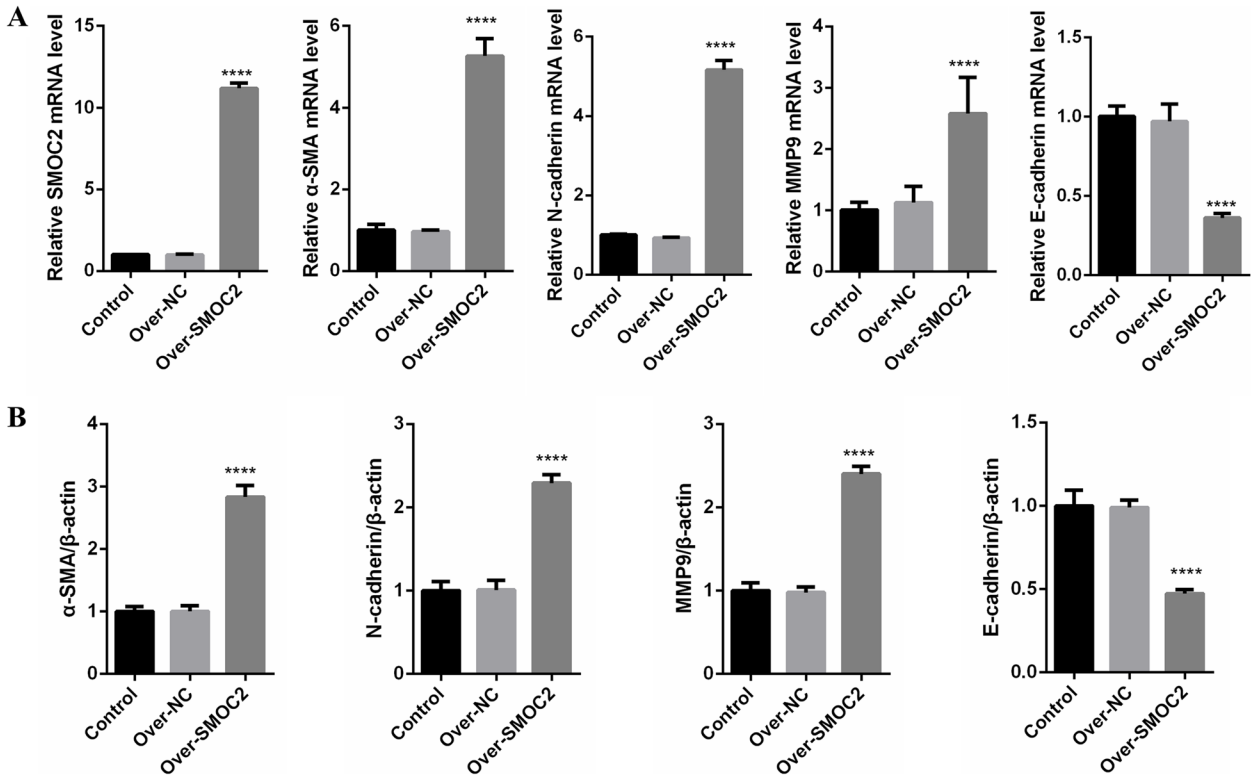

Fig. 4 Evidence of the correlation between SMOC2 overexpression and EMT-related factors in ectopic endometrial cell. **A** The mRNA expression of EMT-related factors and MMP9 in over-SMOC2 cells. The mRNA expression of N-cadherin, α-SMA, MMP9 were increased, but E-cadherin was decreased. **B** The protein expression of EMT-related factors and MMP9 in over-SMOC2 cells. Elevated N-cadherin, α -SMA, and MMP9 protein expression in adenomyosis by boxplots of results of their protein levels in control and ectopic endometrium, but E-cadherin was decreased. ****$p <$ 0.001. Band intensity was quantified using Quantity One software. Target protein expression was normalized to β-actin (target band gray value/ β-actin band gray value). The biological replicates were conducted three times

protein levels compared to controls (****$p < 0.001$, Fig. 4A and 4B). Conversely, SMOC2 knockdown significantly decreased MMP9 mRNA and protein levels compared to controls (***$p < 0.01$, Fig. 5B and C).

These findings suggest that SMOC2 activates the MMP9 signaling pathway, which is crucial for extracellular matrix (ECM) remodeling and may facilitate cell migration and invasion in adenomyosis.

