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Review Article

Moxibustion at Guanyuan (CV4) Inhibits Endometriosis Progression via Regulation of the Wnt/β-catenin Signalling Pathway in a Murine Model.

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Abstract

Objective: To observe the effects of moxibustion on the regulation of the Wnt/β-catenin signal-ling pathway, inhibition of cyst growth, and the expressions of uPA, MMP 9, E-cadherin, Axin, GSK3β, and β-catenin in a mouse model of endometriosis (EMs). The study aimed to explore the mechanism by which moxibustion regulates Wnt/β-catenin signalling to inhibit endometrial adhesion and reconstruction.

Methods: A total of 42 female BALB/c mice, aged 8 weeks, were divided into a control group (Group A) with 8 mice and an experimental group of 34 mice using random distribution. In the experimental group, EMs mouse model was induced by the auto-transplantation method and was further sub-divided into EMs model group (Group B), Cake-separated moxibustion at the *Guanyuan* acupoint (CV4) group (Group C), Agonist group (Group D) and Inhibitor group (Group E), having 8 mice per each sub-group. After successful modelling, the experimental groups underwent respective interventions for 14 consecutive days. Following treatment, the size of the cysts was observed, and the expression of key proteins such as uPA, MMP 9, E-cadherin, Axin, GSK3β, and β-catenin in ectopic endometrial tissues was assessed using west-ern blot.

Results: Relatively to the control group all parameters were considered normal. Comparatively to model Group B and the agonist Group D, the present results in moxibustion Group C and the inhibitor Group E show that: the number of writhing reactions was significantly reduced (both $P < 0.05$); the overall volume of ectopic cysts significantly decreased (both $P < 0.05$); the protein grayscale values of Axin, GSK3β, and β-catenin were significantly lower (all $P < 0.05$); the protein grayscale values of uPA and MMP 9 were significantly lower (all $P < 0.05$); the E-cadherin was significantly higher too (all $P < 0.05$).

Conclusion: Moxibustion at the CV4 acupoint can alleviate dysmenorrhea symptoms, inhibit the growth of endometriosis cysts, regulate the Wnt/β-catenin signalling pathway, suppress the ac-tivation of the Wnt/β-catenin signalling pathway, reduce the expression of downstream factors uPA and MMP 9, inhibit the adhesion and reconstruction of ectopic endometrial cells, promote the expression of E-cadherin, enhance the adhesion between normal endometrial cells, prevent the adhesion and cell migration process, and prevent the reconstruction of the matrix and base-ment membrane.

Keywords: Acupuncture; Moxibustion; Wnt/B-Catenin Signalling Pathway; MMP 9; Endometriosis; uPA; E-Cadherin; Axin; GSK3β; B-Catenin.

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Introduction

EMs is a complex disease characterized by the presence of endometrial glands and stroma outside the uterine cavity. It is reported that the prevalence of EMs in women of reproductive age is 15%, with 50% of infertile patients combined with EMs, and up to 80% of women with pelvic pain also combined with EMs. Clinical symptoms (e.g., dysmenorrhea, infertility, menorrhagia, and pain during sexual intercourse) produced by EMs have a severe impact on the quality of life and mental health of modern women [1]. Studies have shown that EMs has biological behaviours similar to malignant tumours, such as adhesion, invasion, angiogenesis and distant metastasis, which cause great pain to women while imposing a heavy medical and economic burden on society [2]. The specific pathogenesis of this disease has not been elucidated because of its pathogenetic complexity. To date, clinical treatment is mainly based on surgery and medication, which has many adverse effects, while surgery removes the lesions but can still recur and increase the risk of tissue adhesions, leading to impaired ovarian function or even loss [3]. Therefore, finding an effective treatment that aligns with long-term management principles is a hot topic in gynaecological clinical practice and basic research.

Traditional Chinese medicine (TCM) has always played a significant role in the treatment of EMs. Compared with traditional therapies, the use of TCM alone or in combination with traditional therapies can effectively minimize the side effects caused by traditional therapies [4]. Acupuncture and moxibustion, as a specialty treatment in TCM, have also been shown to have excellent therapeutic effects on EMs. Studies have found that acupuncture and moxibustion can effectively improve the pain-related symptoms associated with EMs, reduce the pain manifestations of patients, and improve their quality of life [5]. Clinical studies have shown that herb-partitioned moxibustion can effectively reduce the serum CA125 level in patients with EMs, and lead to the reduction of cyst diameter, improve the quality of life of patients [6]. This drew our attention, so we tried to figure out the specific role played by acupuncture and moxibustion in the treatment of EMs.

So far, the mechanism of EMs is not clear. The attachment-invasion-angiogenesis theory, or 3A theory, is a critical step in the pathological process of EMs. The initial critical step in the formation of EMs is the adhesion between endometrial stromal cells, which involves the remodeling of the extracellular matrix (ECM) by endometrial proteases. Interestingly, acupuncture and moxibustion have been found to effectively regulate ICAM-1, PPAR-γ, MMPs and various angiogenesis regulators, inhibiting the process of 3A pathological progress, thereby improving clinical symptoms of EMs and improving the pelvic microenvironment [7]. Studies have shown that aberrant activation of the Wnt/β-catenin signaling pathway promotes the development of the 3A pathological process in ectopic endometrial tissue. It was found that moxibustion could promote the proliferation of bone marrow cells by interfering Wnt/β-catenin signaling pathway in bone marrow suppression model mice [8]. For ankylosing spondylitis (AS), moxibustion can improve bone cachexia in AS mice by regulating GSK3β, β-catenin involved in Wnt/β-catenin signaling pathway protein expression [9].

Therefore, we hypothesized that herbal cake-separated moxibustion could inhibit the reconstitution of endometrial adhesions in EMs mice model by regulating the mechanism of Wnt/β-catenin signaling pathway. We used cake-separated moxibustion to study EMs mice. By observing the expression levels of key factors in the Wnt/β-catenin signaling pathway and target molecules such as matrix metalloproteinases (MMPs), E-cadherin, uPA, we investigated how cake-separated moxibustion exerts a therapeutic effect on EMs.

Materials and Methods

Experimental Animals and Grouping

Healthy SPF-grade female BALB/c mice (42 in total), aged 8 weeks and weighing 18-22g, were obtained from Hunan Slake Jingda Experimental Animals Co., Ltd. The animals were provided with an animal permit number: SCXK (Xiang) 2019-0004 and met the standards for animal experiments. They were housed in an SPF-grade laboratory barrier environment provided by the Experimental Animal Technology Centre of the Jiangxi University of Chinese Medicine. Housing conditions included 5 mice per cage, 12-hour light/dark cycle, room temperature (22±2°C), relative humidity of 45-65%, ventilation pressure difference >10 Pa, sterilized feed, and free access to food and water. After adaptive feeding for one week, 42 female BALB/c mice were randomly divided into the following groups: a control group (Group A) consisting of 8 mice and an experimental group consisting of 34 *EMs* mice. After modelling for 7 days, two mice were randomly euthanized to observe if the modelling was successful. Following successful modelling, the experimental EMs mice were randomly divided into different groups: *a* model

group (Group B) with 8 mice, a moxibustion with a herbal cake-separated group (Group C) with 8 mice, an agonist group (Group D) with 8 mice, and an inhibitor group (Group E) with 8 mice.

