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Original Research

Endometrial Mesenchymal Stem Cells and Their Role in the Origin and Treatment of Endometriosis

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Abstract

Background: To identify endometrial mesenchymal stem cells (eMSCs) in retrograde menstruation, in various endometriosis lesions, in normal control tissues, and to investigate the association between eMSCs and endometriosis. We also plan to evaluate the effect of gonadotropin-releasing hormone agonists (GnRH-a) on eMSCs. **Methods**: Patients diagnosed with endometriosis were included if they had experienced surgery during the time frame 1 January 2015 to 31 December 2019 in West China Second Hospital, Sichuan University. Immunofluorescence was performed to identify eMSCs in those tissues with cell surface markers PDGFR-β/CD146. The percents of eMSCs in various tissues were calculated, and compared using analysis of variance. A two-sided p value less than 0.05 showed significant difference. **Results**: This study included 508 patients. eMSCs were identified in retrograde menstruation and numerous pathologic specimen but were not detected in normal control tissues. There was no significant difference in the percent of eMSCs between the GnRH-a treatment group and the control group ($p > 0.05$). **Conclusions**: Our study demonstrated that eMSCs played a critical role in the development and recurrence of endometriosis and that GnRH-a did not affect eMSCs. Gynecologists should regard endometriosis as a chronic disease requiring lifetime management, especially for patients with chronic pelvic pain.

Keywords: endometrial mesenchymal stem cells; endometriosis; chronic pelvic pain; gonadotropin-releasing hormone agonists

1. Introduction

Chronic pelvic pain (CPP) and dysmenorrhea are the most common health problems that affect women of childbearing age [1]. There is a variety of causes for CPP which include endometriosis, adenomyosis, chronic infection, vulvodynia, irritable bowel syndrome, and bladder pain syndrome. Endometriosis is the most common cause of CPP [2], accounting for 24–40% of all CPP diagnoses [3]. For the management of endometriosis, surgery is a frequent choice since the efficacy of medical treatment alone is either poorly documented or of limited efficacy. However, owing to the unclear etiology of endometriosis, its recurrence rate following surgery remains high. Reoperation occurs in 51% of patients with endometriosis, often resulting in damage to ovarian reserve [4]. The risk for reoperation, coupled with uncertainty for results and the presence of continued pelvic pain, makes endometriosis a chronic disease.

Reya *et al*. [5] proposed a cancer stem cell (CSC) theory that some rare cell populations that exist in cancer tissues, had the capacity for self-renewal, multipotential differentiation, tumorigenesis, metastasis, relapse and treatment-resistance. Evidence exists that endometrial mesenchymal stem cells (eMSCs) are located within the endometrium [6]. eMSCs are believed to contribute to cyclical changes of human endometrium, including proliferation, differentiation, tissue breakdown and shedding under the influence of estrogen and progesterone during the menstrual cycle [7]. The migration of eMSCs is similar to that of CSCs, with endometriosis possessing biological behaviors of local aggressiveness, distant metastasis and high disease recurrence. eMSCs may play a key role in the development and relapse of endometriosis. A suggested hypothesis is that eMSCs are abnormally shed during menses, present in amniotic fluid and blood. They are capable of gaining access to the peritoneal cavity or an abdominal wall scar, where they establish ectopic implants in those women who develop endometriosis.

This work aimed to identify eMSCs in retrograde menstruation, various endometriosis lesions, and normal control tissues in order to investigate the association between eMSCs and endometriosis. The effectiveness of gonadotropin-releasing hormone agonists (GnRH-a) on endometriosis was also compared using percent of eMSCs between the groups.

2. Materials and Methods

2.1 Study Participants

Patients with the following criteria were enrolled in this study. Criteria included: (1) All subjects aged 20 to 45 underwent surgery after menstruation during the

