Original Article

Iranian Journal of Immunology

September 2024; 21(3):

miR-196b-5p Affects Macrophage Polarization and Inflammation in Endometriosis

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ABSTRACT

Background: miR-196b-5p was found to be significantly reduced in endometriosis, but its function and the mechanisms involved remained unclear.

Objective: To explore the effect of miR-196b-5p on manipulating macrophage phenotype and the underlying mechanisms in endometriosis

Methods: The endometriosis mice and End1/E6E7 cells were used for *in vivo* and *in vitro* experiments, respectively. QRT-PCR was used to detect miR-196b-5p, suppressor of cytokine signaling 1 (SOCS1), high mobility group AT-Hook 1 (HMGA1), and CCL2 expressions. Western blot was used to detect SOCS1 and HMGA1 protein levels while luciferase reporter assay was performed to determine the interaction between miR-196b-5p and SOCS1/HMGA1. ELISA was used to measure CCL2, IL-10, and IL-6 levels and immunohistochemical staining and flow cytometry were used to examine CD86 and CD206 expressions.

Results: Significantly reduced levels of miR-196b-5p, and increased levels of SOCS1, HMGA1, and CCL2 were observed in the ectopic endometrium of mice with endometriosis. The miR-196b-5p mimic significantly reduced the lesion size, increased M1 macrophages, and decreased M2 macrophages in the ectopic endometrium of mice with endometriosis. End1/E6E7 cells transfected with miR-196b-5p mimic significantly increased M1 macrophages, decreased M2 macrophages and reduced the migration in PMA-treated THP-1 cells. Conversely, transfection with a miR-196b-5p inhibitor led to the opposite outcomes. miR-196b-5p targeted SOCS1/HMGA1, and miR-196b-5p inhibitor significantly up-regulated CCL2 and IL-10, and down-regulated IL-6 levels in End1/E6E7 cells. These effects were markedly reversed by si-SOCS1/si-HMGA1.

Conclusion: miR-196b-5p elevates M1 macrophages and decreases M2 macrophages in endometriosis, possibly by targeting SOCS1/HMGA1. This research may provide a novel insight into the pathological mechanisms of endometriosis.

Keywords: Endometriosis, Inflammation, Macrophages, miR-196b-5p

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Cite this article as:
Xue Z, Guo Y, Wang F, Yang Q, Chen Q, Lin T, Lin S. miR-196b-5p Affects Macrophage Polarization and Inflammation in Endometriosis. *Iran J Immunol.* doi:

Received: 2024-05-23
Revised: 2024-08-06
Accepted: 2024-08-13

INTRODUCTION

Endometriosis is a common benign gynecological disease characterized by the presence of endometrial tissue outside the uterine cavity. It affects 6% to 10% of women of reproductive age (1). The growth, infiltration and repeated bleeding of endometriosis result in pain, infertility, and other symptoms. This seriously affects the quality of life and brings significant psychological and economic pressure to patients (2). Most scholars believed that countercurrent flow of menstrual blood was the pathogenesis of endometriosis, but it was difficult to fully explain the specific pathogenetic mechanisms of endometriosis.

Increasing evidence reveals that the countercurrent of menstrual blood is merely an inducement, and immune system imbalance caused by macrophages plays an important role in promoting endometrial adhesion, implantation and growth (3, 4). Macrophages are important inflammatory cells, that are divided into two polarized phenotypes: M1 macrophages phagocytize antigens and participate in immune responses, while M2 macrophages promote tumor development and angiogenesis (5, 6). Lagana et al. (7) reported that M1 macrophages in women with ovarian endometriomas exhibited a continuous decrease as the stage progressed from I to IV, while M2 macrophages showed an opposite trend. Gou et al. (8) reported that lactate-induced M2 macrophages promoted the invasion of endometriotic stromal cells *in vitro* and *in vivo*. - Nanovesicles derived from M1 macrophages repolarized macrophages from an M2 to an M1 phenotype, inhibiting the development of endometriosis (9). However, Wu et al. (10) reported that, compared to normal controls, M2 macrophages were significantly reduced in the ectopic endometrium of women with endometriosis. Thus, the functional role of macrophages in endometriosis has yet to be studied, and the mechanisms for regulating the phenotypic transformation of macrophages are also unclear.

More and more evidence suggests that microRNAs (miRNAs) may play an important role in regulating the phenotypic transformation of macrophages. In glioma, miRNA-1246 derived from hypoxic glioma-derived exosomes induces M2 macrophage polarization through various pathways (11). In myocardial infarction, miRNA-21 triggers a phenotype switch in cardiac macrophages from pro-inflammatory to reparative in the remote myocardium (12). However, there have been few studies on the role of miRNAs in manipulating macrophage phenotype in endometriosis. miR-196b-5p is a recently discovered miRNA that is differentially expressed in various diseases, and is also associated with disease progression (13-15). Although miR-196b-5p was found to be significantly decreased in endometriosis (16), there have been few studies on miR-196b-5p in endometriosis. The ENCORI database predicted the binding sites between miR-196b-5p and suppressor of cytokine signaling 1 (SOCS1) or high mobility group AT-Hook 1 (HMGA1). Considering the single cell sequencing results (17), we speculated that miR-196b-5p might regulate cytokines and macrophage migration to affect macrophages via SOCS1/HMGA1, thus affecting immunosuppressive environments in endometriosis.

MATERIALS AND METHODS

Establishment of an Endometriosis Mouse Model

Female BALB/C mice (8-10 weeks old) were intramuscularly injected with pregnant horse serum gonadotropin, and the uterin tissues were dissected after 42 hours. Next, the endometrial tissues were cut into 1 mm^3 pieces before being placed in normal saline. Subsequently, each recipient mouse was intraperitoneally administered with the tissue suspension. The recipient mice were sacrificed after 18 days, and the lesions were removed and measured for size. The

ethical committee of Fuqing Maternal and Child Health Hospital approved all animal procedures (2021-001), and all experimental procedures were carried out in accordance with the guidelines for the Care and Use of Laboratory Animals published by the National Academic Press.