Chemicals and Reagents

Moxa (Jiangxi PoAi Technology Co., Ltd., China). Oxytocin Injection (H32025280, Nanjing Xinbai Pharmaceutical Co., Ltd., China), Estradiol Valerate Tablets (J20171038, Bayer Healthcare Co., Ltd. Guangzhou Branch, China).

BCA Protein Assay Kit (P0010, Beyotime Biotechnology, China); Recombinant Mouse sFRP-1 Protein (9019-SF, R&D Systems, USA); WNT-7A human (SRP3296, Sigma-Aldrich LLC, Germany); Anti-E Cadherin Antibody (ab231303, Abcam, UK); Anti-uPA Receptor/U-PAR Antibody (ab255815, Abcam, UK); Anti-beta Catenin Antibody (ab6301, Abcam, UK); MMP 9 Antibody (AF5228, Affinity Biosciences, USA); Anti-Axin 2 Antibody (ab109307, Abcam, UK); Anti-GSK3 beta Antibody (ab280376, Abcam, UK); Recombinant Anti-GAPDH antibody (GB15004-100, Servicebio, China). ECL Western Blotting Substrate (PE0010, Solarbio Life Sciences, China).

EMs Model Preparation

In this experiment, an animal model of EMs was established using the autolotransplantation method, following the modelling methods for EMs [10]. Two days before surgery, the mice were orally administered medroxyprogesterone acetate (0.5mg/kg) to synchronize them to the same oestrous cycle phase. Fasting for 12 hours was implemented on the day before modelling. During modelling, mice were anesthetized using a 1% sodium pentobarbital solution (10 mL/kg).

After disinfecting and preparing the abdominal area of the mice, a longitudinal incision was made in the abdominal wall to expose the left uterus. The uterus was ligated at both ends, and adherent fat and surrounding connective tissues were removed. A 1cm segment from the mid-section of the uterus was dissected into 5 mm×5 mm fragments. These uterine fragments were then securely fixed to the inner wall of the right abdominal cavity of the mouse using 6-0 sterile absorbable ophthalmic sutures. Additionally, 0.1 mL of cefuroxime sodium capsule solution was left in the left abdominal cavity before suturing the abdominal cavity and disinfecting the wound.

After modelling, the mice were intramuscularly injected with penicillin (2000 u per mouse) for three consecutive days. On the 7th day, two mice were randomly selected from the modelling group. After sterile laparotomy, the modelling lesions were observed. Successful modelling was confirmed when semi-transparent cysts with a diameter of 1-3 mm appeared at the lesion site, with visible blood vessels on the surface and fluid inside.

Intervention Methods

- Group A (Control group): Mice received 0.2 mL volumes of normal saline via tail vein injection every 3 days for a total of 4 injections.
- Group B (EMs model group): Model of EMs, mice received equal volumes of normal saline via tail vein injection every 3 days for a total of 4 injections.
- Group C (Cake-separated moxibustion group): EMs Mice were subjected to moxibustion with separated herbal cake on the CV4 acupoint. After securing the mice with one hand, the separated herbal cake moxibustion was performed. The moxibustion lasted for about 20 minutes, allowing the moxa stick to burn completely. Starting from the 14th day after modelling, this intervention was performed once a day for 14 consecutive days.
- Group D (Agonist group): EMs Mice were administered WNT-7A human (0.02 μg/mL diluted in 0.1% BSA) via tail vein injection every 3 days for a total of 4 injections [11].
- Group E (Inhibitor group): EMs Mice were administered Recombinant Mouse sFRP-1 Protein (0.05 μg/mL, diluted in DMSO <1:10000) via tail vein injection every 3 days for a total of 4 injections [11].

Sample Collection and Parameter Measurements

Writhing Response in Mice

Mice were administered oxytocin solution (2 U per mouse) via intraperitoneal injection 1 hour after treatment. The number of writhing episodes by the mice within 30 min after injection was recorded.

General observation of mice and volume of cysts

We observed the hair lustre and suppleness, water intake and diet, urination and defecation, and mobility, and recorded their body weights in each group of mice in our study. After euthanasia, ectopic endometrial tissue (cysts) was dissected. The length (mm), width (mm) and height (mm) of the cysts were measured using callipers.

Western Blot Analysis of Key Protein Expression in Ectopic Endometrial Tissue

Ectopic endometrial tissue samples from each experimental group were collected. Approximately 20 mg of tissue was added to 200 μL of RIPA lysis buffer with a ratio of 10 μL PMSF per 1 mL of RIPA lysis buffer. After homogenization based on the sample tissue weight, an appropriate amount of RIPA lysis buffer and PMSF was added to extract proteins. The protein concentration of the samples was determined, followed by denaturation at 100°C for 10 minutes. SDS-PAGE gels were prepared, loaded with samples, subjected to electrophoresis, and transferred onto membranes. The membranes were then blocked with 5 % skim milk for 2h at room temperature (RT) and then incubated overnight at 4°C with primary antibodies (GAPDH, 1:25000; MMP 9, β-catenin, E-cadherin, Axin, GSK3β and uPA,1:1000). After washing the membranes, secondary antibodies (1:3000) were applied for 2 h at RT. Chemiluminescent signals were measured using a gel imaging system (ChemiDoc XRS+, Bio-rad Laboratories, Inc., USA). The grayscale values of protein bands were quantitatively analysed via Image Pro Plus. GAPDH, serves as an internal reference for protein standardization in Western blot experiments. This involves dividing the protein content of each sample by the GAPDH content of that sample to obtain the relative protein content of each target protein in the sample. Then, comparisons are made between samples.

Statistical Analysis

Data analysis was performed using SPSS 25.0 (IBM, New York, USA), the experimental images were analysed using Image Pro Plus 6.0 (Media Cybernetics, USA) and the graph was carried out via Graphpad Prism 8.6 (GraphPad Software, CA, USA) software. Quantitative data are presented as mean ± standard deviation ($\bar{x} \pm s$). One-way analysis of variance (ANOVA) was used to determine intergroup differences, and LSD tests were conducted for post hoc comparisons. A significance level of $P < 0.05$ was considered statistically significant.

Results

Writhing Response in Mice

From Table 1 and Figure 1, it can be observed that the number of writhing was 0 in Group A and 43.14 ± 11.04 in Group B. Compared with Group B, the writhing response counts decreased significantly in Group C to 30.43 ± 4.72 ($P < 0.05$) and in Group E decreased to 22.71±7.52 ($P < 0.01$), while significantly increased in Group D to 59.29±11.19 ($P < 0.01$). When compared with Group D, both Group C and Group E also showed a significant decrease in writhing response count, with statistical significance (both $P < 0.01$).