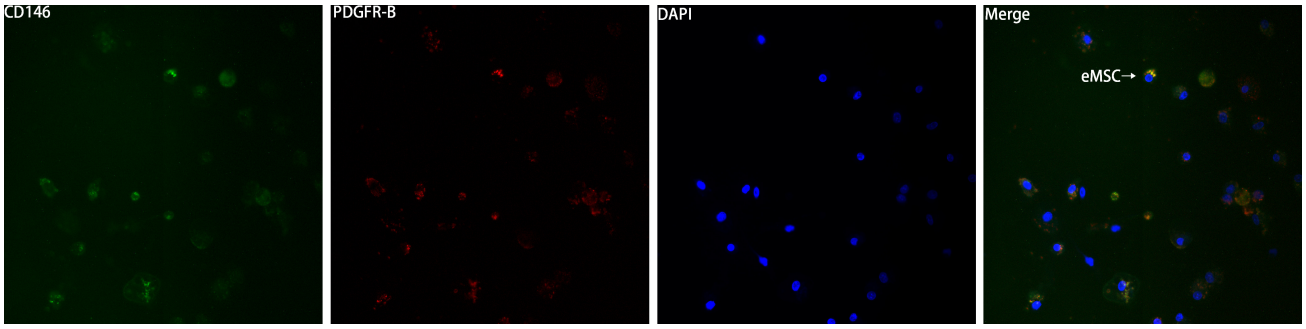

Fig. 1. The eMSCs in retrograde menstruation (×400).

time frame 1 January 2015 to 31 December 2019 at West China Second Hospital, Sichuan University; (2) Diagnoses were endometriosis including ovarian endometriosis, adenomyosis, abdominal wall scar endometriosis (AWSE) and deep endometriosis (DE), all confirmed by experienced pathologists; (3) All subjects had regular menstrual cycles (25–35 days) and were documented as not being pregnant. Patients who received any kind of hormonal therapy (levonorgestrel-releasing intrauterine system or oral contraceptives) for endometriosis before surgery, or suffered from any severe medical or surgical complications were excluded. For postoperative management, all patients were to receive 6 cycles of GnRH-a. Following the treatment, patients were recommended to attempt pregnancy, take oral contraceptives or receive a levonorgestrel-releasing intrauterine system. All patients underwent follow-up for at least 2 years. Relapse of endometriosis-associated dysmenorrhea, dyspareunia, non-menstrual pelvic pain, or a cyst more than 2 cm detected by ultrasound was considered a recurrence. For patients with adenomyosis, postoperative hormonal therapy or follow-up was not required as they underwent a total hysterectomy. To evaluate the effectiveness of GnRH-a on eMSCs, we selected patients who suffered from dysmenorrhea or DE. These patients had indications for preoperative GnRH-a treatment. Before surgery, they received a minimum of 3 cycles of GnRH-a (leuprorelin or triptorelin acetate 3.75 mg subcutaneous injection every 4 weeks). We selected matched control patients who had similar clinical manifestation and were diagnosed with adenomyosis or DE. These patients preferred surgery to GnRH-a and they were matched (1:1) with treatment patients on age, body mass index (BMI), fertility desire and operative approach. Informed consent was obtained from all patients in the study. This study was approved by the ethics committee on human research at West China Second Hospital, Sichuan University.

2.2 Double Immunofluorescence

Retrograde menstruation and tissue sections were collected to detect eMSCs by utilization of immunofluorescence. For immunofluorescence staining to identify eMSCs, primary antibodies included mouse anti-human anti-CD146 and rabbit anti-human anti-PDGFR-β (Abcam) [6]. The secondary antibodies included goat anti-mouse IgG AF488 and goat anti-rabbit IgG AF594 (Beyotime Biotechnology, Shanghai, China). Retrograde menstruation with a volume of 10–20 mL was gathered from the pelvic cavity and stored at 4 °C. Then retrograde menstruation collected was incubated with red blood cell lysis buffer (Beyotime Biotechnology, Shanghai, China) for 5 min. After a 3-min interval of 800 rpm centrifugation, the sample was washed with phosphate buffer solution (Phosphate Buffered Saline (PBS), pH 7.4, Gibco, Shanghai, China) and then was blocked with 1.0% Bovine Serum Albumin (BSA) (Cwbio, Beijing, China). Following washing with PBS (Gibco, Shanghai, China), the cell suspension was incubated with 1:100 initial primary antibody at room temperature for 30 min; the suspension was again washed with PBS and then incubated with 1:100 secondary antibody at room temperature for 30 min. After staining with DAPI (Beyotime Biotechnology, Beyotime, Shanghai, China), the suspension was examined under an OLYMPUS microscope (Olympus Corporation, Tokyo, Japan) and images were captured with a Nikon ECLIPSE 80i (version 4.6, Nikon Corporation, Missouri, WA, USA). Tissue sections (7 um thick) obtained from ovarian endometriosis, adenomyosis, AWSE, DE and normal control tissues (normal ovary, normal myometrium, normal adipose tissue and normal peritoneal membrane), were cut with a cryostat microtome (Leica, Wetzlar, Germany). The sections were fixed in –20 °C methanol for 10 min and blocked in 1.0% BSA for 2 h at room temperature, and then washed with PBS and incubated overnight at 4 °C with 1:200 initial primary antibody. Following three washes for 10 min each, the sections were incubated with 1:200 initial secondary antibody for 1 h at 37 °C and washed with PBS three times for 10 min each. Images were captured using an OLYMPUS microscope fitted with a Nikon ECLIPSE 80i. Four high-power fields were randomly chosen to calculate the mean percent of CD146$^+$/PDGFR-β^+ cells in a section.