Cell Culture and Treatment

End1/E6E7 cells (YS1164C) were obtained from Yaji Biotechnology (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (DMEM, PM150210B, Procell, Wuhan, China) supplemented with 10% fetal calf serum (FBS; Sigma, St. Louis, MO, USA), and 1% penicillin and streptomycin (Sigma). The cells were incubated in a 5% CO_2 atmosphere at 37˚C. The cells were then transfected with miR-196b-5p mimic/mimic NC (RiboBio, Guangzhou, China) or miR-196b-5p inhibitor/inhibitor NC (RiboBio) for 48h. Additionally, the cells were co-transfected with miR-196b-5p inhibitor and si-SOCS1/ si-HMGA1 for 48 h. The transfection was carried out using Lipofectamine 8000 (C0533, Beyotime, Shanghai, China) according to the manufacturer's instructions. The siRNA sequences are listed below:
Si-SOCS1:
5'-GAUUAAGACGGUUGAAACUAG-3'
Si-HMGA1:
5'-GACCCGGAAAACCACCACA-3'
Si-ctrl:
5'-ACGUGACACGUUCGGAGAATT-3'

Enzyme-linked Immunosorbent Assay (ELISA)

The concentrations of CCL2, IL-6, and IL-10 in the supernatant of End1/E6E7 cells were measured using the Human MCP-1 ELISA Kit (E-EL-H6005, Elabscience, Wuhan), the Human IL-6 ELISA Kit (E-EL-H6156, Elabscience, Wuhan), and the Human IL-10 ELISA Kit (E-EL-H6154, Elabscience, Wuhan), respectively.

Immunohistochemistry (IHC) Staining

The ectopic endometrium of mice with endometriosis was incubated with CD86 (1:100, DF6332, Affinity, OH, USA) or CD206 (1:80, DF4149, Affinity) antibodies, and then stained with DAB and hematoxylin. Finally, the sections were photographed under an inverted microscope (DMI 4000B, Leica, Germany).

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

The extracted RNA was reverse-transcribed into cDNA, and the expressions of miR-196b-5p, SOCS1, HMGA1, and CCL2 were detected using the ABI-7500 Real Time PCR System. Data analysis was conducted using the $2^{-\Delta\Delta Ct}$ method and either β-actin or U6 was used for normalization. The primers are listed below
miR-19b-5p (F):
5'-ACACTCCAGCTGGGTAGGTAGTTTC CTGTT-3'
miR-19b-5p (R):
5'-TGGTGTCGTGGAGTCG-3'
SOCS1(F):
5'-GAGACCTTCGACTGCCTTTTC-3'
SOCS1(R):
5'-GGAAGGAACTCAGGTAGTCAC-3'
HMGA1(F):
5'-GGCAGACCCAAGAAACTGG-3'
HMGA1(R):
5'-GGCACTGCGAGTGGTGAT-3'
CCL2 (F):
5'-TGAAGTTGACCCGTAAATCTGAA-3'
CCL2 (R):
5'-AGGCATCACAGTCCGAGTC-3'
U6 (F): 5'-CTCGCTTCGGCAGCACA-3'
U6 (R):
5'-AACGCTTCACGAATTTGCGT-3'
β-actin (F):
5'-GTCCCTCACCCTCCCAAAAG-3'
β-actin (R):
5'-GCTGCCTCAACACCTCAACCC-3'

Western Blot

The proteins were extracted and transferred onto a nitrocellulose membrane. They were then incubated with the following primary antibodies: SOCS1 (1:1000, AF5378, Affinity),

HMGA1 (1:1000, AF5218, Affinity), and CCL2 (1:1000, MA5-17040, Invitrogen). β-actin was used as the internal control. HRP-conjugated goat anti-mouse IgG was used as the secondary antibody. An enhanced chemiluminescence kit was used to visualize the protein bands.

The Co-culture of End1/E6E7 Cells and THP-1 Cells

THP-1 cells (YS295C, Yaji, Shanghai) in the logarithmic growth phase were treated with PMA (100 ng/mL, S1819, Beyotime) to induce macrophages. .The induced macrophages were then co-cultured with End1/E6E7 cells in the logarithmic growth phase at a 1:1 ratio , and cultured for 24-48h for the following experiments.

Bioinformatics Analysis

According to the ENCORI database, binding sites were predicted between miR-196b-5p and SOCS1/HMGA1.