Table 1. Writhing Response Counts of Mice in Each Experimental Group ($\bar{x} \pm s$).

Group	N	Writhing Count
Group A	8	0±0
Group B	8	43.14±11.04 $^{\triangle\blacktriangle}$
Group C	8	30.43±4.72 $^{\triangle\square}$
Group D	8	59.29±11.19 $^{\square\blacktriangle}$
Group E	8	22.71±7.52 $^{\triangle\square}$

Note: Compared with Group B$^{\triangle}$; Compared with Group C$^{\square}$. Compared between Group B$^{\blacktriangle}$ and D$^{\blacktriangle}$.

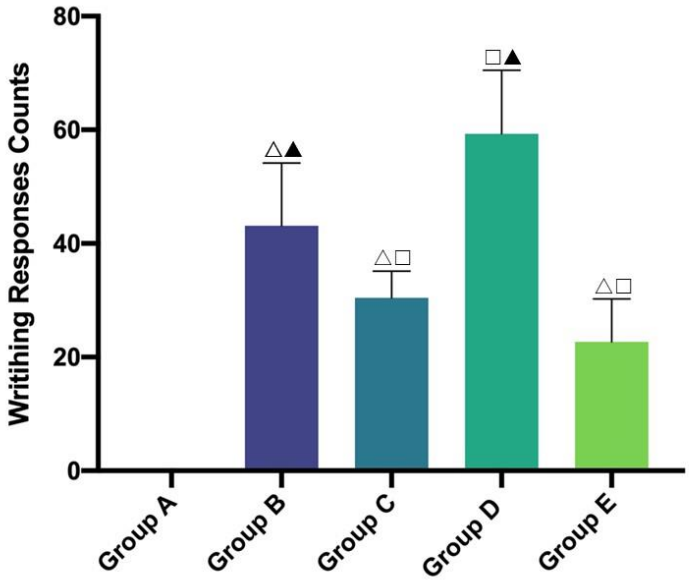

Figure 1. Writhing Response Counts of Mice in Each Experimental Group
Note: Compared with Group B△; Compared with Group C□. Compared between Group B▲ and D▲.

General observation of mice and volume of cysts in each group

We observed the general condition of mice in each group during our treatment. The mice in group A had soft hair, rapid response, normal diet and smooth bowel movements. While the mice in Group B, Group C, Group D and Group E had decreased food intake and defecation and delayed activity due to the operation. With the treatment, Group A remained normal, while mice in Group B, Group C and Group E were less active, preferred to gather in groups under the feed, drank less water, and had dull and unruly hair. In contrast, the mice in Group D were more active than before, with less clumping and clustering than in the other groups, and the abdominal hair was yellowed by the cakes, while the other hairs were smooth and shiny.

Table 2 shows the changes in body weight of mice before modelling, 2 weeks after modelling and 4 weeks after modelling. From the results, it can be obtained that there is no significant difference in the body weight of mice before and after modelling, which is not statistically significant.

Table 2: Weight changes in mice in each group ($\bar{x} \pm s$) (g)

Group	Pre-modelling	Weight after 2 weeks of model-ling	Weight after 4 weeks of modelling
Group A	23.81±1.18	23.995±1.01	22.44±1.07
Group B	23.76±1.12	22.1±1.62	22.46±1.30
Group C	23.87±1.19	22.54±0.78	21.08±1.02
Group D	23.61±1.16	22.66±1.28	22.39±1.22
Group E	23.94±1.18	23.43±0.99	23.2±0.90

Figure 2 shows the pictures of the removed cysts, and Table 3 shows the size of the cysts volume in each group of EMs mice.

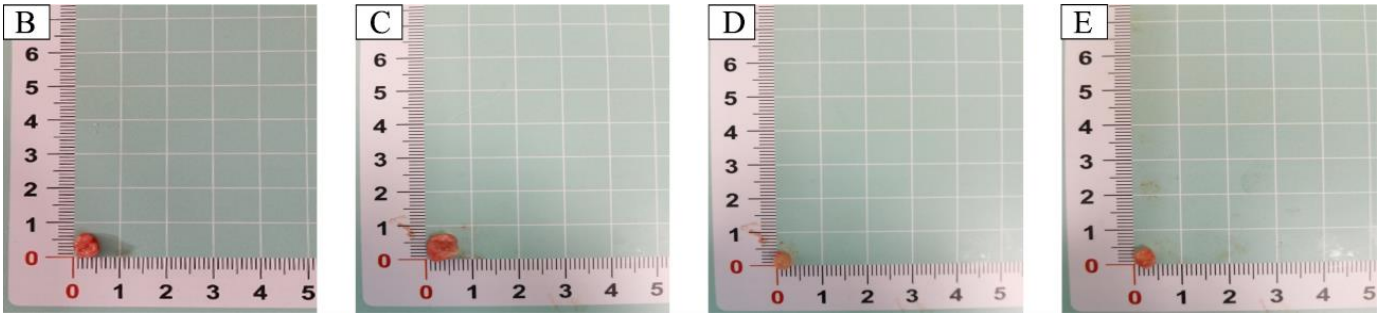

Figure 2. Sample Images of Cysts in Mice from Each Group named B, C, D, E. B: Images of cysts in Group B (EMs model group). C: Images of cysts in Group C (cake-separated moxibustion group). D: Images of cysts in Group D (agonist group). E: Images of cysts in Group E (inhibitor group). Group A is a control group without cysts.

Table 3. Volume of Ectopic Uterine Tissue (Cysts) in Mice ($\bar{x} \pm s$)

Group	N	Total Volume of Ectopic Uterine Tissue (mm^3)
Group A	8	0±0
Group B	8	10.25±2.50△▲
Group C	8	5.50±2.07△□
Group D	8	13.25±4.27□▲
Group E	8	4.55±0.80△□

Note: Compared with Group B△; Compared with Group D □.
Compared between Group B▲ and D▲ Group A is a control group without cysts.

Figure 2B-E shows the pictures of the removed cysts, and Table 3 shows the size of the cysts volume in each group of mice. The volume of the cysts in Group D of 13.25±4.27 was significantly larger than in Group B of 10.25±2.50 mm^3. Compared with Groups B and D, the cysts were significantly smaller in size in Group C with a volume of 5.50±2.07 mm^3 ($P < 0.01$) and in Group E with a volume of 4.55±0.80 mm^3 ($P < 0.01$).