$$\text{Percent} = \frac{\text{CD146} + /\text{PDGFR} - \beta + \text{ cells}}{\text{total cells}} \times 100\%$$

Table 1. Baseline characteristics of the patients.

Group	OE (n = 270)	AM (n = 129)	AWSE (n = 37)	DE (n = 72)
Age (year)	32.7 ± 0.3	39.2 ± 0.5	36.8 ± 0.8	33.4 ± 0.7
BMI (kg/m^2)	20.1 ± 0.1	20.8 ± 0.2	21.1 ± 0.5	20.8 ± 0.3
Nulliparity	175	48	0	41
Laparotomy	91	77	37	37
2-year recurrence	7.4%	NS	2.7%	6.9%

OE, ovarian endometriosis; AM, adenomyosis; AWSE, abdominal wall scar endometriosis; DE, deep endometriosis; BMI, body mass index; NS, not statistics.

Table 2. Baseline characteristics of the patients with GnRH-a evaluation.

	Treatment group (n = 66)	Control group (n = 66)	*p*
Adenomyosis, n (%)	50 (75.76%)	50 (75.76%)	-
DE, n (%)	16 (24.24%)	16 (24.24%)	-
Age (years)	36.0 ± 0.8	37.5 ± 0.6	0.14
BMI (kg/m^2)	20.6 ± 0.2	20.7 ± 0.2	0.92
Nulliparity, n (%)	31 (46.97%)	32 (48.48%)	0.99
Laparotomy, n (%)	29 (43.94%)	33 (50.00%)	0.78
Laparoscopy, n (%)	37 (56.06%)	33 (50.00%)	0.78
2-year recurrence*	2	3	0.62

GnRH-a, gonadotropin-releasing hormone agonists; DE, deep endometriosis; BMI, body mass index; * only statistics for DE group.

2.3 Statistical Analysis

Data was presented as mean ± SD. Baseline characteristics of patients who were enrolled in the GnRH-a treatment or control group, and percent of eMSCs in tissues were calculated with analysis of variance (ANOVA) being utilized. A two-sided p value less than 0.05 showed significant difference with software STATA (version 12.0, Stata Corporation, Urbana, IL, USA).

3. Results

In the period from 1 January 2015 to 31 December 2019, a total of 508 patients, which included 270 cases of ovarian endometriosis, 129 cases of adenomyosis, 37 cases of AWSE, and 72 cases of DE, were enrolled. The age (mean ± SD) of the study population was 34.8 ± 0.3 years and BMI was 20.9 ± 0.1 kg/m^2. Two hundred and sixty-four patients (52.0%) were nulliparous. A total of 242 open surgeries and 266 laparoscopies were performed. The 2-year recurrence rate was 6.9% (26/379), which included 20 cases of ovarian endometriosis, 1 case of AWSE and 5 cases of DE. Baseline data for all patients is shown in Table 1.

For evaluation of GnRH-a, a treatment group consisted of 50 adenomyosis patients and 16 DE patients, with a similar control group. There was no significant difference from baseline between the treatment group and the control group ($p > 0.05$, Table 2).

The operation was carried out after menstruation. Twenty-three cases of retrograde menstruation were found during surgery and gathered for analysis. We detected eMSCs in retrograde menstruation material. As shown in Fig. 1, the eMSCs expressing PDGFR-β^+ and CD146$^+$, showed red and green light, while the nuclei, stained with DAPI, showed blue light.

eMSCs were detected in various endometriotic lesions. However, eMSCs could not be identified in normal ovary, myometrium, adipose tissue or peritoneal membrane. Fig. 2 shows the comparison between endometriosis and normal tissues. The percents (mean ± SD) of eMSCs were 0.88% ± 0.01% of ovarian endometriosis (0.88% ± 0.01% of relapse-free patients vs 0.88% ± 0.03% of relapse patients; $p = 0.88$), 1.01% ± 0.01% of adenomyosis, 0.76% ± 0.02% of AWSE (0.76% ± 0.02% of relapse-free patients vs 0.68% of 1 relapse patient; $p = 0.40$), and 1.06% ± 0.01% of DE (1.06% ± 0.01% of relapse-free patients vs 1.04% ± 0.05% of relapse patients; $p = 0.65$) (Fig. 3). Statistical differences were not found between relapse-free patients and those experiencing a relapse.