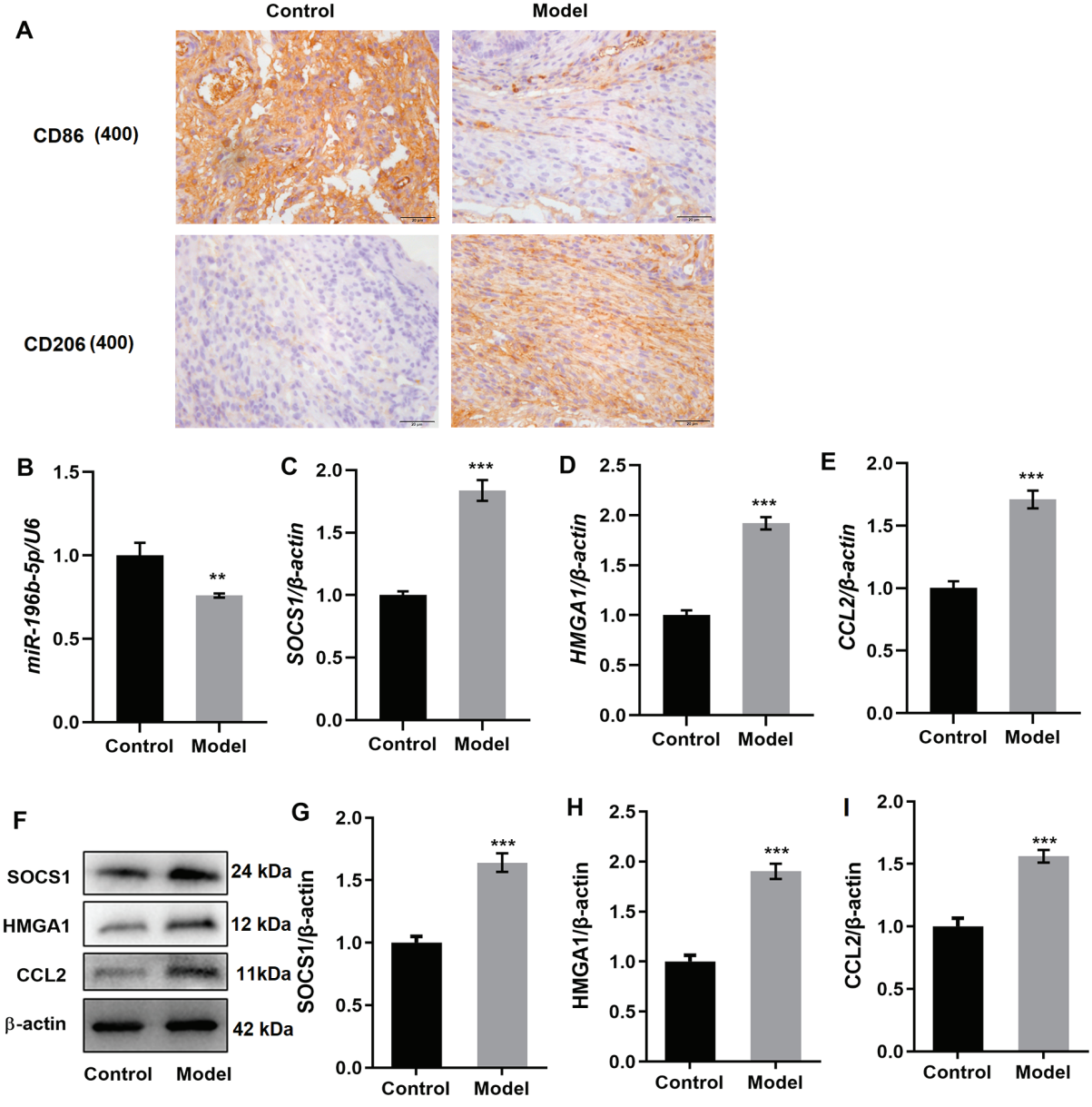

Fig. 1. Down-regulation of miR-196b-5p in ectopic endometrium of mice with endometriosis. Mice with endometriosis were established, and the ectopic endometrium was collected. (A) Immunohistochemical analysis was performed to assess CD86 and CD206 positive cells; (B-E) qRT-PCR was used to examine the expressions of miR-196b-5p, SOCS1, HMGA1, and CCL2 mRNA; (F-I) Western blot was used to examine the levels of SOCS1, HMGA1, and CCL2 protein. **p<0.01, ***p<0.001 *vs* Control. suppressor of cytokine signaling 1 (SOCS1), high mobility group AT-Hook 1 (HMGA1), chemokine ligand 2 (CCL2)

Dual Luciferase Reporter Assay

The SOCS1-3'UTR and HMGA1-3'UTR containing wild-type or mutant binding sites, were cloned into the psiCHECKTM-2 vector. Subsequently, miR-19b-5p mimic and the SOCS1-3'UTR or HMGA1-3'UTR psiCHECKTM-2 plasmid were co-transfected into HEK-293 T cells. The dual luciferase

