The role of macrophage in endometriosis and endometriosis-associated ovarian cancer

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Abstract

Endometriosis affects approximately 176 million women worldwide and carries a high risk of malignant transformation. Understanding the mechanism of endometriosis occurrence and development is crucial for its treatment, particularly in preventing malignant transformation. Macrophages are among the most important immune cells in promoting endometriosis occurrence and development. Defects in their function can result in incomplete clearance of retrograde endometrial debris. M2 macrophages can promote the formation of endometriotic lesions and induce an anti-inflammatory environment that promotes the development and malignant transformation of endometriosis. This review summarizes the role of macrophages in endometriosis and endometriosis-associated ovarian cancer. The review also covers existing treatment methods and strategies of targeted macrophage immunotherapy for endometriosis.

Keywords

Endometriosis; M1; M2; macrophage; endometriosis associated ovarian cancer; immunotherapy.

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1.Overview of endometriosis and endometriosis-associated cancer

1.1 Endometriosis

Endometriosis (EMS), in which endometrial tissue is abnormally located outside the uterus, is a common female inflammatory reproductive disorder, associated with chronic pelvic pain and subfertility. It impacts nearly 5%-10% of fertile women, equivalent to 176 million women worldwide[1]. According to Sampson's theory of retrograde menstruation, EMS results from the reflux of endometrial fragments through the fallopian tubes during menstruation, which then implants on the peritoneum and ovary[2]. The revised American Society for Reproductive Medicine classification is one of the most frequently used systems to classify EMS[3]. In general, the lesion location and infiltration depth of surrounding tissue are bases for staging of EMS, corresponding to superficial peritoneal lesion, deep infiltrating EMS, and endometrioma[4].

EMS is an estrogen-dependent inflammatory disease[5]. In recent years, endocrine/paracrine alterations and immune aspects, such as complements, cytokines, growth factors, hormones, and immune cells, have been proposed to be involved in the pathophysiology of EMS (Figure 1). For example, neutrophils produce biochemical factors that aid in peritoneal immune inflammation and angiogenesis. The cytotoxicity of natural killer (NK) cells in endometriosis is inhibited to eliminate endometrial cells in the abdominal cavity, thereby resulting in immune escape. The imbalance between T helper type 1 (Th1)/ T helper type 2 (Th2) cells causes cytokine secret aberrantly, which induces lesion progression[6-8]. Overall, in the abdominal cavity microenvironment, the dysfunction of the immune system promotes the adhesion and invasion of ectopic endometrial cells, thus contributing to poor clearance, implantation, angiogenesis, and proliferation of endometrial debris [5] [8, 9]. Although there is little information available on the clinical application of immune therapy in EMS, these findings provide novel insights into targeting related immune factors for EMS treatment[10]. The role and transformation of macrophage engaging in EMS will be elaborated in detail in this review.

1.2 Endometriosis-associated cancer

EMS is a benign disease but is potential for malignant transformation. It shares features with cancer, including metastasis-like behavior, tissue invasion, proliferation, angiogenesis, and reduced apoptosis[11]. Growing evidence has suggested that the occurrence and development of various cancers are associated with the malignant progression of EMS, such as endometrioid carcinoma, clear cell carcinoma, non-Hodgkin's lymphoma, brain tumors, and endocrine cancers[12]. Notably, a strong genetic correlation and a positive association between EMS and ovarian cancer have been confirmed by Mortlock and Kvaskoff et al respectively[4, 11]. A study by He ZX et al. showed that 2.9% of women with ovarian endometriosis were found to have endometriosis-associated ovarian cancer[13]. Meanwhile, Hermens's study revealed a significant higher incidence of endometriosis-associated endometrioid ovarian cancer in women with histologically proven endometriosis[14]. Therefore, EMS-related ovarian cancer is the research focus. Atypical EMS, including hyperplasia and/ or atypia type, is associated with ovarian cancer. Pathologic diagnosis of endometriosis is characterized by the presence of 2 of the 3 following histologic features: endometrial stromal cells, endometrial-type glands, and findings consistent with chronic bleeding, such as hemosiderin-laden macrophages[15]. In contrast to the histological features of typical endometriosis, atypical endometriosis is defined as EMS with a localized proliferation of crowded glands lined by atypical epithelium resembling endometrial intraepithelial neoplasia, or alteration in endometriotic cyst lining with stratification, disorganization and cytologic atypia by the World Health Organization[16]. Epidemiological studies have shown that endometriosis is associated with an increased risk of epithelial ovarian cancer (EOC)[4]. The subtypes of ovarian cancer that have a

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high association with endometriosis are endometrioid ovarian cancer (ENOC) and clear cell ovarian cancer (CCOC), whereas high-grade serous ovarian cancer (HGSOC) is considered to be less associated with endometriosis[4]. Concurrent endometriosis is observed in 21%-51% of CCOC patients and 23%-43% of ENOC patients [4]. Studies have shown this association. When ovarian cancer coexists with EMS, there are three possible mechanisms: ① they are caused by the same risk factors; ② cancer cells develop from endometriotic cells; or ③ they are caused by different risk factors. This study focuses on the mechanisms of endometriosis-associated ovarian cancer (EAOC) by which cancer cells develop from EMS[4].

The development of endometriosis-associated ovarian cancer is a multifactorial process that involves genetic and environmental factors, including gene mutation resulting from long-term inflammation in the abdominal cavity, immune disorder, and DNA damage (Figure 1)[5]. EMS lesions in the abdominal cavity periodically and repeatedly bleed, leading to overload of iron in the microenvironment. High iron concentration promotes ferroptosis of normal cells in the microenvironment such as immune cells and stromal cells, thus promotes the development of EMS[17, 18]. This process is associated with intracellular reactive oxygen species(ROS) production, lipid peroxidation, and mitochondrial damage[19]. Additionally, activation of inflammatory pathways and angiogenesis can lead to EMS progression and iron-mediated DNA damage[20].

Shared somatic mutations between benign endometriotic lesions and adjacent tumors suggest that these lesions may serve as the cellular precursors of EAOC. Several genes, including AT-rich interaction domain 1A (ARID1A), Kirsten rat sarcoma viral oncogene homolog (KRAS), phosphatase and tensin homolog (PTEN), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), and P53, which also play important roles in uterine endometrioid carcinomas and EMS-related cancer development, are involved in the development of endometriosis[5, 11, 21]. Mutations in these specific genes have a high potential to trigger the progression of endometriosis to EAOC. Studies have shown that patients with endometriosis have increased peritoneal fluid concentrations of ovarian cancer markers carbohydrate antigen 125 (CA125) and human epididymis protein 4 (HE4) [22]. Estrogen excess is common in both EMS and ovarian cancer, and the decrease in estrogen receptor levels can increase estrogen levels, which may lead to an increase in carcinogenesis[23]. Estrogen-positive is common in endometrioid adenocarcinoma. The use of contraceptives has been demonstrated to decrease the risk of ovarian cancer, regardless of the presence of endometriosis. Hepatocyte nuclear factor 1-beta (HNF1-β) is generally expressed in clear cell and serous carcinomas, as well as in precursor lesions of endometriosis like borderline and atypical endometriosis[24],[25]. This suggests that endometriosis is associated with a higher risk of ovarian cancer due to genetic and epigenetic changes in gene expression, pathways, and other factors.