Discussion

Adenomyosis, though benign, presents significant clinical challenges due to its poorly understood pathogenesis and limited treatment options. In preliminary work, we conducted microarray analysis on ectopic endometrial tissues from adenomyosis patients, revealing 147 upregulated and 324 downregulated differentially expressed genes. Following PCR validation and comprehensive literature review, SMOC2 was selected as the prioritized upregulated factor for subsequent mechanistic exploration due to its established association with extracellular matrix (ECM) remodeling and pathological fibrosis processes. This study investigated the role of SMOC2, an extracellular matrix (ECM) protein, in the progression of adenomyosis, particularly its impact on fibrosis, epithelial-mesenchymal transition (EMT), and cell migration. Our findings provide new insights into the molecular mechanisms underlying adenomyosis and highlight potential therapeutic targets.

SMOC2 and fibrosis in adenomyosis

Masson staining revealed significant fibrosis in adenomyosis tissues, accompanied by elevated SMOC2 expression in the ectopic endometrium. This aligns with previous studies linking SMOC2 to fibrotic processes in renal [12] and pulmonary fibrosis [13]. We propose that SMOC2 promotes ECM deposition and remodeling around ectopic endometrial foci, disrupting myometrial architecture and accelerating fibrosis. This mechanism may explain the progressive nature of adenomyosis and its resistance to conventional treatments. Therefore, targeting SMOC2 could offer a novel approach to mitigating fibrosis in adenomyosis.

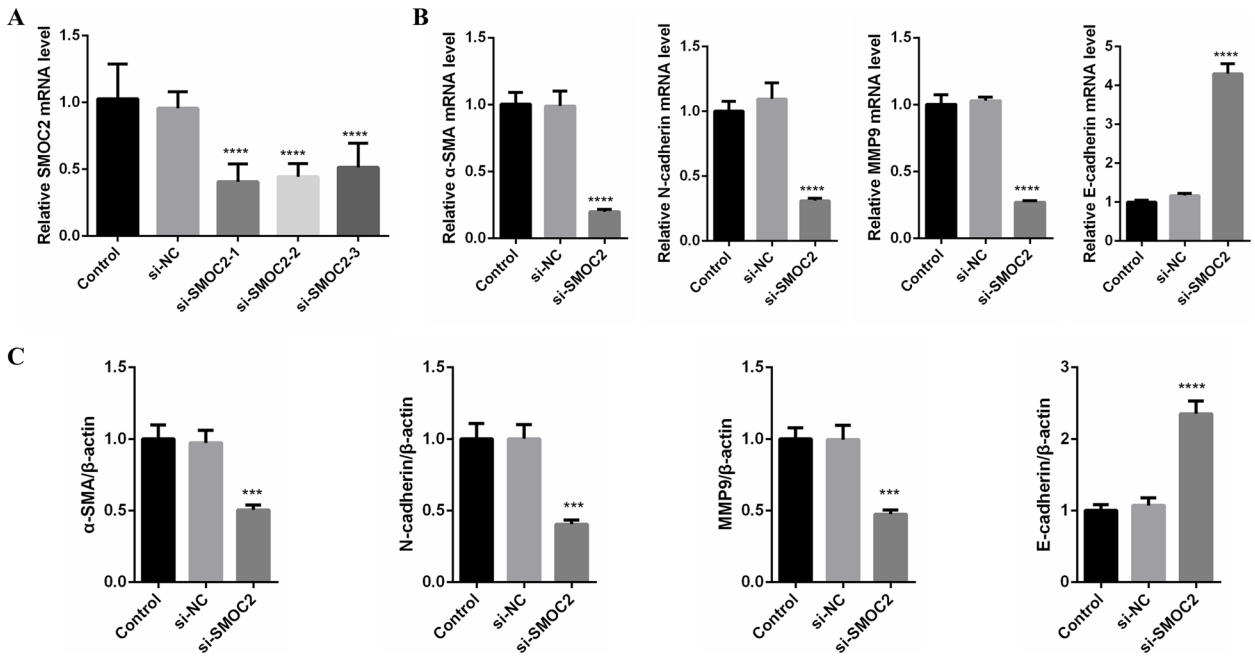

Fig. 5 Evidence of the correlation between si-SMOC2 and EMT-related factors expression in ectopic endometrial cell. **A** The expression level of SMOC2 mRNA was significantly reduced after silencing SMOC2, and the interference efficiency of si-SMOC2 - 1 was the highest by RT-PCR. **B** The mRNA expression of EMT-related factors and MMP9 in si-SMOC2 - 1 cell. The mRNA expression of N-cadherin, α-SMA, MMP9 were decreased, but E-cadherin was increased. **C** The protein expression of EMT-related factors and MMP9 in si-SMOC2 - 1 cell. Reduced N-cadherin, α -SMA, and MMP9 protein expression in adenomyosis by boxplots of results of their protein levels in control and ectopic endometrium, but E-cadherin was increased. ***$p < 0.01$, ****$p < 0.001$. Band intensity was quantified using Quantity One software. Target protein expression was normalized to β-actin (target band gray value/β-actin band gray value). The biological replicates were conducted three times