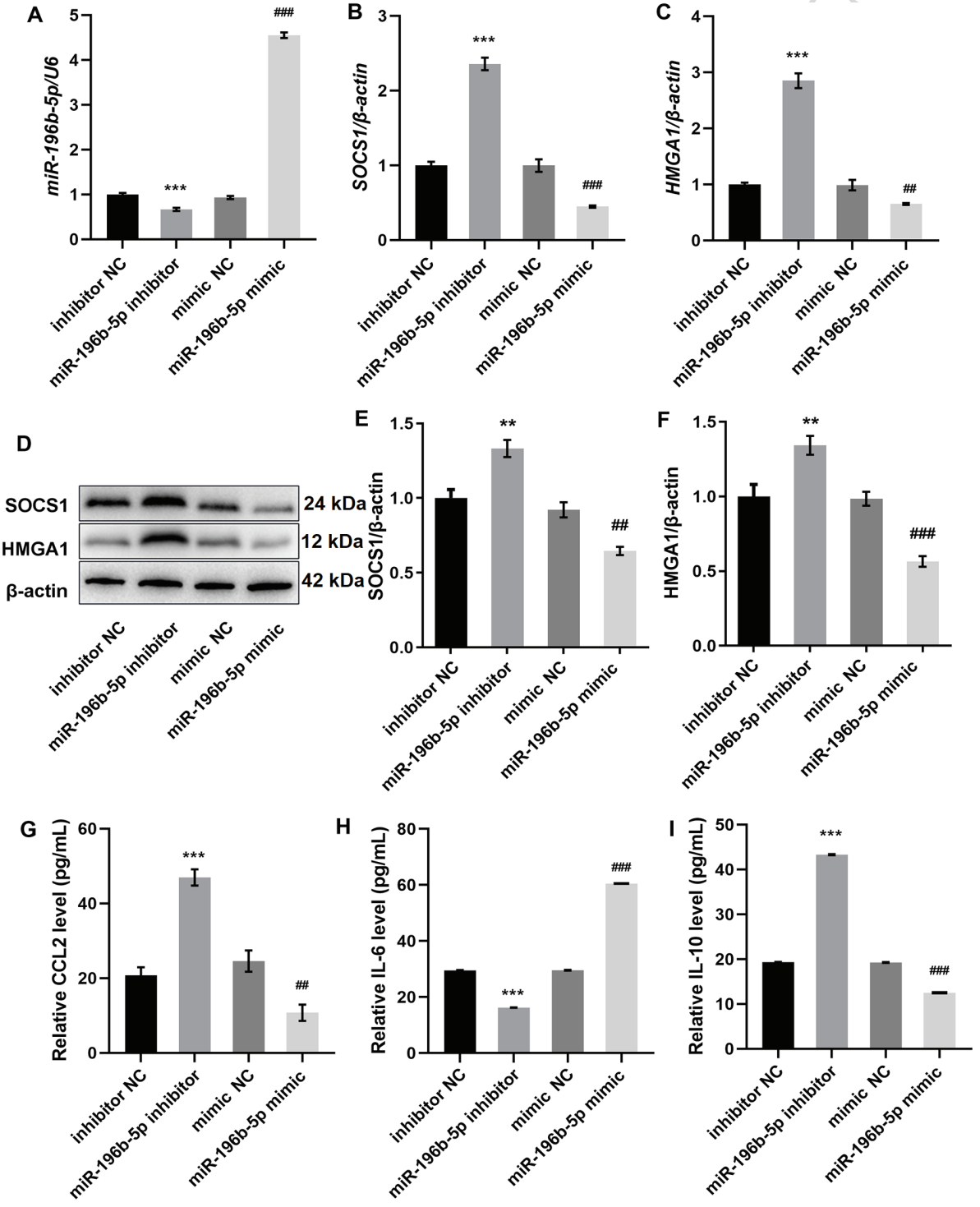

Fig. 2. miR-196b-5p affects inflammatory cytokines and SOCS1/HMGA1 in End1/E6E7 cells. End1/E6E7 cells were transfected with the miR-196b-5p mimic or inhibitor. (A-C) qRT-PCR was used to examine the expressions of miR-196b-5p, SOCS1, and HMGA1; (D-F) Western blot was used to examine the protein levels SOCS1 and HMGA1; (I) ELISA was employed to detect CCL2 (G), IL-6 (H), and IL-10 levels. ***p*<0.01, ****p*<0.001 *vs* inhibitor NC; ##*p*<0.01, ###*p*<0.001 *vs* mimic NC. suppressor of cytokine signaling 1 (SOCS1), high mobility group AT-Hook 1 (HMGA1), chemokine ligand 2 (CCL2), negative control (NC), interleukin (IL)

reporter assay system (E1910, Promega, Beijing, China) was employed to measure luciferase activity.

Statistical Analysis

The differences between groups were analyzed using unpaired Student's t tests or one-way analysis of variance (ANOVA) in GraphPad Prism 7.0 after confirming normal distribution through Shapiro-Wilk tests. A significant *p*-value was considered to be less than 0.05.

RESULTS

The Alterations in Macrophage Polarization and miR-196b-5p Expression in the Ectopic Endometrium of Mice with Endometriosis

The immunohistochemical results revealed a significant decrease in $CD86^+$ cells (M1 macrophages) and an increase in $CD206^+$ cells (M2 macrophages) in the ectopic endometrium of mice with endometriosis, compared to normal mice (Fig. 1A). QRT-PCR results revealed the down-regulation of miR-196b-5p (Fig. 1B) and the up-regulation of SOCS1 (Fig. 1C), HMGA1 (Fig. 1D), and CCL2 (Fig. 1E) in the ectopic endometrium of mice with endometriosis. The western blot results further confirmed the up-regulation of SOCS1, HMGA1, and CCL2 at the protein level (Fig. 1F-I).

miR-196b-5p Affects Inflammatory Cytokines by Targeting SOCS1/HMGA1 in End1/E6E7 Cells

The End1/E6E7 cells were transfected with a miR-196b-5p mimic or inhibitor.