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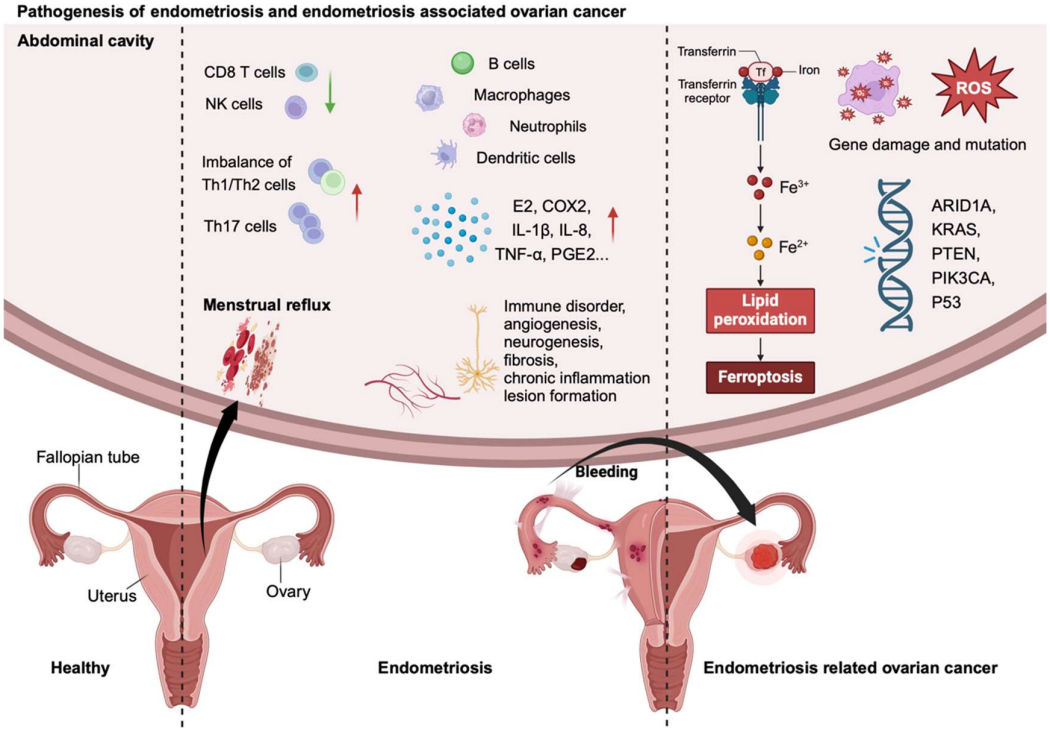

Figure 1 Pathogenesis of endometriosis and endometriosis-associated ovarian cancer.
EMS results from the reflux of endometrial fragments through the fallopian tubes during menstruation and subsequent implantation on the peritoneum and ovary. Estrogen, complements, cytokines, growth factors, hormones, and immune cells interact to influence the peritoneal microenvironment, leading to poor clearance, implantation, angiogenesis, and proliferation of endometrial debris. Endometriosis-associated ovarian cancer is caused by complicating factors including gene mutations, immune dysregulation, and DNA damage. Mutations in some specific genes have a high potential to trigger the progression of endometriosis to EAOC. In the peritoneal cavity, periodically and repeatedly bleeding EMS lesions lead to ferroptosis, which is associated with intracellular ROS production, lipid peroxidation, and mitochondrial damage. In addition, activation of inflammatory pathways and angiogenesis may lead to EMS progression and iron-mediated DNA damage. NK cells, natural killer cells; Th1 cells, T helper type 1 cells; Th2 cells, T helper type 2 cells; Th17 cells, T helper type 17 cells; E2, estradiol; COX2, cyclooxygenase 2; IL-1β, interleukin-1 beta; IL-8, interleukin-8; TNF-α, tumor necrosis factor alpha; PGE2, prostaglandin E2; Tf, Transferrin; ROS, reactive oxygen species; ARID1A, AT-rich interaction domain 1A; KRAS, Kirsten rat sarcoma viral oncogene homolog; PTEN, phosphatase and tensin homolog; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha. (Created by BioRender).

2. Overview of macrophage in endometriosis

Macrophages are typically classified as either classically activated 'M1' macrophages or alternatively activated 'M2' macrophages based on their function (Figure 2). Surface markers including CD68, toll-like receptor 4 (TLR4), CD14, human leukocyte antigen DR (HLA-DR), CD80, and CD86 express on human M1 macrophages (F4/80, major histocompatibility complex Ⅱ (MHC II), CD86, and inducible nitric oxide synthase (iNOS) on mouse macrophages) [26, 27]. These macrophages express pro-inflammatory markers, secrete pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), prostaglandin E2 (PGE2), hepatocyte growth factor (HGF), interleukin-12

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(IL-12), interleukin-23 (IL-23), and NOS, and promote inflammation[26]. The surface markers for human M2 macrophages are CD68, TLR4, CD163, CD40, CD206, $CD14^{low}$, $HLA\text{-}DR^{low}$, $CD80^{low}$, and $CD86^{low}$ (for mice are $F4/80^{+}$, MHC II^{+}, $CD206^{+}$ and arginase1 $(Arg1)^{+}$)[27, 28]. Conversely, M2 macrophages secrete anti-inflammatory cytokines, such as interleukin-10 (IL-10), transforming growth factor beta (TGF-β), and vascular endothelial growth factor (VEGF), and are involved in homeostasis, wound healing, and immune regulation[29]. According to physiological or pathological conditions, macrophages alter their transcription factors and phenotype in response to signals from the local microenvironment.

Although the M1/M2 classification system is a useful tool for studying macrophage activation, it is now recognized that macrophages in vivo exhibit a broad spectrum of tissue- and disease-specific phenotypes. Therefore, the M1/M2 system cannot fully represent the diversity and complexity of macrophage phenotypes. With the in-depth study of macrophages, they are classified as resident macrophages or recruited macrophages based on their origin (Figure 2). Resident macrophages originate from the yolk sac, fetal liver, and erythrocyte-marrow progenitor (EMP) cells produced during primitive hematopoiesis in embryos at embryonic stages 7.5 and 8.25[30]. After the formation of the blood circulation, macrophages derived from EMPs are implanted in fetal tissues. These macrophages are partially or completely replaced by monocytes from the fetal liver (excluding microglia) and differentiate into tissue macrophages[31]. Resident macrophages derived from fetal liver monocytes can persist through out adulthood and undergo self-renewal with specific tissue macrophage characteristics, such as microglia, and langerhans cells[32]. In certain organs, such as the intestine and dermis, tissue macrophages that originate from fetal liver monocytes are gradually replaced by monocytes that originate from bone marrow[33].

The macrophages involved in the development of EMS are primarily endometrial macrophages, peritoneal macrophages, and monocyte-supplemented macrophages[28]. Abdominal cavity macrophages are one of the most studied macrophage populations in mice and one of the most important cell populations involved in the development of EMS due to their ease of isolation. Abdominal cavity macrophages can be classified according to their origin into resident macrophages (peritoneal macrophages) and monocyte-derived macrophages (Figure 2). Peritoneal macrophages in mice are classified into two subpopulations, "large" (larger size) peritoneal macrophages (LpM) and "small" (smaller size) peritoneal macrophages (SpM), based on differential expression of F4/80 and MHC II. LpM is characterized by $F4/80^{high}$ and MHC II^{low}[34]. The LpM is the most abundant macrophage subpopulation in peritoneal luminal homeostasis and plays an important role in immunosurveillance, B1b cell recruitment and maintenance, and intestinal immunoregulation. It has a specific transcription factor, GATA-6, which controls self-renewal, proliferation, and phenotype[34]. The SpM is a subpopulation of $F4/80^{low}$ MHC II^{high} macrophages and conventional dendritic cells. Their differentiation is dependent on interferon regulatory factor 4 (IRF4), and they are replaced over time by lymphocyte antigen 6 complex $(Ly6C)^{high}$ classical monocytes in a cysteine-cysteine motif chemokine receptor 2 (CCR2)-dependent manner[34]. SpM have been implicated in the inflammatory response, but their role in the homeostatic abdominal cavity remains unclear. Chronic inflammation, fibrosis and angiogenesis are inherent features of EMS in which macrophages play an important role. EMS lesions are formed through tissue repair, regeneration, angiogenesis, and nerve fiber reconstruction involving macrophages[35]. Learning the complex characterization of macrophage is

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vital for us to learn the development of EMS and better intervene in it.