Gray Values of Key Proteins in the Wnt/β-catenin Signalling Pathway in Ectopic Endometrial Tissues of Mice

The results in Figure 3 show the expressions of key proteins in the Wnt/β-catenin signalling pathway in each group of mice. The results showed that the expression of Axin, GSK3β, and β-catenin proteins was significantly increased in Group B mice compared to Group A ($P < 0.05$). Compared with Group B, the protein expression levels of Axin, GSK3β, and β-catenin in Groups C and E were decreased ($P< 0.05$). Compared with Group B, Group D showed increased levels of Axin, GSK3β, and β-catenin protein expression ($P<0.05$). After herbal cake-separated moxibustion in Group C and inhibitor effect in Group E, the protein expression levels of Axin, GSK3β, and β-catenin decreased compared with Group D ($P < 0.05$).

Comparison of E-cadherin, MMP-9, and uPA, Gray Values in Mice in Each Group

Next, the protein content of E-cadherin, MMP 9 and uPA in the cyst tissues of EMs mice in each group was examined. The results showed that compared with Group A, Group B tissues expressed increased levels of MMP 9 and uPA, while E-cadherin levels decreased ($P < 0.05$). Compared with Group B, Groups C and E showed decreased levels of MMP 9 and uPA expression, while E-cadherin levels increased ($P < 0.05$). In contrast, compared with Group B, the expression levels of E-cadherin were decreased, while the expressions of MMP 9 and uPA were significantly increased in Group D Comparing with Group D, the expression contents of E-cadherin increased, while MMP 9 and uPA in both Group C and E were significantly decreased than both ($P < 0.05$).

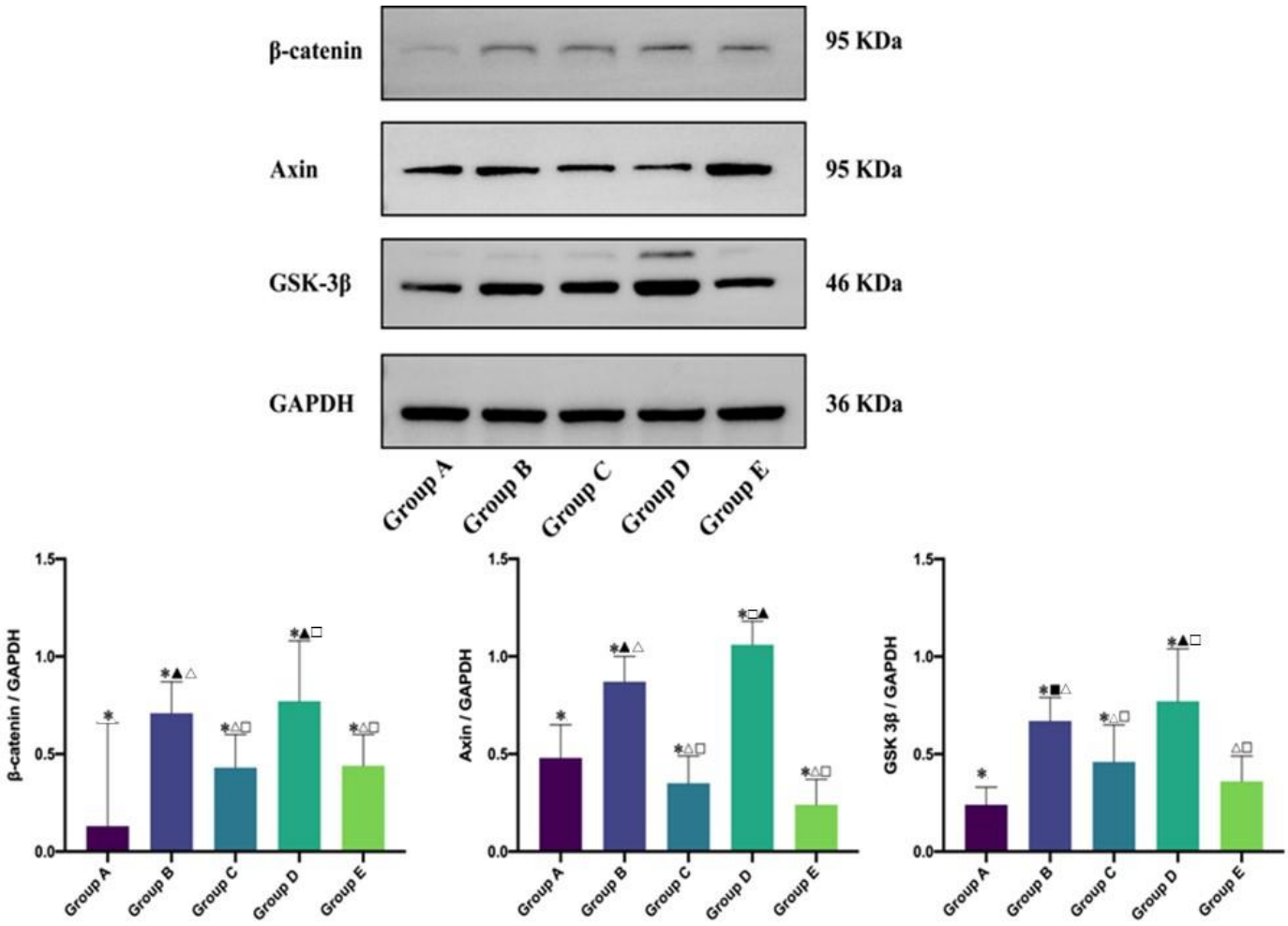

Figure 3. Comparison of Gray Values of Key Proteins in the Wnt/β-catenin Signalling Pathway
Note: Compared with Group A*; Compared with Group B△; Compared with Group D□. Compared between Groups B▲ and D▲.

Discussion

The prerequisite for the formation of EMs is the need for shed endometrial cells to break through three barriers: peritoneal fluid or ascites, peritoneal cells, and the extracellular matrix of peritoneal cells. These cells then implant and grow in the pelvic cavity, peritoneum, and other areas, forming ectopic lesions. Moxibustion therapy for EMs effectively reduces pelvic pain, shrinks lesion size, decreases recurrence, promotes and improves fertility, and has few adverse reactions, making it suitable for long-term management and showing promising prospects. However, the exact mechanism by which moxibustion inhibits the adhesion of endometrial cells is not yet fully understood. In our previous research [12], our team found that herbal cake-separated moxibustion could inhibit ICAM-1 expression, and promote PPAR-γ expression, thereby inhibiting the adhesion of endometrial cells and preventing angiogenesis.