Specimens obtained from the GnRH-a treatment and control groups were tested to quantitatively compare eMSCs in both groups. As shown in Fig. 4, eMSCs were found in both adenomyosis and DE in the two groups. There was no statistical difference in percents of eMSCs between the treatment group and the control group for adenomyosis patients (1.00% ± 0.03% vs 1.05% ± 0.03%, $p = 0.27$, Fig. 5). Similar to patients with DE, no statistical difference was found for eMSCs between the groups (1.13% ± 0.06% vs 1.15% ± 0.05%, $p = 0.82$, Fig. 5). Evaluation of the 2-year recurrence rate demonstrated no statistical difference ($p = 0.64$, Table 2).

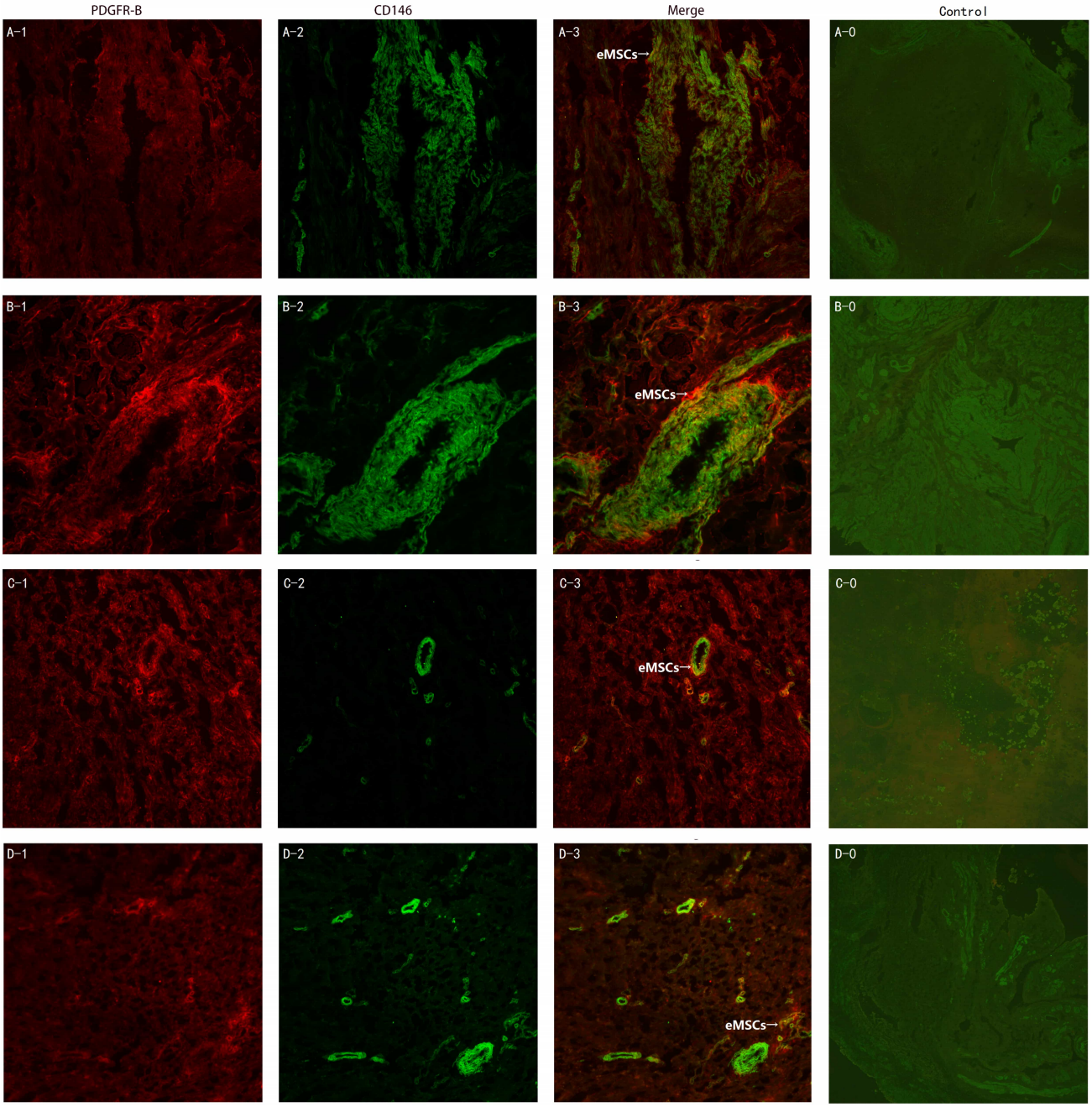

Fig. 2. The eMSCs in different endometriosis lesions and control normal tissues (×100). A-1,2,3 ovarian endometriosis, A-0 ovary tissue. B-1,2,3 adenomyosis, B-0 myometrium of uterine. C-1,2,3 abdominal wall scar endometriosis, C-0 abdominal adipose tissue. D-1,2,3 deep endometriosis, D-0 peritoneal membrane.

4. Discussion

CPP and dysmenorrhea are common health problems and affect women of reproductive age. Women with CPP, regardless of a diagnosis of endometriosis, experience significant negative impact across a range of life issues including education, work, social, and sexual relationships [8]. Although endometriosis is one of the most common gynecological disorders, the etiology of endometriosis still remains unclear, resulting in unsatisfactory management and high risk of recurrence after surgery. Theories include