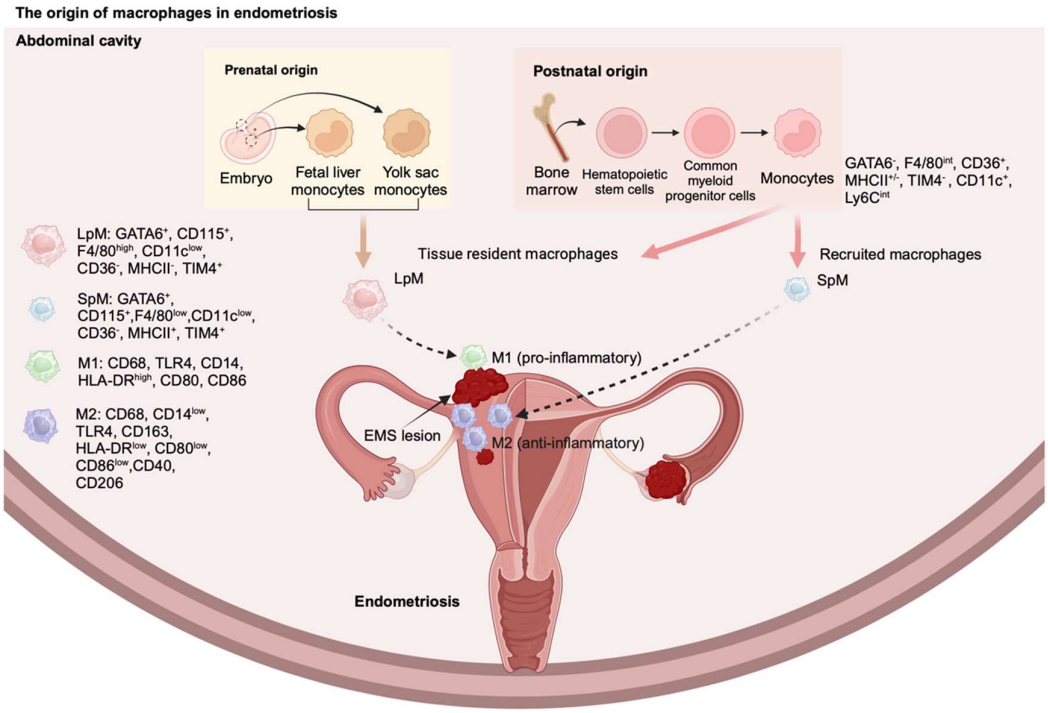

Figure 2 The origin of macrophages in endometriosis.

Macrophages are typically classified as 'M1' macrophages and 'M2' macrophages based on their function. M1 macrophages express pro-inflammatory markers, secrete pro-inflammatory cytokines and promote inflammation. Conversely, M2 macrophages secrete anti-inflammatory cytokines, and are involved in homeostasis, wound healing, and immune regulation. Depending on the origin, macrophages in the abdominal cavity were classified as tissue resident macrophages and recruited macrophages. Tissue resident macrophages originate from the yolk sac, fetal liver, and erythrocyte-marrow progenitor cells produced during primitive hematopoiesis in embryos. Resident macrophages differentiate into "large" (larger size) peritoneal macrophages (LpMs) in the abdominal cavity, which mainly exhibit M1 phenotype. SpMs are smaller size peritoneal macrophages derived from monocytes, which tend to be M2 phenotype. LpM, "large" (larger size) peritoneal macrophages; SpM, "small" (smaller size) peritoneal macrophages; MHC Ⅱ, major histocompatibility complex Ⅱ; TIM4, T-cell immunoglobulin mucin-4; EMS, endometriosis; Ly6C, lymphocyte antigen 6 complex; TLR4, toll-like receptor 4; HLA-DR, human leukocyte antigen-DR. (Created by BioRender).

2.1 Endometrial macrophages

The human endometrium is highly regenerative and can undergo approximately 400 menstrual cycles[36]. The menstrual cycle consists of four phases: menstrual, regenerative, proliferative, and secretory phase. In the menstrual phase, the functional layer of the endometrium is shed, and an inflammatory microenvironment is formed in the uterus, recruiting various immune cells[37]. Macrophages play a crucial and irreplaceable role in this process[37].

Macrophages in the endometrium are mainly divided into resident and recruited macrophages. Tissue-resident macrophages are present in the three layers of the uterus, including the endometrium, myometrium, and periuterine[26]. They are particularly abundant in the endometrium. Monocyte influx occurs during the secretory and menstrual phases in the normal circulating endometrium (Figure 3). They are regulated by cysteine-cysteine motif chemokine ligand 2 (CCL2) and C-X3-C motif chemokine

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receptor 1 (CX3CR1) and influenced by sex hormones, particularly estrogen and progesterone[38]. Ultimately, these monocytes differentiate into macrophages in the uterus[28]. The number of macrophages in the endometrium, regulated by estrogen and progesterone, fluctuates during the natural menstrual cycle[26]. They account for approximately 1-2% of all cells in the endometrium during the proliferative phase, increasing slightly during the secretory phase to approximately 3-5%, and reaching a peak of 6-15% during the menstrual phase[26]. The duration of resident and recruited macrophages and their immune response during the menstrual cycle determine the outcome of the endometrial healing process, including the degree of fibrosis and tissue regeneration. During menstruation, macrophages play an important role in removing uterine tissue debris and secreting inflammatory cytokines and growth factors[39]. This process is predominantly carried out by the M1 macrophages in the first phase of the immune response. During the proliferative phase of the adaptive immune response, macrophages secrete anti-inflammatory cytokines and growth factors to promote tissue regeneration[26]. This process mainly involves the M2 macrophages. Additionally, macrophages in the endometrium perform regulatory repair of the endometrium without scar formation to maintain tissue integrity during the natural menstrual cycle. Endometrial macrophages may play a role in various processes of normal pregnancy, as well as in the maintenance of immune tolerance. However, their function in the uterus has not been clearly demonstrated.

The inflammatory features of endometriosis are not limited to the pelvic and abdominal cavities or the vicinity of the ectopic lesion but also affect the native endometrium. A previous study found that the number of M1 macrophages was higher in stage I-II endometriosis than in healthy controls, while the number of M2 macrophages was elevated in the eutopic endometrium of women with stage III-IV endometriosis. Additionally, a study reported that M2 macrophages were the predominant phenotype in healthy endometrium, whereas the population of endometrial M2 macrophages in women with endometriosis was lower than in controls at all cycle stages[6].

The predominance of M2 macrophages in the endometrium of healthy women is due to the need for the uterus to maintain an anti-inflammatory environment that facilitates embryo implantation[40]. In contrast, endometrial macrophages from EMS patients exhibited a more pro-inflammatory phenotype[41]. They secreted higher levels of IL-1, IL-6, IL-8, and HGF compared to normal controls. Monocyte chemoattractant protein-1 (MCP-1) and macrophage inhibitory factors are involved in the formation of the pro-inflammatory environment. Endometrial $CD91^{+}$ macrophages overexpressed signal regulatory protein α (SIRPα), a phagocytosis inhibitor, and CD64, which is associated with inflammation environment[42]. The incomplete clearance of endometrial debris during menstruation and decreased phagocytosis by macrophages allow tissue debris to "reflux" outside the uterine cavity, leading to the formation of endometriosis-associated lesions (Figure 4)[39].

Studying the effect of endometrial resident macrophages on EMS can be challenging due to their low numbers and the fact that immune cells often infiltrate the uterus during menstrual cycle and are non-uniformly distributed in both the epithelium and stromal layer[43, 44]. So, it is important to consider these factors when conducting research on this population. Further accumulation of evidence is essential to gain a clear and comprehensive understanding of the precise role of uterine macrophages involved in these physiological and pathological events.