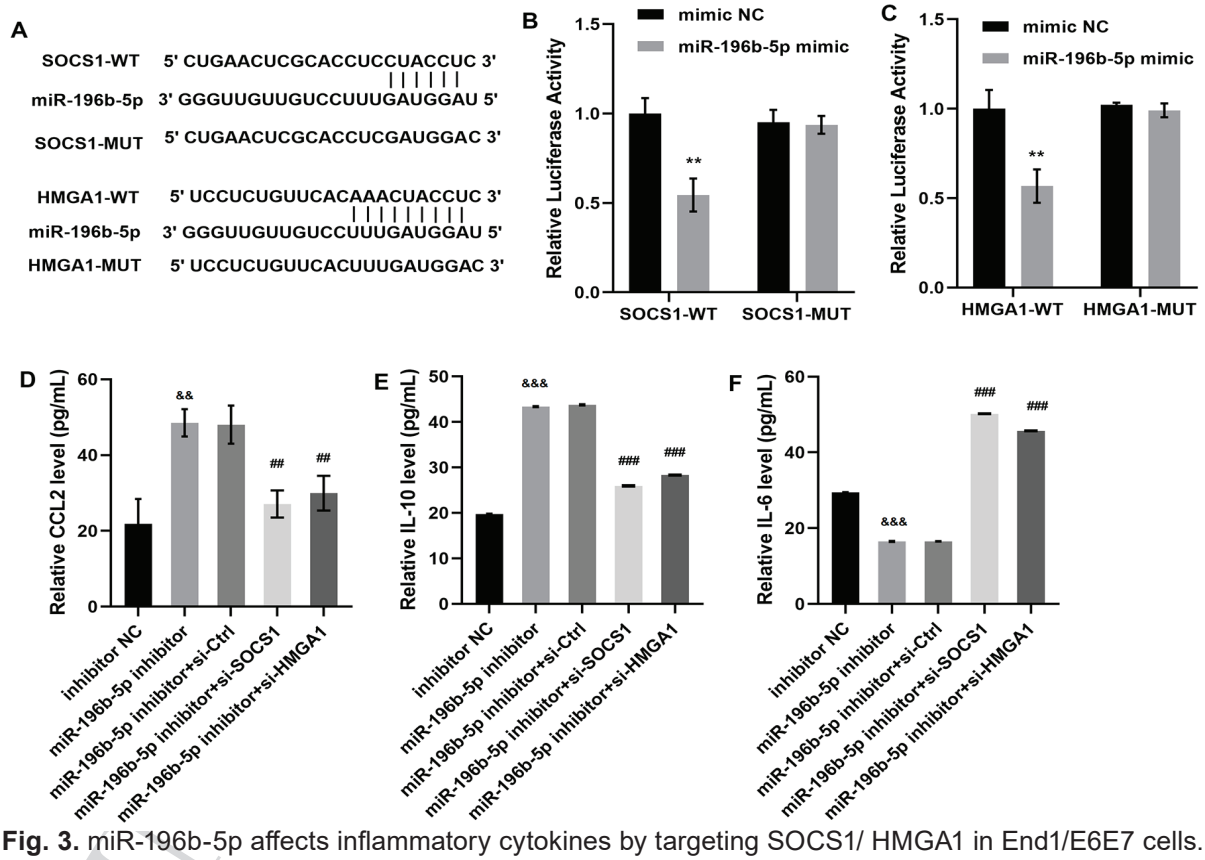

Fig. 3. miR-196b-5p affects inflammatory cytokines by targeting SOCS1/ HMGA1 in End1/E6E7 cells. (A) The binding sites between miR-196b-5p and SOCS1/HMGA1 were investigated. The dual luciferase reporter assay was conducted to evaluate the interaction between miR-19b-3p and SOCS1 (B) or HMGA1 (C); End1/E6E7 cells were co-transfected with the miR-196b-5p inhibitor and si-SOCS1 or si-HMGA1, ELISA was used to measure CCL2 (D), IL-6 (E), and IL-10 (F) levels. ***p*<0.01 *vs* mimic NC; &&*p*<0.01, &&& *p*<0.001 *vs* inhibitor NC; ##*p*<0.01, ###*p*<0.001 *vs* miR-196b-5p inhibitor+ si-Ctrl. suppressor of cytokine signaling 1 (SOCS1), high mobility group AT-Hook 1 (HMGA1), chemokine ligand 2 (CCL2), negative control (NC), interleukin (IL)

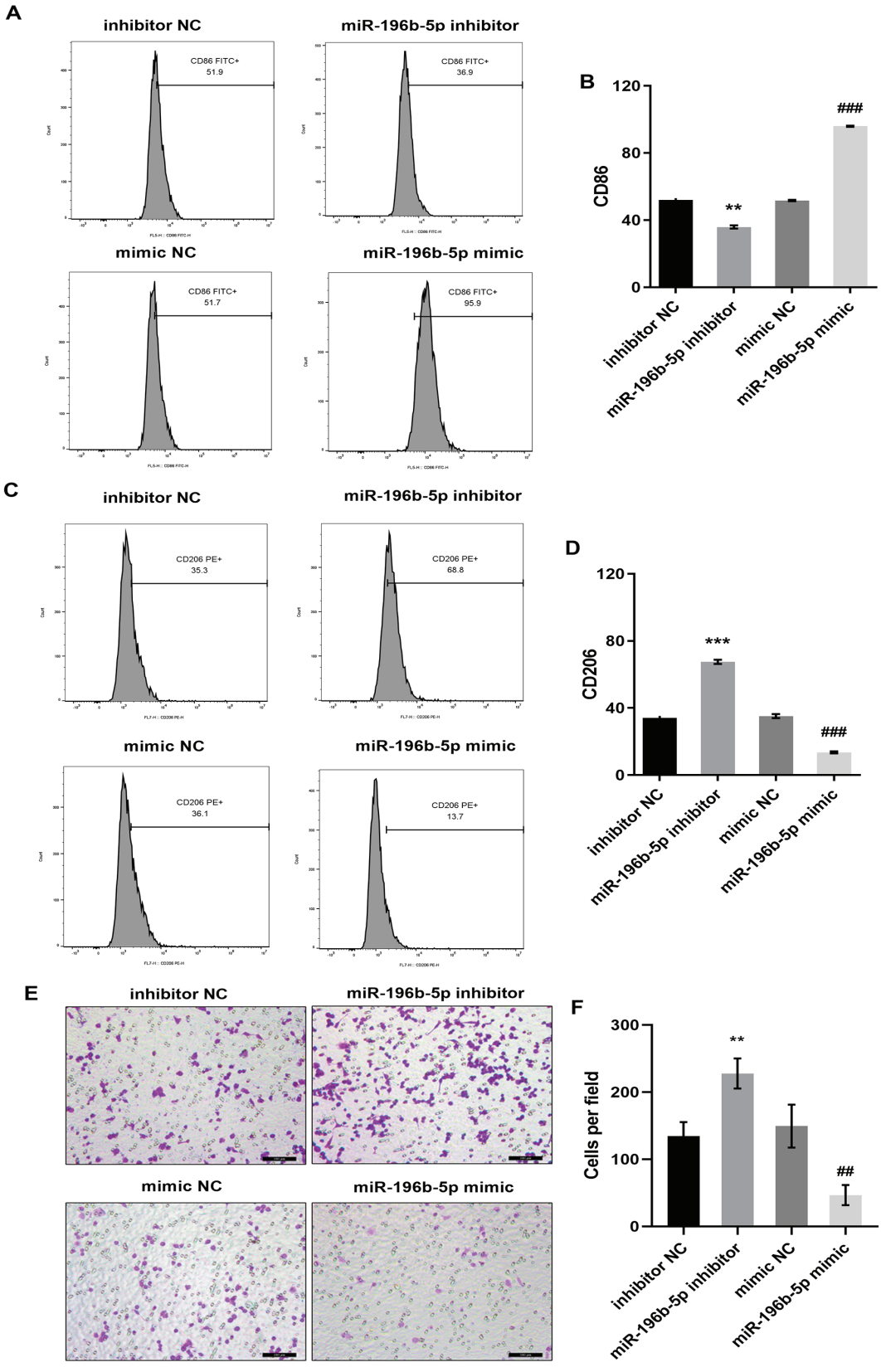

Fig. 4. The miR-196b-5p mimic facilitates M1 polarization and inhibits M2 polarization *in vitro*. THP-1 cells were treated with PMA to induce M0 macrophages, which were then co-cultured with End1/E6E7 cells transfected with either the miR-196b-5p mimic or inhibitor. Flow cytometry was used to detect CD86 (A, B) and CD206 (C, D) expressions; (E, F) A transwell assay was conducted to assess the migration of macrophages. ***p*<0.01, ****p*<0.001 *vs* inhibitor NC; ##*p*<0.01, ###*p*<0.001 *vs* mimic NC. negative control (NC)