implantation theory, metaplasia theory of coelomic epithelium and induction theory, none of which clearly explain the etiology of endometriosis resulting in non-ideal treatments [9–11]. To improve therapeutic effect, the etiology of endometriosis needs to be clarified. Previous studies have suggested that the recurring endometriotic lesions arise from lesions or cells not completely removed during the primary surgery [12]. Thus, the recurrence may be unavoidable until the etiology is clarified and targeted therapy developed. The CSCs theory proposes that both tumor

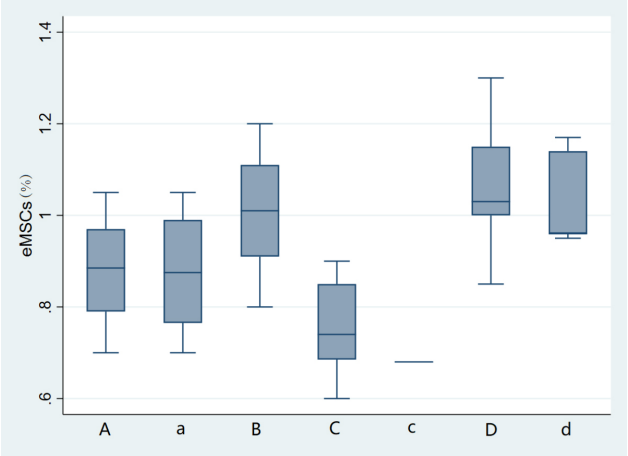

Fig. 3. Percent of eMSCs in different endometriosis lesions. (A) Ovarian endometriosis relapse-free. (a) Ovarian endometriosis with relapse. (B) Adenomyosis. (C) Abdominal wall scar endometriosis relapse-free. (c) Abdominal wall scar endometriosis with relapse. (D) Deep endometriosis relapse-free. (d) Deep endometriosis with relapse.

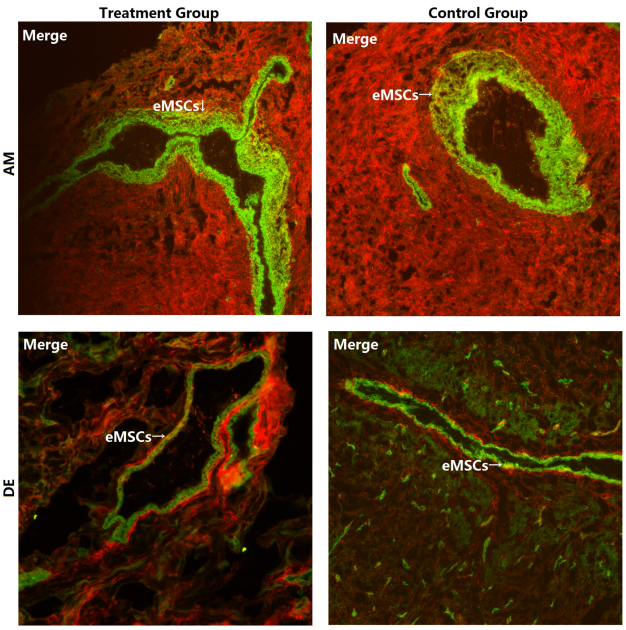

Fig. 4. The eMSCs in the GnRH-a treatment group and the control group (×100). AM, adenomyosis; DE, deep endometriosis.

development and progression are driven by undifferentiated stem cells capable of self-renewal and tumor-initiation. Considering the migration of eMSCs being similar to that of CSCs, and with endometriosis possessing biological behaviors of local aggressiveness, distant metastasis and high disease recurrence, similar to those of ovarian cancer, eMSCs may play a key role in development and relapse of endometriosis.