2.2 Abdominal cavity macrophages

As previously mentioned, one of the pathogenic mechanisms of EMS is retrograde menstruation. Senescent erythrocytes accumulate and die in the pelvic cavity, leading to iron overload[45]. The iron overload in peritoneal macrophages exacerbated phagocytosis and clearance of erythrocytes, which

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affects macrophage activation[2]. Macrophage dysfunction, which includes reduced antigen presentation, phagocytosis, and cytotoxic activity, as well as activation of the nuclear factor-κB (NF-κB) pathway, ultimately leads to immune escape of refluxing endometrial debris and an increased inflammatory microenvironment and chemotaxis in the abdominal cavity.

The two subpopulations of abdominal cavity macrophages, LpM and SpM, play different role in EMS development. It has been demonstrated that macrophages from EMS lesions in mice are derived from LpMs and monocytes in eutopic endometrial tissue. In addition, abdominal cavity inflammation due to EMS triggers sustained recruitment of monocytes and increased $CCR2^+$ LpM[28]. However, it has also been shown that the proportion of SpMs rises immediately after abdominal cavity injections of endometrial tissues, whereas LpMs show the opposite trend[46]. Thus, the effect of macrophages in the abdominal cavity, which are typed according to their origin, on the development of EMS is controversial and needs to be verified by more experiments.

In EMS peritoneal macrophages, M1 and M2 macrophages have been studied in more detail. The M2 phenotype, required for angiogenesis and ectopic lesion growth, is a peritoneal macrophage type involved in the development of EMS (Figure 4)[47]. Compared with healthy women, patients with EMS have decreased M1 macrophages and increased M2 macrophages in the abdominal cavity, while the number of M1/M2 macrophages change contrarily in the endometrium. In EMS patients, anti-inflammatory M2 macrophages are the predominant phenotype in the abdominal cavity, whereas pro-inflammatory M1 macrophages play a leading role in the ectopic lesion. Research has shown that ectopic endometrial homogenates or sera can increase the percentage of $CD163^+$ macrophages and IL-10, while decreasing the percentage of $CD86^+$ macrophage cells and IL-12. This is achieved by up-regulating suppressor of mother against decapentaplegic family member 2 (SMAD2)/SMAD3 in macrophages[48]. Activation of estrogen receptor beta (ERβ) in endometrial stromal cells (ESCs) is involved in macrophage recruitment through NF-κB/CCL2 signaling and contributes to the pathogenesis of EMS by promoting macrophage differentiation into the M2 phenotype[47]. The presence of M2 macrophages in the abdominal cavity and ectopic lesions indicates that this cell population plays a role in creating an anti-inflammatory environment that allows for lesion establishment and growth.

The polarization and function of M2 macrophages are affected by many factors. Research has shown that patients with EMS exhibit an increased proportion of T helper type 17 (Th17) cells[46, 49, 50]. Th17 cells promote M2 macrophage polarization and lead to the recruitment of bone marrow-derived SpM precursors and $CD206^+$ SpM[51]. Additionally, in vitro, it enhances the expression of IL-8, which promotes inflammation and fibrosis in EMS by inducing neutrophil migration and MCP-1 secretion, making macrophage migrate towards EMS lesions[52, 53]. Via the transporter legumain pseudogene 1 (LGMNP1), ectopic endometrial stromal cell-derived extracellular vesicular can act as intercellular messengers to mediate upregulation of legumain (LGMN) mRNA expression in macrophages, thus reprogramming macrophages into M2 phenotype in vitro[54]. Lactate was found to be a key factor driving macrophage M2 polarization to promote ESC invasion in vitro and in vivo, and activation of methyltransferase 3 (Mettl3) and its target gene, tribbles pseudokinase 1 (Trib1), promotes M2 macrophage polarization through the extracellular regulated protein kinases (ERK)/ signal transducer and activator of transcription 3 (STAT3) signaling pathway when inhibition of glycolysis significantly reduces EMS progression[55]. Nanovesicles derived from M1 macrophages directly or indirectly inhibited the migration and invasion of ESCs obtained from patients with endometriosis and reduced the formation of tubular structures and inhibited the development of EMS by reprogramming M2 macrophages[56].

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In addition, M2 macrophages in the abdominal cavity and lesions may exacerbate pain in women with endometriosis by promoting the growth of nerve fibers. Ectopic lesions in the abdominal cavity trigger the immune system and induce subsequent inflammation, which supports the survival and growth of ectopic endometrial tissue fragments. Netrin-1, an extracellular guidance cue for neuronal navigation, promotes neurovascularization in endometriosis. Studies have shown that netrin-1 expression peaks in peritoneal macrophages in endometriosis-associated infertility. Netrin-1 induces the formation of ovarian endometriotic angiogenesis by interacting with CD146 in vascular endothelial cells[57]. Additionally, netrin-1 promotes endometriotic protrusions by up-regulating microtubule-associated protein 4 (MAP4), tau protein, and calcitonin gene-related peptide (CGRP) through another receptor, neogenin, which contributes to the growth and sensitization of endometriotic lesions[57].

Cytokines and chemokines secreted by macrophages also play a role in EMS development (Figure 4). It has been found that IL-33 secreted by macrophages can accelerate the progression of EMS[58]. Co-culturing with macrophages or using IL-33/ suppression of tumorigenicity 2 (ST2) can stimulate the viability and migration of ectopic endometrial stromal cells (eESCs). Macrophage-derived IL-33 upregulates solute carrier family 7 member 11 (SLC7A11) in eESCs through the p38/ c-Jun N-terminal kinase (JNK)/ activating transcription factor 3 (ATF3) pathway, reduce intracellular iron levels and lipid peroxidation, which ultimately result in protection against ferroptosis in eESCs[58]. Exosome from EMS patients' peritoneal macrophages transports long non-coding RNA (lncRNA) CHL1-AS1 to promote eESC proliferation, migration, and invasion, inhibits apoptosis by downregulating mir-610 and upregulating Murine double minute 2 (MDM2)[59]. Additionally, ESC can influence macrophages to promote EMS progress. Co-culturing with ESC, macrophage secreted cysteine-cysteine motif chemokine ligand 20 (CCL20) and activated cysteine-cysteine motif chemokine receptor 6 (CCR6), which induced the proliferation and migration of ESC as well as impaired the function of lysosome, thereby blocking the autolysosomal degradation of ESC[60].

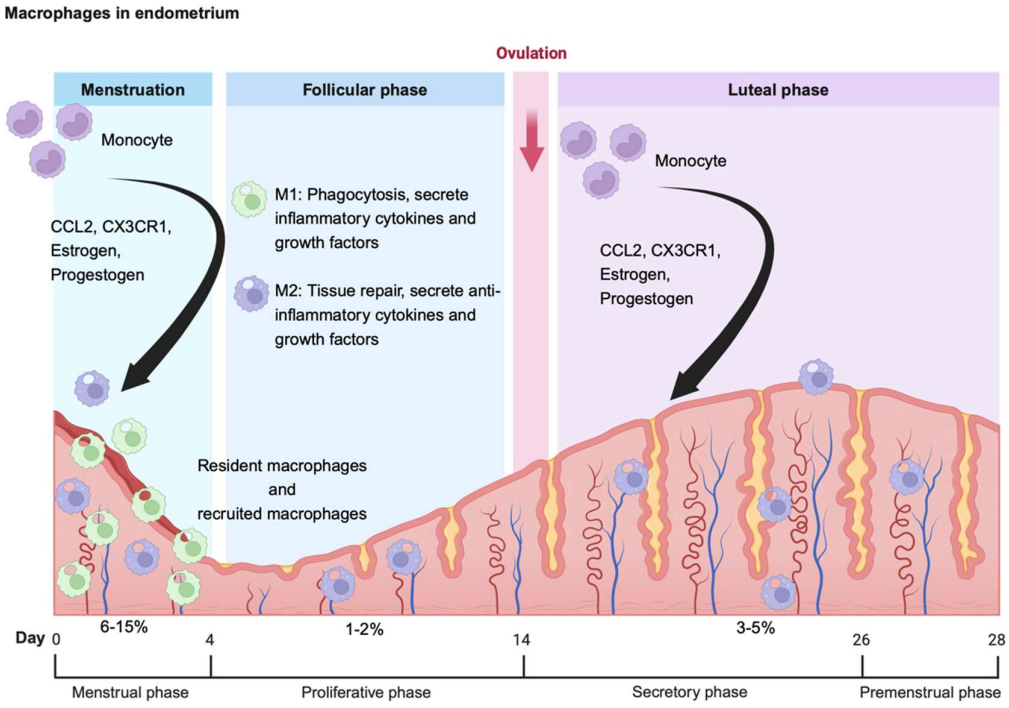

Figure 3 Macrophages in endometrium.