Subsequent qRT-PCR results confirmed the successful transfection (Fig. 2A). The miR-196b-5p mimic reduced SOCS1 and HMGA1 expression in End1/E6E7 cells, while the miR-196b-5p inhibitor produced the opposite results, at both mRNA (Fig. 2B, C) and protein levels (Fig. 2D-F). Additionally, the miR-196b-5p mimic increased IL-6 level, and decreased CCL2 and IL-10 levels in End1/E6E7 cells, whereas the miR-196b-5p inhibitor produced the opposite results (Fig. 2H-I). Bioinformatic analysis revealed the binding

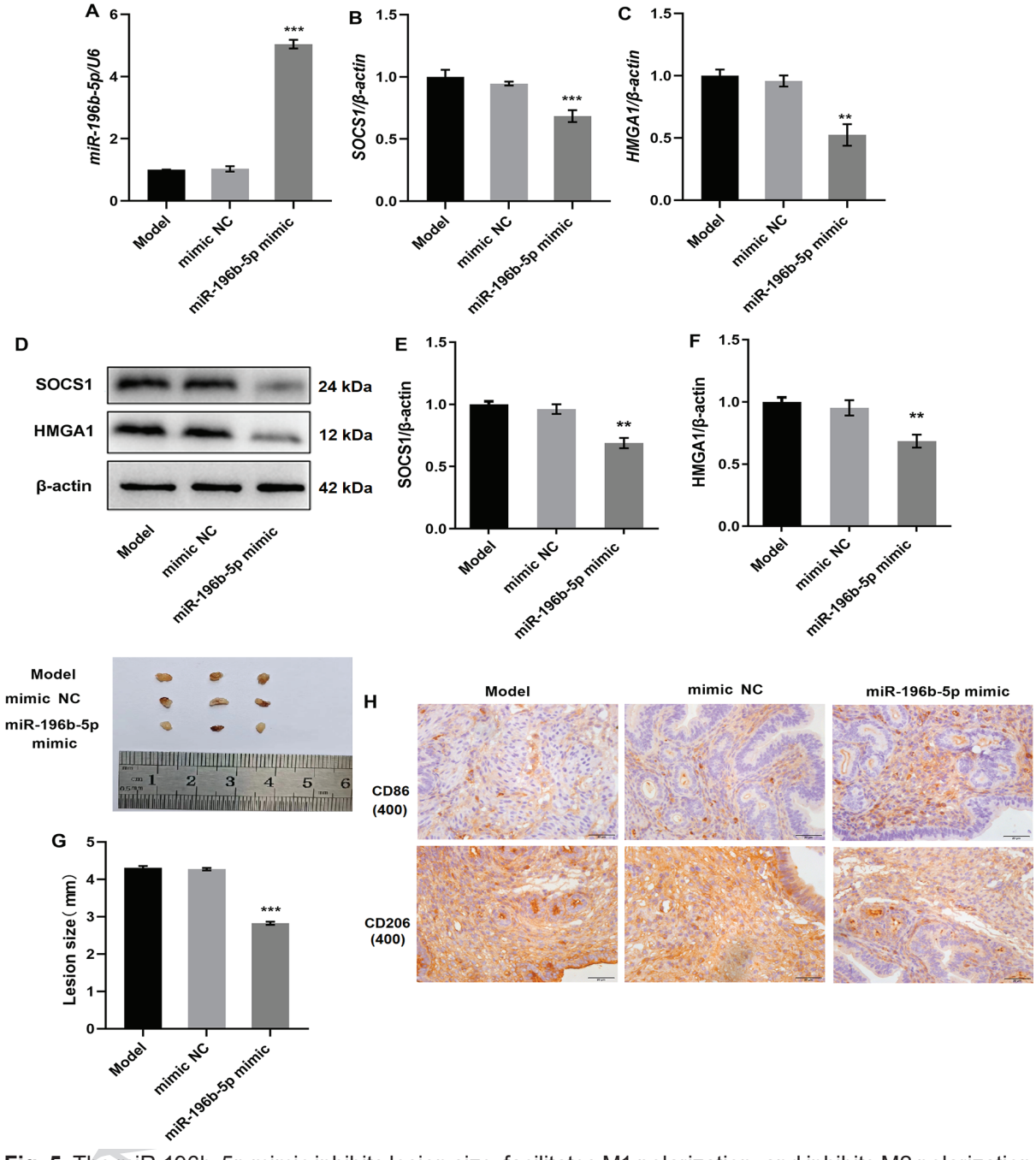

Fig. 5. The miR-196b-5p mimic inhibits lesion size, facilitates M1 polarization, and inhibits M2 polarization *in vivo*. The mice with endometriosis were injected with the miR-196b-5p mimic, and the ectopic endometrium was collected. (A-C) qRT-PCR was employed to detect the expressions of miR-196b-5p, SOCS1, and HMGA1 mRNA; (D-F) Western blot was used to measure the levels of SOCS1 and HMGA1 protein; (G) The size of the lesions was measured using the vernier caliper; (H) Immunohistochemical analysis was performed to identify CD86 and CD206 positive cells. ***p*<0.01, ****p*<0.001 *vs* mimic NC. suppressor of cytokine signaling 1 (SOCS1), high mobility group AT-Hook 1 (HMGA1), chemokine ligand 2 (CCL2), negative control (NC), interleukin (IL)

sites that connect miR-196b-5p with SOCS1/HMGA1 (Fig. 3A). Dual luciferase reporter assay confirmed that both SOCS1 (Fig. 3B) and HMGA1 (Fig. 3C) were targets of miR-196b-5p. Furthermore, si-SOCS1 or si-HMGA1 reversed the changes in CCL2 (Fig. 3D), IL-6 (Fig. 3E), and IL-10 (Fig. 3F) induced by the miR-196b-5p inhibitor in End1/E6E7 cells.