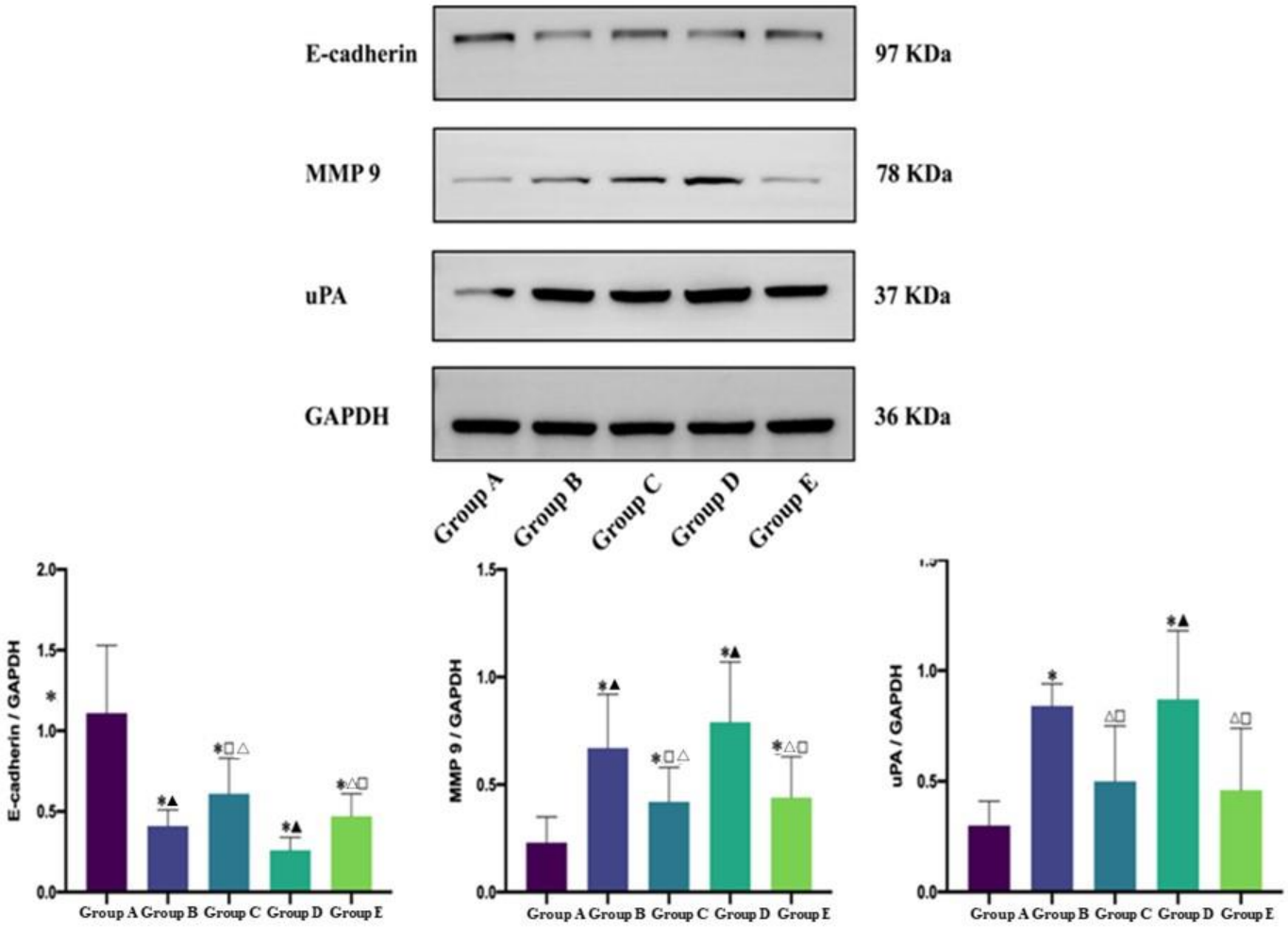

Figure 4: Gray Values of E-cadherin, uPA and MMP-9 Proteins in Each Group

Note: Compared with Group A*; Compared with Group B△; Compared with Group D□. Compared between Groups B▲ and D▲.

Possible Mechanisms of Moxibustion at the CV4 Acupoint in Treating EMs

Herbal cakes are composed of ingredients such as cinnamon, aconite, and safflower, ground in specific proportions and mixed with yellow wine to form uniform cake-like structures. They possess the functions of promoting blood circulation, removing blood stasis, warming and nourishing the lower abdomen, regulating Qi, and alleviating symptoms. The CV4 acupoint is a vital point in the human body. Moxibustion at the CV4 acupoint can tonify the lower abdomen, nourish the kidneys, invigorate the yang, activate blood circulation, and relieve pain. Research has shown that the CV4 acupoint is the most frequently used acupuncture point in the treatment of endometriosis [13,14]. Studies have also shown that herbal cake-separated moxibustion applied to the CV4 acupoint combines both the effects of moxibustion and herbal effects [15]. This combination stimulates the acupuncture point, generates moxibustion biological effects, affects tissue cell metabolism, and achieves the goals of promoting Qi circulation, alleviating pain, and activating blood circulation while eliminating stasis. Research has found that the sensory nerves entering CV4 converge and overlap in the spinal ganglia between the third lumbar and fifth sacral vertebrae [16,17]. These findings may be one of the effective mechanisms by which herbal cake-separated moxibustion applied to the CV4 acupoint treats endometriosis.

Reduction in Writhing Response Frequency and Inhibition of Cyst Growth by Herbal Cake-separated Moxibustion

A study has shown that the dysmenorrhea model induced by surgery, oestrogen, and oxytocin from a pathological perspective meets the diagnostic criteria for EMs and, from a behavioural perspective, aligns with the standards for dysmenorrhea models [18,19]. Research has indicated that herbal cake-separated moxibustion at the CV4 acupoint has achieved good results in treating EMs by alleviating dysmenorrhea and inhibiting cyst growth [20]. In this study, both the writhing response and the number of writhing mice in the herbal cake group and inhibitor group were significantly lower than those in the model-control group and the agonist group. The cyst volume in the moxibustion group was 5.50 ± 2.07 mm^3, significantly lower than the cyst volumes in the other groups. This suggests that herbal cake-separated moxibustion can alleviate dysmenorrhea symptoms and inhibit cyst growth.

Inhibition of the Activation of the Canonical Wnt/β-catenin Signalling Pathway by Herbal Cake-separated Moxibustion

β-catenin is a crucial "switch" that activates the canonical Wnt/β-catenin signalling pathway, determining whether this signalling pathway is active or inactive. Wnt proteins serve as the "keys" to this signalling pathway. The Wnt/β-catenin signalling pathway consists of Wnt proteins, a degradation complex (mainly composed of Axin, Adenomatous polyposis coli (APC) protein, glycogen synthase kinase 3β (GSK3β), and casein kinase 1 (CK1)), and β-catenin. Under physiological conditions where Wnt protein is absent or in the presence of its inhibitor, Recombinant Mouse sFRP-1 Protein, the complex directly binds to Wnt protein, preventing its binding to its receptor. The intracellular degradation complex then mediates the phosphorylation and subsequent ubiquitin-mediated degradation of β-catenin. Consequently, β-catenin in the cells is maintained at a low concentration and low level, preventing its translocation into the cell nucleus and inhibiting the expression of downstream target genes, effectively "closing" the Wnt/β-catenin signalling pathway [21]. Under pathological conditions or in response to its agonist Wnt-7a, Wnt binds to its primary receptor, Frizzled, and low-density lipoprotein receptor-related protein (LRP), activating the intracellular Dsh protein, releasing GSK-3β, leading to the dissociation of the APC/Axin/GSK-3β/CK1 complex, inhibiting β-catenin phosphorylation and degradation [22]. Accumulated β-catenin then enters the cell nucleus, recruits a series of co-activators by binding to the transcription factor TCF/LEF, activates the transcription of downstream target genes, and promotes cell proliferation and differentiation.