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During the menstrual cycle, monocytes in the uterus differentiate into macrophages. This process is regulated by CCL2 and CX3CR1 and influenced by sex hormones, especially estrogen and progesterone. In the physiological process, M1 macrophages remove uterine tissue debris and secrete inflammatory cytokines and growth factors in the first phase of the immune response. M2 macrophages secrete anti-inflammatory cytokines and growth factors to promote tissue regeneration during the proliferative phase of the adaptive immune response. CCL2, cysteine-cysteine motif chemokine ligand 2; CX3CR1, C-X3-C motif chemokine receptor 1. (Created by BioRender).

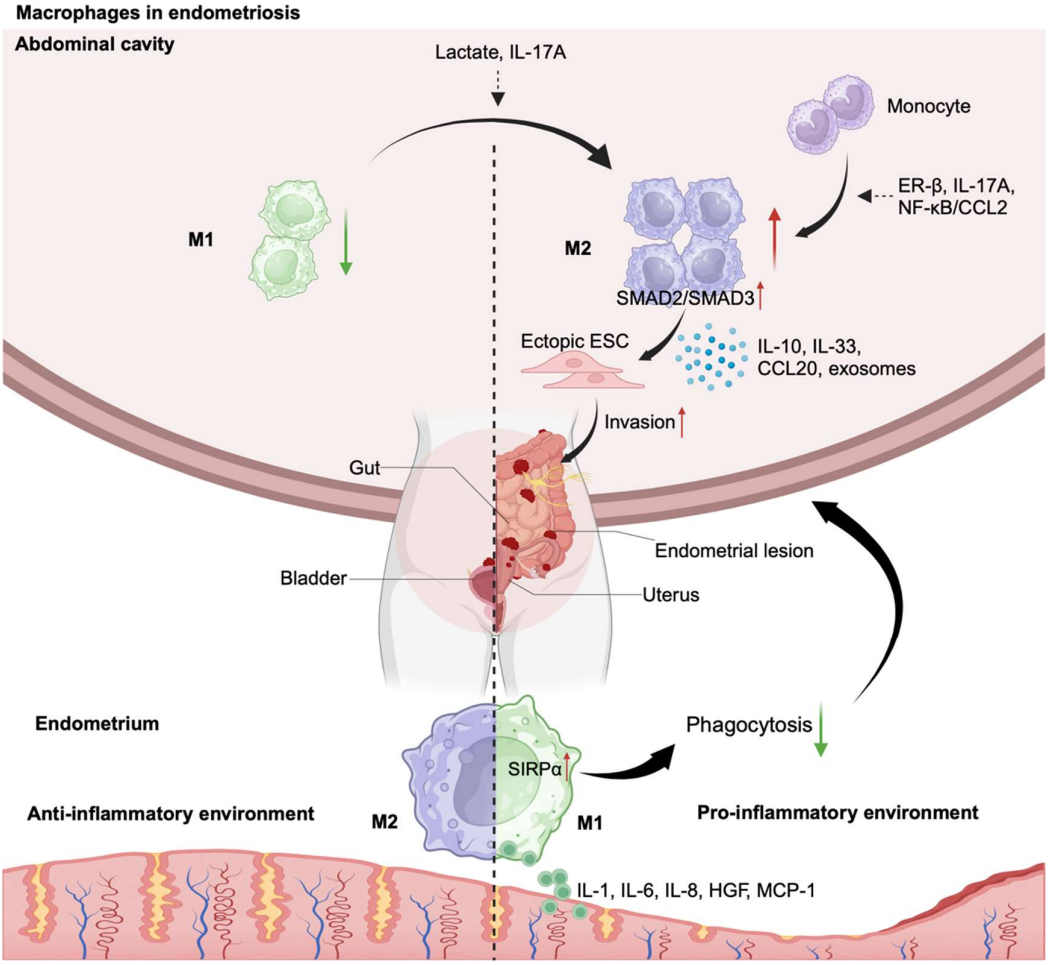

Figure 4 Macrophages in endometriosis.

In the abdominal cavity, lactate and IL-17A drive M1 macrophages to polarize to the M2 phenotype via the SMAD2/SMAD3 pathway. Activation of ERβ in ESCs leads to macrophage recruitment through the NF-κB/CCL2 pathway, which promotes macrophage differentiation into the M2 phenotype. M2 macrophages secrete cytokines, exosomes, and chemokines targeting ESCs, which promote ESC invasion. In the endometrium, M1 macrophages secrete a number of pro-inflammatory cytokines, including IL-1, IL-6, IL-8, HGF, and MCP-1. Additionally, they overexpress SIRPα, which contributes to the formation of a pro-inflammatory environment. IL-17A, interleukin-17A; SMAD2/SMAD3, SMAD family member 2/3; ERβ, estrogen receptor beta; NF-κB, nuclear factor-kappa B; CCL2, cysteine-cysteine motif chemokine ligand 2; IL-10, interleukin-10; IL-33, interleukin-33; CCL20, cysteine-cysteine motif chemokine ligand 20; SIRPα, signal regulatory protein α; IL-1, interleukin-1; IL-6, interleukin-6; IL-8, interleukin-8; HGF, hepatocyte growth

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factor; MCP-1, monocyte chemoattractant protein-1. (Created by BioRender).

3 Macrophages in EAOC

EOC typically spreads through the abdominal cavity, attaching to the abdominal wall and spreading easily within the cavity[61]. It is noteworthy that the abdominal cavity contains a significant number of macrophages. Macrophages play an essential role in the development of EMS into EAOC[62]. However, it is unclear how EOC evades macrophage-mediated anti-tumor immune surveillance.

3.1 The evolution of macrophages in EOC

Tumor macrophage's functional phenotype and distribution are regulated by the dynamic nature of the tumor microenvironment (TME), responding to tissue-specific and tumor-specific stimuli. During the early stages of carcinogenesis, macrophages exhibit a higher degree of similarity to pro-inflammatory M1-like phenotype when they infiltrate into the tumor from the periphery, and participate in tumor antigen capturing, phagocytosis, and production of inflammatory factors[63]. As the tumor grows, stimuli in the TME change, leading to alterations in the infiltration and polarization of macrophages. In ovarian cancer, the TME predominantly drives the polarization of macrophages to an M2-like phenotype, which were known as tumor associated macrophages (TAMs)[64]. A higher proportion of M2 macrophages and a lower proportion of M1 macrophages are linked to a poor prognosis in ovarian cancer patients[64]. In human EOC and ascites, TAMs constitute the most abundant infiltrating immune cell population[65]. TAMs exhibit limited tumoricidal activity and promote immunosuppression, tumor cell invasion and metastasis, and angiogenesis. TAMs of ovarian cancer can also directly mediate cell-cell interactions via immune checkpoint receptors and their ligands, leading to functional impairment of T cell responses. Furthermore, TAMs in ovarian cancer promote extracellular stromal remodeling, which contributes to the invasion of various tumor cells.