SMOC2 and EMT in adenomyosis

Adenomyosis displays the characteristic of invading the myometrium, similar to the invasive behavior of tumors, and is clinically well-known as the process of epithelial-mesenchymal transition (EMT) [14].Our study demonstrated that SMOC2 overexpression in primary adenomyosis cells upregulated mesenchymal markers (N-cadherin, α-SMA) and downregulated epithelial markers (E-cadherin), while SMOC2 knockdown reversed these effects. These findings suggest that SMOC2 drives EMT in adenomyosis, enhancing cell migration and invasion. This is consistent with studies in other cancers (e.g., renal cell carcinoma, lung adenocarcinoma, colon cancer, hepatocellular carcinoma et al.), where SMOC2 has been implicated in EMT-mediated metastasis [15–18]. The cyclic nature of EMT, regulated by hormonal fluctuations, may further exacerbate adenomyosis progression by promoting recurrent tissue damage and fibrosis.

SMOC2 and MMP9 signaling

MMP9 is a key member of the MMP family and plays a crucial role in remodeling the extracellular matrix (ECM) by promoting degradation. Our study identified a potential link between SMOC2 and MMP9, a key enzyme in ECM remodeling. Overexpression of SMOC2 upregulated MMP9, while SMOC2 knockdown reduced its expression. This suggests that SMOC2 may promote adenomyosis progression by activating MMP9-mediated ECM degradation, facilitating cell migration and invasion. MMP9 has been widely studied in cancer for its role in metastasis [19–21], and its involvement in adenomyosis underscores the disease's invasive nature. Many studies have demonstrated that suppressing MMP9 expression can inhibit the progression of epithelial-mesenchymal transition (EMT), thereby reducing local invasion and distant metastasis of malignant tumors, findings that are corroborated with our research results [22–25]. Further, Wang H et al. [26]found that MMP9 interacts with cell surface integrins, modulating adhesion and migration via integrin/FAK/MAPK signaling. Feng D et al. [27] demonstrated that SMOC2 binds integrin β3, activates FAK and paxillin downstream, and promotes EMT through this pathway. The interaction between SMOC2 and MMP9 highlights a potential signaling axis

via integrin/FAK/MAPK signaling that could be targeted for therapeutic intervention.

Therapeutic implications

Our findings suggest that SMOC2 plays a central role in adenomyosis progression by promoting fibrosis, EMT, and MMP9-mediated ECM degradation and remodeling. Targeting SMOC2 could disrupt these processes, offering a novel therapeutic strategy. For instance, small-molecule inhibitors or siRNA-based therapies could be developed to suppress SMOC2 expression or activity. Additionally, the link between SMOC2 and MMP9 opens avenues for combination therapies targeting both proteins simultaneously.

Limitations and future directions

While this study provides valuable insights, further research is needed to fully elucidate the mechanisms of SMOC2 in adenomyosis. In vivo studies are necessary to validate the role of SMOC2 in disease progression and to explore its potential as a therapeutic target. Additionally, the interplay between SMOC2, MMP9, and other ECM components warrants further investigation (e.g., cell migration/invasion assays et al.) to develop comprehensive treatment strategies.

Conclusions

In summary, our study suggests that the upregulation of SMOC2 in ectopic endometrial tissue of adenomyosis patients is closely related to the development of epithelial-mesenchymal transition (EMT) and may be mediated through the MMP9 pathway. This finding provides a theoretical framework for understanding the pathogenesis of adenomyosis and suggests that SMOC2 may serve as a valuable molecular biomarker for clinical diagnosis and personalized therapeutic strategies. However, further studies with larger sample sizes and additional experimental data are required to validate and extend these findings.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12905-025-03700-8.

Supplementary Material 1.

Supplementary Material 2.

Acknowledgements
All authors read and approved the final manuscript.

Authors' contributions
L.N.W wrote the main manuscript text. L.P.H designed this study. L.R, L.L, H.J.L and M.L.G prepared tables and figures. S.L.L edited and review the manuscript. X.M.N, S.V, S.N are responsible for editing the revised manuscript. All authors read and approved the final manuscript.

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Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
Ethical approval was provided by The Third People's Hospital of Luoyang in accordance with the Declaration of Helsinki. Approval number 2020–03 - 002-F01. All participants gave informed consent before taking part.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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