This study demonstrates that β-catenin expression is low in the normal-control (A) group, and Axin expression in the EMs model group (B) and the agonist group (D) is significantly higher than in the other groups, consistent with the results reported by Virivskaya *et al.* [23] and Pazhohan *et al.* [24]. The β-catenin expression in the moxibustion group (C) with herbal cakes is significantly lower than in the EMs model group and the agonist group, similar to the findings reported by Liu *et al.* [25]. The results of this study suggest that herbal cake-separated moxibustion can regulate the "switch" of the Wnt signalling pathway, β-catenin, and the expression levels of other key factors in the Wnt signalling pathway, inhibiting the activation of the Wnt signalling pathway and preventing the adhesion process of ectopic endometrium.

Inhibition of Uterine Endometrial Adhesion Reconstruction in EMs Mice by Herbal Cake-separated Moxibustion

The adhesion of endometrial cells to the extracellular matrix (ECM) marks the occurrence of endometriosis in situ. Most researchers believe that a prerequisite for the formation of in-situ endometriosis is a decrease in the adhesion force between in-situ endometrial cells and an increase in their adhesion to the ECM. When uterine endometrial tissue adheres to the peritoneal surface, endometrial proteolytic enzymes begin to remodel the ECM. The detachment of endometrial cells from each other follows, along with the adhesion between endometrial cells and the ECM [26,27], leading to the invasion of endometrial tissue into the peritoneal stroma. The remodelling of the ECM requires the involvement of two families of proteolytic enzymes, namely MMPs, uPA, and tPA [28,29]. Therefore, herbal cake-separated moxibustion can reduce the occurrence of endometriosis by inhibiting the reconstruction of endometrial adhesion in EMs mice.

Promotion of E-Cadherin Expression by Herbal cake-separated moxibustion to Prevent Cell Adhesion

E-cadherin is a calcium-dependent transmembrane glycoprotein and an important member of the cadherin family that specifically participates in cell adhesion between epithelial cells [30]. The connection between epithelial cells must be maintained

by E-cadherin. When E-cadherin levels increase, the connections between cells become tighter, and cell migration is inhibited. Conversely, when there is an abnormal increase in processes like protein hydrolysis, E-cadherin levels decrease, reducing the adhesion force between endometrial cells and enhancing their migratory ability, ultimately leading to the migration and recurrence of tumour cells [31-33]. Endometrial cells in EMs patients also exhibit abnormal expression of E-cadherin. Studies have shown that the expression of E-cadherin in the endometrial glandular epithelium of EMs patients is significantly reduced [34]. The results of this study show that the expression level of E-cadherin in the normal group is relatively low, similar to previous research results [35]. The expression of E-cadherin in the model group (B) and the agonist group (E) is significantly lower than that in the normal group, similar to the results of Scotti *et al.* [34]. However, the expression of E-cadherin in the herbal cake-separated moxibustion group (C) and in the inhibitor group (E) is significantly higher than that in the model group and the agonist group, indicating that herbal cake-separated moxibustion can promote the expression of E-cadherin, increase the adhesion force between normal endometrial cells, and prevent the migratory movement of ectopic endometrial cells.

Inhibition of MMP 9 Expression by Herbal Cake-separated Moxibustion to Prevent the Formation of Ectopic Endometrial Cell Skeleton

Matrix Metalloproteinases (MMPs) are a highly conserved family of proteolytic enzymes belonging to the zinc-dependent endopeptidase family and play a crucial role in regulating the dynamic balance of the ECM [29]. MMPs can effectively degrade almost all components of the Extracellular Matrix (ECM), including glycosaminoglycans, cell adhesion molecules, and others [36]. Under normal physiological conditions, MMPs maintain the normal structure and function of the ECM, participating in cell migration and ECM remodelling, with very low levels of MMP expression. In pathological conditions, MMP 9 can degrade components such as type IV and type V collagen, and gelatine in the ECM, disrupting the ECM and basement membrane. It cleaves the extracellular segment of E-cadherin to generate soluble E-cadherin, regulating cell adhesion among endometrial tissue cells [37]. Huang *et al.* [38] and Collette *et al.* [39]state that the expression of MMP 9 in the serum of EMs patients was significantly higher than that in the normal group, indicating that MMP 9 is involved in the hydrolysis of the ECM in ectopic endometrium. In this study, the normal group (A) exhibited low-level expression of MMP 9. The expression of MMP 9 in the herbal cake-separated moxibustion group and the inhibitor group was significantly lower than that in the model group (B) and the agonist group (D), indicating that herbal cake-separated moxibustion can regulate the expression of MMP 9, inhibit the hydrolysis of the ECM in ectopic endometrium, and halt the process of endometrial reconstruction in uterine endometriosis. Research has shown that blocking the Wnt/β-catenin signalling pathway significantly inhibits the expression of the downstream factor MMP 9 [25]. The results of this study suggest that herbal cake-separated moxibustion may inhibit the activation of the Wnt signalling pathway, suppress the expression of the downstream factor MMP 9, and prevent the adhesion and reconstruction of ectopic endometrial tissue.

Inhibition of uPA Expression by Herbal Cake-separated Moxibustion to Prevent Matrix and Basement Membrane Reconstruction

Urokinase-type plasminogen activator (uPA) is a serine protease that can bind to uPA receptors (uPAR) on the cell surface to become active uPA. It then catalyses plasminogen into plasmin, which, in turn, degrades fibronectin, laminin, type III collagen, and other components of the ECM and basement membrane. This process generates biologically active molecules, including those involved in signalling and adhesion [40-42]. Studies by Bai *et al.* [43] and Chen *et al.* [44] have found that the expression of uPA in ectopic endometrial tissue of EMs patients is higher than in eutopic endometrium. This suggests that uPA may be involved in the pathogenesis of EMs, specifically, the elevated expression of uPA degrades the extracellular matrix and basement membrane surrounding cells, providing the necessary conditions for adhesion, invasion, migration, and the formation of ectopic lesions in endometriosis.