3.2 Function of TAMs in TME

TAMs promote the development of ovarian cancer through a variety of ways. Inhibitory monocytes and macrophages were recruited to the TME by producing cytokines, chemokines, growth factors, and other molecules, including IL-6, leukemia inhibitory factor, CCL2, colony stimulating factor 1 (CSF1), tumor necrosis factor (TNF), cysteine-cysteine motif chemokine ligand 2 (CCL22), C-X-C motif chemokine ligand 12 (CXCL12), VEGF, periostin (POSTN), and Semaphorin 4D[63]. To promote tumor growth and metastasis, macrophages are promoted to differentiate into M2 macrophages. Ovarian cancer cells secrete macrophage colony-stimulating factor (M-CSF) to polarize TAMs towards the M2 phenotype. In EOC, the AT-rich interaction domain 1A (ARID1A)^{644delG}/ E4F transcription factor 1 (E4F1) complex induces the expression of histone deacetylase 6 (Hdac6) through GATA3 activation. The overexpression of Hdac6 regulates the trafficking of IL-10 to the extracellular environment, promoting M2 polarization of macrophages[66]. TAMs promote tumor growth by releasing cytokines, enzymes, and chemokines that enhance the formation of ovarian cancer spheroids and the adhesion of cancer cells to metastatic sites, allowing them to evade immune cell attack. TAMs secrete CCL18 to induce epithelial mesenchymal transition (EMT) of ovarian cancer by upregulating the expression of Zinc finger E-box binding homeobox 1 (ZEB1) and other EMT-related transcription factors. ZEB1, in turn, promotes the transcription of M-CSF[67]. TAMs secrete epidermal growth factor (EGF) and upregulate αMβ2 integrin on TAMs and intercellular cell adhesion molecule-1 (ICAM-1) on ovarian tumor cells, promoting binding between tumor cells and TAMs. Additionally, TAM-secreted EGF activates EGFR on tumor cells, which upregulates VEGF/ vascular endothelial growth factor receptor (VEGFR) signaling in surrounding tumor cells, supporting tumor cell proliferation and metastasis[68]. M2 macrophages regulate vascular permeability through the very late antigen 4 (VLA4)/ vascular cell adhesion molecule 1 (VCAM1)

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pathway, which determines ovarian tumor ascites development. The study found that M2 macrophages reduce VCAM1 levels in endothelial cells and regulate VLA4 expression when in direct contact with them. However, overexpression of VLA4 or VCAM1 leads to increased vascular permeability[69]. Macrophages can promote POSTN expression in ovarian cancer cells through TGF-β. POSTN released by ovarian cancer cells can recruit macrophages. POSTN enhances integrin/ERK/NF-κB signaling through autocrine effects on cancer cells to produce macrophage-attracting and mobilizing cytokines, including macrophage inflammatory protein 1 beta (MIP-1β), MCP-1, tumor necrosis factor alpha (TNF-α), and regulated on activation normal T cell expressed and secreted (RANTES), leading to the polarization of Tohoku Hospital Pediatrics-1 (THP-1) to M2 macrophages in vitro. This process may potentially promote tumor growth[70, 71].

3.3 Important molecules shared by EMS and EAOC

C-X-C motif chemokine receptor 3 (CXCR3) is expressed in various immune cell subsets, stromal cells (including vascular endothelial cells), and some types of tumor cells[72]. CXCR3B is a variant of CXCR3, and C-X-C motif chemokine 4 (CXCL4) is its major functional ligand. CXCL4 directly interacts with VEGF and fibroblast growth factor-2 (FGF-2) to exert inhibitory effects on these angiogenic factors. CXCL4 and its receptor CXCR3B have been found to be down-regulated in clear-cell ovarian cancer. The evidence indicates that the CXCL4-CXCR3B axis may be disrupted in this type of cancer. In a separate study, CXCL4 and its variant CXCL4L1 were significantly down-regulated in EAOC compared to endometriosis[72]. CXCL4 was strongly expressed in $CD68^{+}$ -infiltrating macrophages of endometriosis. CXCL4 deficiency may be involved in the specific inflammatory microenvironment of ovarian cancer, which arises from endometriosis. However, the inhibition of CXCL4 in cancer lesions may be partially attributed to TAMs[72].

Heme oxygenase 1 (HO-1) is a type II detoxifying enzyme that catalyzes the rate-limiting step in heme degradation, producing carbon monoxide (CO), free iron, and biliverdin[73]. As a substrate for HO-1, heme has been shown to be a potent pro-oxidant. It has been demonstrated to accelerate inflammatory damage and promote cell death. However consequently, HO-1 is regarded as a pivotal negative mediator of inflammatory cell and tissue damage[74]. HO-1 has been demonstrated to confer an anti-inflammatory protective effect by modulating necroptosis and pyroptosis. On the contrary, in the context of ferroptosis, HO-1 may play a role in promoting cell death by enhancing iron release. Furthermore, HO-1 has been implicated in the co-regulation of autophagy[74-76]. HO-1 is mainly expressed in macrophages. Macrophage recruitment and interaction with endometriotic cells were thought to play a key role in the initiation and progression of endometriosis. Endometriotic cells secret TGF-β1 to promote HO-1 expression in macrophage, thus protecting endometriotic cells from oxidative damage and promoting cell survival[77]. Meanwhile, dysregulation of heme metabolism mediated by HO-1 may be a crucial factor in the initiation and growth of EAOC. Compared to endometriosis tissues, HO-1 is increasing expressed in ovarian clear cell carcinoma tissues. The expression of HO-1 in macrophage favors M2 differentiation, promotes fibrosis of mature endometriosis lesions, decreases heme levels in the tumor microenvironment, thereby supporting ovarian tumor growth[78]. In addition, HO-1 expression has been observed in tumor-infiltrating macrophages and has been associated with cancer progression, invasiveness, and aggressiveness[79]. Although HO-1 plays a promoting role in EMS and EAOC, in principle, the downregulation of HO-1 results in elevated intracellular reactive oxygen species (ROS) levels and DNA damage, which may contribute to tumorigenesis. In a subset of cancers, such as lung cancer, HO-1 expression in macrophages has been observed to be reduced[80]. Yamada et al observed a lower number of M2 macrophages expressing HO-1 in EAOC patients compared to those

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with ovarian endometriosis. The sustained decrease of HO-1 expressing M2 macrophages may have an important role in promoting the malignant transformation of endometriosis[81]. Consequently, the role of HO-1 in the pathogenesis of tumor progression remains a subject of debate.

CD47 is a glycoprotein expressed on nearly all cell types. This molecule is referred as the "do not eat me signal" as it functions by binding to SIRPα as a ligand, thereby inhibiting phagocytosis by phagocytes, particularly macrophages[82]. In the current clinical landscape, the exploration of strategies that target the SIRPα-CD47 innate immune checkpoint has emerged as a promising avenue for cancer treatment[83, 84]. According to a study, the high expression of CD47 in ovarian tissue is associated with the tumor's stage and grade[85]. The study found that CD47 was overexpressed in ovarian clear cell carcinoma cells, which led to a reduction in macrophage phagocytosis and promoted the growth and migration of human endometrioid (TOV-112D) and clear cell (TOV-21G) cancer cell lines by binding to SIRPα[85].

Oncogenic miR-1246 plays a significant role in chemotherapy resistance by inhibiting caveolin 1 (Cav1) in M2 macrophages. Additionally, exosomal miR-1246 promotes tumor progression in the tumor microenvironment through M2 oncogenic macrophages[86]. Research has shown that miR-21, an oncogene, partially regulates M2 macrophage polarization, promoting chemoresistance and inhibiting apoptosis in ovarian cancer cells[64]. Additionally, ovarian cancer patients with high expression of hypoxia-inducible factor 1a (HIF-1a) had higher $CD163^+$ cell infiltration and inter-tumor miR-223 levels. Exosomes-enriched miR-223 released from hypoxic macrophages promoted drug resistance of EOC cells both in vivo and in vitro through the PTEN- phosphatidylinositol 3-kinase (PI3K)/ protein kinase B (PKB/AKT) pathway[87].

Under certain conditions, EMS can transform into EAOC. In the occurrence and development of EMS and EOC, macrophages tend to polarize to M2 macrophages, weakening their phagocytic ability and promoting disease progression[81]. Therefore, macrophages may play a key role in the transformation of EMS into EAOC. Studying the functions of macrophages between EMS and EAOC can aid in comprehending the mechanism of malignant transformation of EMS and developing new immunotherapy methods.