End1/E6E7 Cells Overexpressing miR-196b-5p Facilitate M1 Polarization, and Inhibit M2 Polarization in Vitro

Subsequently, End1/E6E7 cells transfected with the miR-196b-5p mimic or inhibitor were co-cultured with PMA-treated THP-1 cells (M0 macrophages). The miR-196b-5p mimic significantly promoted M1 polarization and inhibited M2 polarization, while the miR-196b-5p inhibitor resulted in the opposite effects (Fig. 4A-D). In addition, the migration of macrophages was inhibited by the miR-196b-5p mimic, but promoted by the miR-196b-5p inhibitor (Fig. 4E, F).

Overexpression of the miR-196b-5p Reduces Lesion Size, Promotes M1 Polarization, and Inhibits M2 Polarization in Vivo

Finally, the mice with endometriosis were injected with the miR-196b-5p mimic for the *in vivo* experiments. The miR-196b-5p mimic significantly increased miR-196b-5p mRNA expression (Fig. 5A), while decreasing SOCS1 and HMGA1 expression in the ectopic endometrium of mice with endometriosis, at both mRNA (Fig. 5B, C) and protein levels (Fig. 5D-F). The size of lesions in mice with endometriosis was also reduced by the miR-196b-5p mimic (Fig. 5G). Immunohistochemical results revealed an increase in M1 macrophages and a decrease in M2 macrophages in the ectopic endometrium of endometriosis mice injected with miR-196b-5p mimic (Fig. 5H).

DISCUSSION

Due to their easy availability, consistent repeatability and relative stability in the same individuals, miRNAs have received extensive attention as potential biomarkers in various diseases, including endometriosis (18). Here, miR-196b-5p was significantly reduced in ectopic endometrium of mice with endometriosis,, consistent with the findings of a previous study (16). Furthermore, the *in vivo* experiments revealed that the miR-196b-5p mimic reduced the lesion size of mice with endometriosis. Additionally, SOCS1 and HMGA1 were significantly up-regulated in the ectopic endometrium of mice with endometriosis. Furthermore, both SOCS1 and HMGA1 were identified as targets of miR-196b-5p. Given that SOCS1 plays an important role in modulating inflammation within the body (19), we were curious to investigate whether miR-196b-5p also has an impact on the inflammatory response. To our delight, miR-196b-5p significantly impacted the inflammatory cytokines (CCL2, IL-6, IL-10), and this effect was further reversed by si-SOCS1 or si-HMGA1. These findings suggest that miR-196b-5p may influence inflammation by targeting SOCS1/HMGA1 in endometriosis.

It has been reported that the involvement of macrophages, particularly M2 macrophages, in immune dysfunction is one of the important causes of endometriosis (20, 21). As expected, M1 macrophages were decreased, while M2 macrophages were increased in the ectopic endometrium of mice with endometriosis, suggesting the involvement of macrophages in endometriosis. In addition, activated macrophages help facilitate the degradation of erythrocytes, leading to iron overload in endometrial lesions (22, 23). Additionally, excess iron in endometriotic tissues was associated with periodic hemorrhage and retrograde menstruation, both of which induced inflammatory environments (24). Therefore, blocking M2 macrophages or inducing M1 macrophages represents a novel research direction for the prevention and treatment of endometriosis.

Based on emerging evidence of miRNAs

regulating macrophage phenotype (25-27), the miR-196b-5p mimic significantly induced M1 polarization, and inhibited M2 polarization, both *in vitro* and *in vivo*. Unfortunately, the impact of miR-196b-5p on iron metabolism in endometrial lesions remains unclear.

CONCLUSION

miR-196b-5p reduced lesion size and M2 macrophages in endometriosis, which may depend on targeting SOCS1/HMGA1. This research may provide a novel insight into the pathological mechanisms of endometriosis as well as a new treatment strategy.

ACKNOWLEDGEMENT

This study received funding support from the Fujian Provincial Natural Science Foundation (No. 2021J01421, No. 2021J01414) and the Fujian Provincial Health Technology Project of China (No. 2022GGB004).