In 2007, Schwab *et al.* [6] harvested

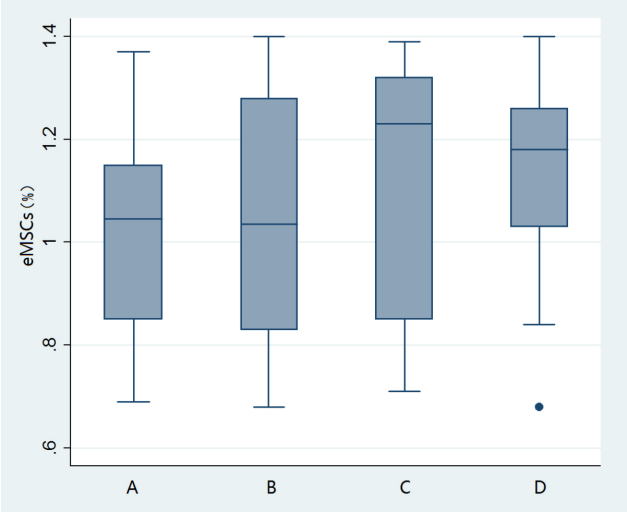

Fig. 5. Percents of eMSCs in the GnRH-a treatment group and the control group. (A) Treatment group of adenomyosis. (B) Control group of adenomyosis. (C) Treatment group of deep endometriosis. (D) Control group of deep endometriosis.

CD146$^+$/PDGFR-β^+ cells from endometrial biopsy samples, which resided in both basalis and functional layers of the endometrium, indicating that eMSCs are likely to be shed in menstrual blood [13,14]. CD146$^+$/PDGFR-β^+ cells, which play a key role in regeneration of the endometrium after menstruation, participated in endometrial cell proliferation, angiogenesis, and implantation [15]. Masuda *et al.* [7] transplanted these cells onto the kidney capsule of NOD/SCID mice whose ovaries were removed, and induced these mice with estrogen and progestin. The cells developed endometrial like structure containing gland and stroma, changing cyclically by estrogen and progestin, indicating these cells owned characteristics of stem cells. Retrograde menstruation, a common phenomenon in women of reproductive age, is a widely accepted risk factor for developing endometriosis. Approximately 76–90% of patients experience retrograde menstruation, particularly those with stenosis or atresia of the cervix [16,17]. In addition, typical endometriosis lesions developed in animal models when menstrual blood was injected [18]. Membrane surface markers, PDGFR-β and CD146, as well as nuclei marker DAPI, were chosen to identify eMSCs in retrograde menstruation [19]. We hypothesized that eMSCs might be shed by menstrual blood into the peritoneal cavity and being induced by sex hormones and microenvironment to evolve into endometriosis, which is similar to the implantation theory. We analyzed various specimens using immunofluorescence and found eMSCs existing in endometriosis lesions. The mean percents of eMSCs varied between a low of 0.76% to a high of 1.06%, slightly below 1.50% in normal endometrium as reported by Schwan *et al.* [13] We did not identify eMSCs in control normal tissue from the same case, indicating

that eMSCs found in endometriosis may arise from other tissues or pathways. We also detected eMSCs in AWSE, indicating that eMSCs might shed with amniotic fluid when patients underwent a cesarean section, differentiate by being exposed to sex hormones and microenvironment, resulting in the development of endometriosis.

GnRH-a, a man-made gonadotropin-releasing hormone, can suppress adenohypophysis and reduce estradiol. GnRH-a could be an adjuvant treatment for endometriosis after surgery [20]. However, the effect of GnRH-a is reversible and recurrence may occur if treatment ceases [21]. A multicenter randomized controlled trial demonstrated that compared with surgery alone, postoperative GnRH-a could prolong the period of recurrence but not effect fertility or reduce recurrence rate (p = 0.08) [22]. In our research, percents of eMSCs showed no statistical difference between the GnRH-a treatment group and the control group, whether adenomyosis (p = 0.27) or DE (p = 0.82) was present. We postulated that GnRH-a may only affect mature endometrial cells except for eMSCs. When treatment is completed or interrupted, the patients' hormone concentrations return and eMSCs are activated, resulting in recurrence. This suggests that the effect of GnRH-a may be limited for endometriosis.