The results of this study show that uPA is expressed at low levels in the normal group (A), while its expression in the model group (B) and the agonist group (D) is significantly higher than in the herbal cake-separated moxibustion (C) and inhibitor groups (E). It can be inferred that herbal cake-separated moxibustion may regulate and inhibit the expression of uPA in ectopic endometrium, reduce adhesion between ectopic endometrium and the ECM, and block the transmission of relevant signalling pathways, similar to the results of Bai *et al.* [43] and Chen *et al.* [44]. Research has shown that the activation of the Wnt signalling pathway can promote the expression of uPA [45,46]. The results of this study suggest that herbal cake-separated moxibustion may

potentially inhibit the activation of the Wnt signalling pathway, suppress the expression of the downstream factor uPA, prevent ectopic endometrial adhesion, and halt the reconstruction of the matrix and basement membrane.

Conclusion

This study supports and suggests that herbal cake-separated moxibustion can significantly inhibit the growth of EMs cysts, regulate the switch β-catenin of the Wnt signalling pathway, suppress the activation of the Wnt/β-catenin signalling pathway, reduce the expression of downstream factors uPA and MMP 9, promote the expression of E-cadherin, enhance the adhesion between normal endometrial cells, and prevent ectopic endometrial adhesion, as well as block the reconstruction of the matrix and basement membrane.

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References

1. Lin YH, Chen YH, Chang HY, Au HK, Tzeng CR, Huang YH. Chronic Niche Inflammation in Endometriosis-Associated Infertility: Current Understanding and Future Therapeutic Strategies. International journal of molecular sciences. 2018;19(8). doi: https://doi.org/10.3390/ijms19082385
2. Smolarz B, Szyllo K, Romanowicz H. Endometriosis: Epidemiology, Classification, Pathogenesis, Treatment and Genetics (Review of Literature). International journal of molecular sciences. 2021;22(19). doi: https://doi.org/10.3390/ijms221910554
3. Bedaiwy MA, Alfaraj S, Yong P, Casper R. New developments in the medical treatment of endometriosis. Fertil Steril. 2017;107(3):555-65. doi: https://doi.org/10.1016/j.fertnstert.2016.12.025
4. Lin Y, Wu L, Zhao R, Chung PW, Wang CC. Chinese Herbal Medicine, Alternative or Complementary, for Endometriosis-Associated Pain: A Meta-Analysis. Am J Chin Med. 2023;51(4):807-32. doi: https://doi.org/10.1142/S0192415X23500386
5. Wang Y, Coyle ME, Hong M, He S, Zhang AL, Guo X, et al. Acupuncture and moxibustion for endometriosis: A systematic review and analysis. Complement Ther Med. 2023;76:102963. doi: https://doi.org/10.1016/j.ctim.2023.102963
6. Hu H, Chen L, Jin X, Li R, Fang J. Effect of herb-partitioned moxibustion on pain and quality of life in women with endometriosis: a protocol for a randomized clinical trial. Journal of traditional Chinese medicine = Chung i tsa chih ying wen pan. 2020;40(2):324-32.
7. Xi J, Cao XX, Gao YL, Cheng J, Xia YB. [Research progress on the mechanism of acupuncture and moxibustion in treatment of endometriosis]. Zhen ci yan jiu = Acupuncture research. 2019;44(10):772-6. doi: https://doi.org/10.13702/j.1000-0607.190023
8. Zhu T, Cheng YT, Ma YZ, Zhao S, Li X. [Effect of wheat-grain moxibustion on Wnt/beta-catenin signaling pathway in bone marrow cell in mice with bone marrow inhibition]. Zhongguo zhen jiu = Chinese acupuncture & moxibustion. 2023;43(1):67-71. doi: https://doi.org/10.13703/j.0255-2930.20220527-0001
9. Xu X, Yang H, Chen JN, Hua LJ, Wang RY, Liu T, et al. Moxibustion attenuates inflammation and alleviates axial spondyloarthritis in mice: Possible role of APOE in the inhibition of the Wnt pathway. J Tradit Complement Med. 2022;12(5):518-28. doi: https://doi.org/10.1016/j.jtcme.2022.04.002

10. Burns KA, Pearson AM, Slack JL, Por ED, Scribner AN, Eti NA, et al. Endometriosis in the Mouse: Challenges and Progress Toward a 'Best Fit' Murine Model. Front Physiol. 2021;12:806574. doi: https://doi.org/10.3389/fphys.2021.806574
11. Gogolla N, Galimberti I, Deguchi Y, Caroni P. Wnt signaling mediates experience-related regulation of synapse numbers and mossy fiber connectivities in the adult hippocampus. Neuron. 2009;62(4):510-25. doi: https://doi.org/10.1016/j.neuron.2009.04.022
12. Miao FR, Zhang P, Zhao CJ. [Effect of Herbal-cake-separated moxibustion on macrophage phagocytosis and activation of VEGF/VEGFR pathway in endometriosis rats]. Zhen ci yan jiu = Acupuncture research. 2022;47(2):115-20. doi: https://doi.org/10.13702/j.1000-0607.201146
13. Zhao J, Liang Y, Wang X. Study on the Application Law of Comprehensive Acupuncture and Moxibustion Therapy for Endometriosis Based on Data Mining. Chinese General Practice. 2018;21(1):104-8.
14. Xiao X, Zhou Z, Wang P. Meta-Analysis of Acupuncture and Moxibustion in the Treatment of Endometriosis. Jiangxi Traditional Chinese Medicine. 2017;48(2):50-3.
15. Zhou Z, Xiao XW, Wang P. Effects of Moxibustion with Herbal Cake Separation on Estrogen-Related Factors in Rats with Endometriosis. Journal of Shanghai University of Traditional Chinese Medicine. 2017;31(6):80-5.
16. Keast JR, De Groat WC. Segmental distribution and peptide content of primary afferent neurons innervating the urogenital organs and colon of male rats. J Comp Neurol. 1992;319(4):615-23. doi: https://doi.org/10.1002/cne.903190411
17. Zhou J, Jin Z, Tao Z. Segmental Distribution of Primary Afferent Neurons in the Spinal Ganglia. Shanghai Journal of Acupuncture and Moxibustion. 2001(3):40-1.
18. Hong F, He G, Zhang M, Yu B, Chai C. The Establishment of a Mouse Model of Recurrent Primary Dysmenorrhea. International journal of molecular sciences. 2022;23(11). doi: https://doi.org/10.3390/ijms23116128
19. SUN H-Y. The establishment of the dysmenorrhea model in mice. Chinese Pharmacological Bulletin. 2002:233-6.
20. Qin X, Liu Q, Lu S. Effect of Moxibustion with Herbal Cake Separation Combined with Leuprorelin on Serum VEGF Pathway in Patients with Endometriosis. Global Traditional Chinese Medicine. 2019;12(4):598-600.
21. Feng Q, Gao N. Keeping Wnt signalosome in check by vesicular traffic. Journal of cellular physiology. 2015;230(6):1170-80. doi: https://doi.org/10.1002/jcp.24853
22. Aristizabal-Pachon AF, Carvalho TI, Carrara HH, Andrade J, Takahashi CS. AXIN2 Polymorphisms, the beta-Catenin Destruction Complex Expression Profile and Breast Cancer Susceptibility. Asian Pac J Cancer Prev. 2015;16(16):7277-84. doi: https://doi.org/10.7314/apjcp.2015.16.16.7277
23. Virivskaya E, Ignatko I, Snimshchikova I, Bakhtiyarov K, Zueva A, Kapyrina T. Wnt/β-catenin signaling pathway role in the endometriosis pathogenesis. Obstetrics, Gynecology and Reproduction. 2025;19(4):534-44.
24. Pazhohan A, Amidi F, Akbari-Asbagh F, Seyedrezazadeh E, Farzadi L, Khodarahmin M, et al. The Wnt/β-catenin signaling in endometriosis, the expression of total and active forms of β-catenin, total and inactive forms of glycogen synthase kinase-3β, WNT7a and DICKKOPF-1. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2018;220:1-5.
25. Liu R, Liu Y, Yu L. The Impact of Targeted siRNA Inhibition of the Wnt/β-Catenin Signaling Pathway on the Expression of VEGF and MMP-9 in Eutopic Endometrial Stromal Cells of Patients with Endometriosis. Journal of Huazhong University of Science and Technology (Medical Sciences). 2016;45(1):6-11.
26. Gonzalez-Ramos R, Van Langendonckt A, Defrere S, Lousse JC, Colette S, Devoto L, et al. Involvement of the nuclear factor-kappaB pathway in the pathogenesis of endometriosis. Fertil Steril. 2010;94(6):1985-94. doi: https://doi.org/10.1016/j.fertnstert.2010.01.013
27. Soares SR, Martinez-Varea A, Hidalgo-Mora JJ, Pellicer A. Pharmacologic therapies in endometriosis: a systematic review. Fertil Steril. 2012;98(3):529-55. doi: https://doi.org/10.1016/j.fertnstert.2012.07.1120
28. McKinnon BD, Kocbek V, Nirgianakis K, Bersinger NA, Mueller MD. Kinase signalling pathways in endometriosis: potential targets for non-hormonal therapeutics. Hum Reprod Update. 2016;22(3):382-403. doi: https://doi.org/10.1093/humupd/dmv060
29. Balkowiec M, Maksym RB, Wlodarski PK. The bimodal role of matrix metalloproteinases and their inhibitors in etiology and pathogenesis of endometriosis (Review). Mol Med Rep. 2018;18(3):3123-36. doi: https://doi.org/10.3892/mmr.2018.9303