AUTHORS' CONTRIBUTION

ZX designed the study and wrote the manuscript. ZX, FW, QY, QC, TL performed all the experiments. YG and SL aggregated the data and analyzed the data. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Chapron, Charles, Marcellin, Louis, Borghese, Bruno, et al. Rethinking mechanisms, diagnosis and management of endometriosis. Nat Rev Endocrinol. 2019;15(11):666-82.
2. Bulun SE, Yilmaz BD, Sison C, Miyazaki K, Wei JJ. Endometriosis. Endocrine Reviews. 2019;40(4):1048-79.
3. Xiaocui L, Wei H, Yunlang C, Zhenzhen Z, Min A. CSF-1-induced DC-SIGN+ macrophages are present in the ovarian endometriosis. Reproductive Biology and Endocrinology. 2022;20(1):48.
4. Huang Y, Yan S, Dong X, Jiao X, Wang S, Li D, et al. Deficiency of MST1 in endometriosis related peritoneal macrophages promoted the autophagy of ectopic endometrial stromal cells by IL-10. Frontiers in Immunology. 2022;13:993788.
5. Li X, Xue S, Zhan Q, Sun X, Chen N, Li S, et al. Sequential Delivery of Different MicroRNA Nanocarriers Facilitates the M1-to-M2 Transition of Macrophages. ACS omega. 2022;7(9):8174-83.
6. Anderson NR, Minutolo NG, Gill S, Klichinsky M. Macrophage-Based Approaches for Cancer Immunotherapy. American Association for Cancer Research (AACR). 2021;81(5):1201-8.
7. Lagana AS, Francesca MariaFrangez, Helena BanGhezzi, FabioVrtacnik-Bokal, EdaGranese, Roberta. Evaluation of M1 and M2 macrophages in ovarian endometriomas from women affected by endometriosis at different stages of the disease. Gynecological endocrinology: the official journal of the International Society of Gynecological Endocrinology. 2020;36(5):441-4.
8. Gou Y, Wang H, Wang T, Wang H, Wang B, Jiao N, et al. Ectopic endometriotic stromal cells-derived lactate induces M2 macrophage polarization via Mettl3/Trib1/ERK/STAT3 signalling pathway in endometriosis. Immunology. 2022;168(3):389-402.
9. Li Q, Yuan M, Jiao X, Huang Y, Li J, Li D, et al. M1 Macrophage-Derived Nanovesicles Repolarize M2 Macrophages for Inhibiting the Development of Endometriosis. Frontiers in Immunology. 2021;12:707784.
10. Wu XG, Chen JJ, Zhou HL, Wu Y, Wang W. Identification and Validation of the Signatures of Infiltrating Immune Cells in the Eutopic Endometrium Endometria of Women With Endometriosis. Frontiers in immunology. 2021;12:671201.
11. Qian M, Wang S, Guo X, Wang J, Zhang Z, Qiu W, et al. Hypoxic glioma-derived exosomes deliver microRNA-1246 to induce M2 macrophage polarization by targeting TERF2IP via the STAT3 and NF-kappa B pathways. Oncogene. 2020;39(2):428-42.
12. Bejerano, Tzlil, Etzion, Sharon, Elyagon, Sigal, et al. Nanoparticle Delivery of miRNA-21 Mimic to Cardiac Macrophages Improves Myocardial Remodeling after Myocardial Infarction. Nano Letters. 2018;18(9):5885-91.
13. A JYM, B PD, B YQ, A XFM, A HWM, A FC. NEAT1 Knockdown Inhibits Keloid Fibroblast

Progression by miR-196b-5p/FGF2 Axis. Journal of Surgical Research. 2021;259:261-70.
14. Xie Y, Zhou J, Tian L, Dong Y, Yuan H, Zhu E, et al. miR-196b-5p Regulates Osteoblast and Osteoclast Differentiation and Bone Homeostasis by Targeting SEMA3A. Journal of Bone and Mineral Research. 2023;38(8):1175-91.
15. de Keizer ROB, Vriends ALM, Hötte GJ, Paridaens DA, Wiemer EAC, Verdijk RM. miR-196b-5p and miR-107 Expression Differentiates Ocular Sebaceous Carcinoma from Squamous Cell Carcinoma of the Conjunctiva. International Journal of Molecular Sciences. 2022;23(9):4877.
16. Ohlsson Teague EMC, Van der Hoek KH, Van der Hoek MB, Perry N, Wagaarachchi P, Robertson SA, et al. MicroRNA-Regulated Pathways Associated with Endometriosis. Molecular Endocrinology. 2009;23(2):265-75.
17. Tan Y, Flynn WF, Sivajothi S, Luo D, Bozal SB, Dave M, et al. Single-cell analysis of endometriosis reveals a coordinated transcriptional programme driving immunotolerance and angiogenesis across eutopic and ectopic tissues. Nat Cell Biol. 2022 Aug;24(8):1306-18. PubMed PMID: 35864314. Epub 2022/07/22.
18. A EP, Mehrdad Noruzinia A, B LK, C WGF. Identification of candidate microRNA markers of endometriosis with the use of next-generation sequencing and quantitative real-time polymerase chain reaction. Fertility and Sterility. 2020;113(6):1232-41.
19. Yinghua Z, Fei P, Jiayi H, Yilun Q, Huiming N, Lingling W, et al. SOCS1 Peptidomimetic Alleviates Glomerular Inflammation in MsPGN by Inhibiting Macrophage M1 Polarization. Inflammation. 2023;46(6):2402-14.
20. Hsieh TH, Hsu CY, Wu CW, Wang SH, Yeh CH, Cheng KH, et al. Vorinostat decrease M2 macrophage polarization through ARID1A6488delG/HDAC6/IL-10 signaling pathway in endometriosis-associated ovarian carcinoma. Biomedicine & pharmacotherapy. 2023;161:114500.
21. Stratopoulou CA, Cussac S, D'Argent M, Donnez J, Dolmans MM. M2 macrophages enhance endometrial cell invasiveness by promoting collective cell migration in uterine adenomyosis. Reproductive biomedicine online. 2023;46(4):729-38.
22. Adamyan L, Pivazyan L, Krylova E, Tarlakyan V, Murvatova K. Iron metabolism markers in peritoneal fluid of patients with endometriosis: systematic review and meta-analysis. Journal of Endometriosis and Uterine Disorders. 2024;5:100061.
23. Ng SW, Norwitz SG, Taylor HS, Norwitz ER. Endometriosis: The Role of Iron Overload and Ferroptosis. Reproductive Sciences. 2020;27(7):1383-90.
24. Pascolo L, Pachetti M, Camillo A, Cernogoraz A, Rizzardi C, Mikus KV, et al. Detention and mapping of iron and toxic environmental elements in human ovarian endometriosis: A suggested combined role. Science of The Total Environment. 2023;864:161028.
25. Shin CH, Byun J, Lee K, Kim B, Noh YK, Tran NL, et al. Exosomal miRNA-19a and miRNA-614 Induced by Air Pollutants Promote Proinflammatory M1 Macrophage Polarization via Regulation of ROR alpha Expression in Human Respiratory Mucosal Microenvironment. The Journal of Immunology: Official Journal of the American Association of Immunologists. 2020;205(11):3179-90.
26. Wang C, Wang X, Zhang D, Sun X, Wu Y, Wang J, et al. The macrophage polarization by miRNAs and its potential role in the treatment of tumor and inflammation. Oncology Reports. 2023;50(4):190.
27. Yu W, Wang S, Wang Y, Chen H, Nie H, Liu L, et al. MicroRNA: role in macrophage polarization and the pathogenesis of the liver fibrosis. Frontiers in Immunology. 2023;14:1147710.