CSCs also have the capacity to affect treatment-resistant tumors [5]. The traditional therapy might not affect CSCs, since residual cells developed homologous ovarian cancer lesions near the primary site [23,24]. Evidence has revealed that CSCs are not only responsible for primary tumor growth, metastasis and relapse of disease, but also for the development of chemoresistance [25]. Considering that the migration of eMSCs is similar to that of CSCs and that endometriosis possesses biological behaviors of local aggressiveness, distant metastasis and high disease recurrence similar to those of ovarian cancer, eMSCs may also play a leading role in the limited efficacy of various drug treatments. Effects of drugs and conservative surgery have a limited role for endometriosis [4]. More than half of patients who underwent conservative surgery required a second surgical procedure. Repeat surgery may damage the patient's ovary harming fertility as well as endocrine function [26]. Recurrent dysmenorrhea and infertility also reduce the patient's quality of life. Our results have demonstrated that peritoneal endometriosis (ovarian endometriosis and DE) might be associated with eMSCs in retrograde menstruation while AWSE is associated with their presence in amniotic fluid at the time of caesarean section. Regardless of whether patients received postoperative GnRH-a, the recurrence rate of AWSE is far below that of peritoneal endometriosis following complete removal of lesions [27]. This may suggest that retrograde menstruation is closely related to the inevitable recurrence of endometriosis within the pelvic cavity. Hence, we postulate that even though surgery could remove every lesion, recurrence cannot be prevented. We suggest that gynecologists regard endometriosis as a chronic disease and develop an individual lifetime management plan for patients based on age, clinical manifestations, fertility desire and quality of life.

5. Conclusions

Our study demonstrated that eMSCs played a critical role in the development and recurrence of endometriosis and that GnRH-a did not affect eMSCs. Gynecologists may regard endometriosis as a chronic disease resulting in lifetime management, especially for patients with CPP.

Author Contributions

JZ—protocol development, data collection, data analysis, manuscript writing. XL—data analysis, manuscript editing. TY—protocol development, manuscript editing. AT—data collection, data analysis, manuscript writing. RP—data collection, data analysis, manuscript writing. GZ—data analysis, manuscript writing. SL—data analysis, manuscript writing. XZ—protocol development, manuscript editing. CB—protocol development, manuscript editing. GS—protocol development, manuscript editing. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

This study was approved by the ethics committee on human research at West China Second Hospital, Sichuan University (No. 2020074). All subjects gave their informed consent for inclusion before they participated in the study.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Mowers EL, Lim CS, Skinner B, Mahnert N, Kamdar N, Morgan DM, *et al*. Prevalence of Endometriosis During Abdominal or Laparoscopic Hysterectomy for Chronic Pelvic Pain. Obstetrics & Gynecology. 2016; 127: 1045–1053.

[2] Hickey M, Ballard K, Farquhar C. Endometriosis. British Medical Journal. 2014; 348: g1752–g1752.

[3] Whitaker LH, Reid J, Choa A, McFee S, Seretny M, Wilson J, *et al*. An Exploratory Study into Objective and Reported Characteristics of Neuropathic Pain in Women with Chronic Pelvic Pain. PLoS ONE. 2016; 11: e0151950.

[4] Cheong Y, Tay P, Luk F, Gan HC, Li T-, Cooke I. Laparoscopic surgery for endometriosis: how often do we need to re-operate? Journal of Obstetrics and Gynaecology. 2008; 28: 82–85.

[5] Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001; 414: 105–111.