30. Wangmo C, Charoen N, Jantharapattana K, Dechaphunkul A, Thongsuksai P. Epithelial-Mesenchymal Transition Predicts Survival in Oral Squamous Cell Carcinoma. Pathol Oncol Res. 2020;26(3):1511-8. doi: https://doi.org/10.1007/s12253-019-00731-z
31. Sang L, Fang QJ, Zhao XB. A research on the protein expression of p53, p16, and MDM2 in endometriosis. Medicine (Baltimore). 2019;98(14):e14776. doi: https://doi.org/10.1097/MD.0000000000014776
32. Zhang MF, Zhang ZY, Fu J, Yang YF, Yun JP. Correlation between expression of p53, p21/WAF1, and MDM2 proteins and their prognostic significance in primary hepatocellular carcinoma. J Transl Med. 2009;7:110. doi: https://doi.org/10.1186/1479-5876-7-110
33. Zhang M, Xu T, Tong D, Li S, Yu X, Liu B, et al. Research advances in endometriosis-related signaling pathways: A review. Biomed Pharmacother. 2023;164:114909. doi: https://doi.org/10.1016/j.biopha.2023.114909
34. Scotti S, Regidor PA, Schindler AE, Winterhager E. Reduced proliferation and cell adhesion in endometriosis. Mol Hum Reprod. 2000;6(7):610-7. doi: https://doi.org/10.1093/molehr/6.7.610
35. Hu XY, Hou PF, Li TT, Quan HY, Li ML, Lin T, et al. The roles of Wnt/beta-catenin signaling pathway related lncRNAs in cancer. Int J Biol Sci. 2018;14(14):2003-11. doi: https://doi.org/10.7150/ijbs.27977
36. Butler GS, Overall CM. Updated biological roles for matrix metalloproteinases and new "intracellular" substrates revealed by degradomics. Biochemistry. 2009;48(46):10830-45. doi: https://doi.org/10.1021/bi901656f
37. Grabowska MM, Day ML. Soluble E-cadherin: more than a symptom of disease. Front Biosci (Landmark Ed). 2012;17(5):1948-64. doi: https://doi.org/10.2741/4031
38. Huang Q, Song Y, Lei X, Huang H, Nong W. MMP-9 as a clinical marker for endometriosis: a meta-analysis and bioinformatics analysis. Frontiers in endocrinology. 2024;15:1475531. doi: https://doi.org/10.3389/fendo.2024.1475531
39. Collette T, Maheux R, Mailloux J, Akoum A. Increased expression of matrix metalloproteinase-9 in the eutopic endometrial tissue of women with endometriosis. Hum Reprod. 2006;21(12):3059-67. doi: https://doi.org/10.1093/humrep/del297
40. Braungart E, Magdolen V, Degitz K. Retinoic acid upregulates the plasminogen activator system in human epidermal keratinocytes. J Invest Dermatol. 2001;116(5):778-84. doi: https://doi.org/10.1046/j.1523-1747.2001.01310.x
41. Fortenberry Y. The role of serpins in tumor cell migration. Biol Chem. 2015;396(3):205-13. doi: https://doi.org/10.1515/hsz-2014-0254
42. Duffy MJ, McGowan PM, Harbeck N, Thomssen C, Schmitt M. uPA and PAI-1 as biomarkers in breast cancer: validated for clinical use in level-of-evidence-1 studies. Breast Cancer Res. 2014;16(4):428. doi: https://doi.org/10.1186/s13058-014-0428-4
43. Bai Z, Lu Y, Yang M. Expression and Significance of uPA Protein and mRNA in Endometriosis. Ningxia Medical Journal. 2015;37(9):769-70.
44. Chen L, Shen A, Hu M. Expression of uPA and PAI-1 Proteins in Endometrial Tissues of Patients with Endometriosis. Journal of Zhengzhou University (Medical Edition). 2014;49(2):251-4.
45. Yepes M. The uPA/uPAR system in astrocytic wound healing. Neural Regen Res. 2022;17(11):2404-6. doi: https://doi.org/10.4103/1673-5374.338991
46. Asuthkar S, Gondi CS, Nalla AK, Velpula KK, Gorantla B, Rao JS. Urokinase-type plasminogen activator receptor (uPAR)-mediated regulation of WNT/beta-catenin signaling is enhanced in irradiated medulloblastoma cells. J Biol Chem. 2012;287(24):20576-89. doi: https://doi.org/10.1074/jbc.M112.348888