4. Therapeutic strategy targeting macrophages in EMS and EAOC

In EMS lesions, endometriotic cells inhibit its apoptotic progression, while promoting invasion, adhesion and proliferation pathways. In the meantime, angiogenesis and neurogenesis also participate in pathogenesis of EMS[3]. Though a number of studies had unraveled the critical mechanisms of how macrophages involve in the sophisticated progression from occurrence of EMS lesions to malignancy transformation, whether targeting macrophages or macrophage-related markers could be applicable options under clinical conditions remains in doubt[88, 89]. However, for the multiple drawbacks of existing treatments of EMS, such as limited indications, adverse effects, unsatisfactory potencies and long-term medication, the exploration of advanced macrophage-related therapies is extremely demanding[3]. Here, we summarize the mainstream therapies of EMS and make a brief illustration of their indications and drawbacks. Then we focus on reported macrophage-related therapeutic strategies and propose its future perspective (Figure 5).

4.1 Existing therapies for EMS

The prevalently applied therapies of EMS include hormonal treatments, non-hormonal treatments and surgery. Hormonal treatments are the mainstay of EMS therapies. By perturbing hormonal fluctuations, hormonal treatments create amenorrhea and prevent inflammation from deterioration[90, 91]. Patients who were diagnosed with EMS and not desiring for pregnancy immediately would be

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prescribed hormonal treatments, which include combined contraceptives, progestogens, Gonadotropin releasing Hormone (GnRH) agonists, GnRH antagonists and aromatase inhibitors[3]. However, all these drugs have distinct side effects to some extents, like nausea for combined contraceptives, weight gain for progestogens as well as bone marrow density decline for GnRH agonists, GnRH antagonists and aromatase inhibitors. More importantly, hormonal treatments only relieve the pain rather than radically cure the EMS lesions, making it a long-term remedy[3, 90].

Non-hormonal treatments mainly consist of conventional analgesics, like non-steroidal anti-inflammatory drugs (NSAIDS), and neuro-modulatory drugs, like analgesic tricyclic antidepressant, selective serotonin uptake inhibitors and anticonvulsants[3].Clinicians only use these drugs to soothe short-term sense of pain. On the other hand, the efficacy of NSAIDS on pain caused by EMS lacks high-quality evidence[92]. Also, long-term administration of NSAIDS and neuro-modulatory drugs may lead to some severe adverse effects, like gastrointestinal bleeding and perforation for NSAIDS as well as central nervous system and circulatory system toxicity for neuro-modulatory drugs.

Surgery treatment is offered when patients cannot tolerate medication treatments or have a plan for pregnancy in the near future. For those suffering from ureteric or bowel obstruction due to EMS, surgery is an inevitable option[91]. However, patients who accepted surgery have high rates of symptom and lesion recurrence. This probably attributes to the continuous reflux of menstruation and sensitization of central nervous system. What's more, patients could also be afflicted with some surgery-related complications like postoperative infection, rectovaginal fistula, neurogenic bladder and so on[90].

4.2 Existing therapies for EAOC

According to several comprehensive reviews, the correlation between the morbidity of EMS and ovarian cancer has been well corroborated[1, 93]. The major subtypes of EAOC include clear-cell ovarian cancer and endometrioid ovarian cancer, which have relatively worse prognosis with chemoresistance[94]. The standard treatment of EAOC is debulking operation followed by adjuvant chemotherapy with paclitaxel and carboplatin or neoadjuvant chemotherapy with interval debulking surgery, resembling that of common EOC, and the efficacy is apparently unsatisfying, especially for patients with recurrence or advanced stage lesions[94, 95]. Efforts have been made in recent years to ameliorate therapies by application of hyperthermic intraperitoneal chemotherapy (HIPEC)[96], adjustment in the cycle and frequency of chemotherapies, combined use of antiangiogenic drugs like bevacizumab[97], poly-adenosine diphosphate ribose polymerase (PARP) inhibitors like Olaparib and rucaparib[98] and immunotherapies represented by checkpoint inhibiting[94]. However, a latest published clinical trial found that addition of HIPEC to cytoreductive surgery did not improve progression-free and overall survival in patients with advanced epithelial ovarian cancer[99]. Also, whether dose-dense or conventional three-weekly paclitaxel administration would be more beneficial to patients still entails further verification[100]. As two of the most promising drugs, bevacizumab and PARP inhibitors display several drug-related toxic effects on the hematological system, gastrointestinal system and urinary system[101]. With limited potency in $BRCA^{wt}$ and homologous recombination proficient ovarian cancer, PARP inhibitors are restricted in use to some extents[95]. Furthermore, two individual clinical studies on avelumab, a PD-L1 monoclonal antibody, do not support its use in the frontline treatment setting[102, 103]. To conclude, though improvements have been witnessed in clinical trials regarding these emerging solutions, more advanced and convincing approaches are still demanding.

4.3 Macrophage-related therapeutic strategies in EMS

There is an altered immune and inflammatory milieu in EMS lesions, where macrophages, the critical modulator in EMS pathology, are recruited and produce an array of adhesion molecules

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(fibronectin, intercellular adhesion molecule1), growth factors (insulin-like growth factor I, platelet-derived growth factor, vascular endothelial growth factor) and pro-inflammatory cytokines (IL-1, IL-6, IL-8, IL-2, tumor necrosis factor)[1]. Ultimately, the aberrant inflammatory environment causes the incomplete apoptosis and phagocytosis of shed endometrial cells during menses as well as implantation, survival and proliferation of ectopic endometrium, resulting in chronic pain and infertility[26]. Throughout the years, many therapeutic strategies targeting specific molecules or functions of macrophages have been reported.

4.3.1 Strengthening phagocytosis of macrophages

The inefficient phagocytosis of macrophages in ectopic EMS lesions and peritoneal fluid is necessary for the survival, adhesion and invasion of endometriotic cells in retrograde menstruation[26, 104]. Thus, the recovery of phagocytosis by macrophages may be a promising therapeutic strategy for EMS patients.

CD47 blockade

SIRPα is an inhibitory receptor commonly expressed by macrophages and upregulated by ectopic endometrial stromal cells during EMS[105]. By conjugating with CD47, which is also enriched in EMS milieu, the immunoreceptor tyrosine-based inhibition motifs (ITIM) of SIRPα transduce inhibitory signals, curb macrophage activation and suppress phagocytosis[106]. Li et al. found that CD47 blockade not only significantly strengthened phagocytosis of macrophages towards human ESCs both in vitro and in vivo, but abrogated the apoptosis tolerance of Human endometrial stromal cells (HESCs)[105, 107]. Furthermore, CD47 blockade could alter the macrophage polarization by reducing M2 phenotype[105]. Therefore, immunotherapy based on the CD47-SIRPα signaling pathway holds potential for treating endometriosis.

Elevating cytosolic Ca^{2+}concentration

Elevation of Ca^{2+}concentration in cytoplasm is indispensable for macrophage efferocytosis, which is critical for instant clearance of apoptosis cells[108, 109]. Another study designed a multifunctional nanoparticle concomitantly loaded with calcium carbonate ($CaCO_3$) and a specialized anti-inflammatory mediator[110]. With concrete enhancement of efferocytosis by increasing the expression of T cell immunoglobulin and mucin domain containing 4 (TIM4) and CD36, this nanoparticle also significantly inhibited the formation of ectopic lesions in vivo[110]. Another study incubated mesoporous silica nanoparticle immobilized with glucosaminyl muramyl dipeptide (GMDP) on the surface with macrophage and observed significant higher expression of membrane scavenger proteins including CD36 and CD204[111]. Nevertheless, whether these treatments could be qualified therapeutic strategies for EMS patients requires further investigation in clinical conditions.

Herbal extracts

Ferulic acid, ligustrazine and tetrahydropalmatine are separately isolated from components of a traditional Chinese formula, which is commonly used for irregular menses. The combination use of ferulic acid, ligustrazine and tetrahydropalmatine evidently enhance the phagocytosis ability of macrophages in vitro and repress the growth of ectopic endometrial lesions[112].