[6] Schwab KE, Gargett CE. Co-expression of two perivascular cell markers isolates mesenchymal stem-like cells from human endometrium. Human Reproduction. 2007; 22: 2903–2911.
[7] Masuda H, Maruyama T, Hiratsu E, Yamane J, Iwanami A, Nagashima T, *et al*. Noninvasive and real-time assessment of reconstructed functional human endometrium in NOD/SCID/gamma c(null) immunodeficient mice. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104: 1925–1930.
[8] Armour M, Sinclair J, Ng CHM, Hyman MS, Lawson K, Smith CA, *et al*. Endometriosis and chronic pelvic pain have similar impact on women, but time to diagnosis is decreasing: an Australian survey. Scientific Reports. 2020; 10: 16253.
[9] Sampson JA. Metastatic or Embolic Endometriosis, due to the Menstrual Dissemination of Endometrial Tissue into the Venous Circulation. The American Journal of Pathology. 1927; 3: 93–110.43.
[10] Ferguson BR, Bennington JL, Haber SL. Histochemistry of mucosubstances and histology of mixed mullerian pelvic lymph node glandular inclusions. Evidence for histogenesis by mullerian metaplasia of coelomic epithelium. Obstetrics & Gynecology. 1969; 33: 617–625.
[11] Steele RW, Dmowski WP, Marmer DJ. Immunologic Aspects of Human Endometriosis. American Journal of Reproductive Immunology. 1984; 6: 33–36.
[12] Guo S-. Recurrence of endometriosis and its control. Human Reproduction Update. 2009; 15: 441–461.
[13] Schwan WR, Shibata S, Aizawa S, Wolfe AJ. The two-component response regulator RcsB regulates type 1 piliation in Escherichia coli. Journal of Bacteriology. 2007; 189: 7159–7163.
[14] Cui C, Uyama T, Miyado K, Terai M, Kyo S, Kiyono T, *et al*. Menstrual Blood-derived Cells Confer Human Dystrophin Expression in the Murine Model of Duchenne Muscular Dystrophy via Cell Fusion and Myogenic Transdifferentiation. Molecular Biology of the Cell. 2007; 18: 1586–1594.
[15] Cervelló I, Mas A, Gil-Sanchis C, Peris L, Faus A, Saunders PT, *et al*. Reconstruction of endometrium from human endometrial side population cell lines. PLoS ONE. 2011; 6: e21221.
[16] Barbieri RL. Stenosis of the external cervical os: an association with endometriosis in women with chronic pelvic pain. Fertility and Sterility. 1998; 70: 571–573.
[17] Song X, Zhu L, Ding J, Xu T, Lang J. Clinical characteristics of congenital cervical atresia and associated endometriosis among 96 patients. International Journal of Gynecology & Obstetrics. 2016; 134: 252–255.
[18] Dehoux J, Defrère S, Squifflet J, Donnez O, Polet R, Mestdagt M, *et al*. Is the baboon model appropriate for endometriosis studies? Fertility and Sterility. 2011; 96: 728–733.e3.
[19] Gargett CE, Schwab KE, Deane JA. Endometrial stem/progenitor cells: the first 10 years. Human Reproduction Update. 2016; 22: 137–163.
[20] Vercellini P, Aimi G, Panazza S, De Giorgi O, Pesole A, Crosignani PG. A levonorgestrel-releasing intrauterine system for the treatment of dysmenorrhea associated with endometriosis: a pilot study. Fertility and Sterility. 1999; 72: 505–508.
[21] Dunselman GAJ, Vermeulen N, Becker C, Calhaz-Jorge C, D'Hooghe T, De Bie B, *et al*. ESHRE guideline: management of women with endometriosis. Human Reproduction. 2014; 29: 400–412.
[22] Vercellini P, Crosignani PG, Fadini R, Radici E, Belloni C, Sismondi P. A gonadotrophin-releasing hormone agonist compared with expectant management after conservative surgery for symptomatic endometriosis. BJOG: an International Journal of Obstetrics and Gynaecology. 1999; 106: 672–677.
[23] Li X, Lewis MT, Huang J, Gutierrez C, Osborne CK, Wu M, *et al*. Intrinsic Resistance of Tumorigenic Breast Cancer Cells to Chemotherapy. Journal of the National Cancer Institute. 2008; 100: 672–679.
[24] Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, *et al*. Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature. 2009; 458: 780–783.
[25] Bishnu A, Sakpal A, Ghosh N, Choudhury P, Chaudhury K, Ray P. Long term treatment of metformin impedes development of chemoresistance by regulating cancer stem cell differentiation through taurine generation in ovarian cancer cells. The International Journal of Biochemistry & Cell Biology. 2019; 107: 116–127.
[26] Exacoustos C, Zupi E, Amadio A, Amoroso C, Szabolcs B, Romanini ME, *et al*. Recurrence of endometriomas after laparoscopic removal: Sonographic and clinical follow-up and indication for second surgery. Journal of Minimally Invasive Gynecology. 2006; 13: 281–288.
[27] Marras S, Pluchino N, Petignat P, Wenger J, Ris F, Buchs NC, *et al*. Abdominal wall endometriosis: an 11-year retrospective observational cohort study. European Journal of Obstetrics & Gynecology and Reproductive Biology: X. 2019; 4: 100096.