4.3.2 Reducing infiltration of macrophages

Several studies have corroborated the correlation between the increase of macrophage infiltration and severity of EMS[47, 113, 114]. These excessively recruited macrophages induce angiogenesis, fibrosis and neuroinflammation rather than phagocyting ectopic endometriotic debris. Over the years, researchers have been seeking for adequate methods to reduce infiltrated macrophages in EMS lesion.

S1PR1 inhibitors

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Sphingosine 1-phosphate receptor 1 (S1PR1), a kind of G protein-coupled receptor involved in numerous immune-related diseases including multiple sclerosis, autoimmune encephalitis and diffuse large B-cell lymphoma, was found significantly upregulated in endometriotic lesions[115, 116]. To explore if S1PR1 blocking could inhibit EMS, Zhang et al. administered a broad-spectrum S1P modulator and the specific S1PR1 modulator in EMS mice and observed strongly suppressed infiltration of macrophages as well as attenuated local inflammation in lesions[117].

Vitamin D receptor agonists

Vitamin D receptor (VDR) is a nuclear ligand-inducible transcription factor that interacts with the active version of vitamin D, $1,25(OH)_2D$ and regulates a vast number of physiological processes[118]. With the prevalent expression on endometrial cells and all arrays of immune cells, VDR mediates distinct anti-inflammatory effects by inhibiting NF-κB pathway. Endometrial cells with deficient NF-κB signaling downregulate IL-8 production and reduce recruitment of macrophages, while peritoneal macrophages with repressed NF-κB signaling decrease excretion of inflammatory cytokines[118, 119].

Mariani et al. created an EMS model based on Balb/c mice and administered a VDR agonist called elocalcitol, which possesses low calcemic liability and anti-inflammatory properties well defined in benign prostatic hyperplasia[120]. Consequently, mice treated with elocalcitol have lower lesion weight, lower macrophage recruitment in abdominal cavity, lower levels of IL-1α and IL-1β in collected peritoneal fluid as well as lower inflammatory cytokines production by peritoneal macrophages[119]. However, no relevant clinical trials on EMS are currently available.

4.3.3 Suppressing inflammatory capacities of macrophages

Estrogen receptor-inflammatory axis modulation

Estrogen receptor (ER) is expressed both by endometrial cells and mature macrophages in endometrium as well as peritoneal fluid. In EMS patients, ER expression in endometriotic lesions is significantly higher[121-123]. Endometrial tissue with enhanced ER activity recruit $CD163^+$ monocyte/macrophage cells to promote ectopic lesion growth[121]. Endometrial stromal cells with elevated ER signaling upregulate CD200 production in ectopic endometrial tissue and curb macrophage phagocytosis[122]. On the other hand, the expression of ER on macrophages in peritoneal fluid has a positive correlation with production of inflammatory cytokines both in healthy individuals and EMS patients[124]. The activation of ER signaling of macrophages also stimulates the production of brain-derived neurotrophic factor and neurotrophin 3, increasing the infiltration of nerve fibers in ectopic lesions[125]. Protopanaxadiol, an aglycone of ginsenosides, was reported to induce downregulation of ER in eutopic endometrium of EMS patients and alleviates interferon-gamma (IFN-γ) and IL-12 production from macrophage in abdominal cavity at the same time, which collaboratively restricted the growth of ectopic lesions[126]. In 2015, two ER ligands called chloroindazole (CLI) and oxabicycloheptene sulfonate (OBHS) that displayed potent antiestrogenic and anti-inflammatory capacities were introduced. By conjugating with ER on endometrial cells and macrophages, CLI and OBHS restrained the endometrial production of cytokines including IL-6, CCL2, CCL5 and TNF-α, reduced the recruitment of macrophages in endometriotic lesions and finally suppressed the endometriotic-like lesion formation in C57BL/6 mouse concurrently treated with uterine fragments and estradiol[127]. However, no relevant clinical trials have been reported.

Neuron-inflammatory axis modulation

In the early 2000's, people found that stimulation of the vagus nerve could suppress inflammatory response in Lewis rats[128]. Following studies on neuroimmune nexus established an intrinsic model of cholinergic anti-inflammatory pathway that precisely modulates systemic inflammatory state[129].

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In brief, vagus nerve controls a subpopulation of sympathetic neurons in the celiac and superior mesenteric ganglia, which in turn sends messages to splenic nerve. The noradrenergic nerves in spleen stimulate $CD4^+$T cells by excreting norepinephrine (NE) and promote the production of acetylcholine (Ach) by $CD4^+$T cells. Finally, Ach diffuses towards macrophages, attaches to α7 -nicotinic acetylcholine receptor (α7nAChR) on the surface of macrophages and inhibits the synthesis and release of pro-inflammatory cytokines.

The strategies modulating catabolite gene activator protein (CAP) activation mainly include vagus nerve stimulation (VNS) and pharmacological stimulation of α7nAChR. Interestingly, women with EMS tend to have lower vagal activity as compared with controls[130]. In an EMS Balb/c mouse model, VNS reduces total EMS lesion weight while vagotomy ablates this beneficial effect[130]. A study examining IL-1β expression in human peritoneal mononuclear cells (PFMC) stimulated by lipopolysaccharide (LPS) with or without α7nAChR agonists found a significant decrease of IL-1β expression in α7nAChR agonist group[131]. The following study on EMS Balb/c mice model also observed a significant shrinkage of EMS lesions when α7nAChR agonist was administered[131]. Though there is currently no reported clinical trial concerning VNS and α7nAChR agonist, it could possibly be one of the promising therapeutic strategies for patients with EMS in the future.

PGE2 receptor inhibition

The concentration of PGE2 is largely increased in peritoneal fluid of EMS patients, leading to the growth and survival of ectopic endometriotic cells[132, 133]. NSAIDS was designed to inhibit the activity of cyclooxygenase 2 (COX2), the rate limiting enzyme of PGE2 production. However, its concurrent inhibition of COX1 may cause some adverse effects, restricting the dosage of NSAIDS. Thus, researchers have resorted to PGE2 receptor antagonization in recent years and revealed myriad benefits of concurrent inhibition of PGE2 receptor 2 (EP2) and PGE2 receptor 4 (EP4) including decreasing angiogenesis and innervation of EMS lesions, alleviating pelvic pain by suppressing inflammation state of dorsal root ganglia as well as restoring endometrial functional receptivity[134]. In the latest study, Tomako et al. demonstrated that the combination of EP2 and EP4 antagonist also significantly decreased PGE2-induced IL-6, VEGF, CXCL2 and CXCL3 production in peritoneal macrophages in vitro, making it a potential therapeutic scheme[135].

Herbal extracts

Throughout the years, people from different regions tried using processed extract from special local plants to cure EMS and found these methods efficacious. For example, *Euterpe oleracea* is a common plant found in the Amazon region of Brazil known for its high level of myriad beneficial phytochemicals, which endow *Euterpe oleracea* with antioxidant, antinociceptive, anti-inflammatory and anti-cancer activities[136]. In an EMS Sprague-Dawley rat model, *Euterpe oleracea* extract was administered through gastric tube daily for 30 consecutive days and a significant decline of macrophage infiltration in endometriotic lesion was observed. Simultaneously, the inflammatory profile measured by COX2, PGE2 and NO as well as angiogenesis process gauged by matrix metalloproteinase-9 (MMP-9), VEGF and VEGFR-2 were ablated, leading to regression of lesion sites[136]. Another study on a Japan-derived herbal implied that its ingredients, represented by ferulic acid, could suppress IL-8 production of patient-derived peritoneal macrophages[137]. Moreover, dehydrocostus lactone, an active compound extracted from the roots of *Aucklandia lappa*, was found to promote lesion regression by inhibiting the expression of M2 markers and NF-κB pathway activity in EMS-associated macrophages (EAMs)[138]. However, a further exploration of latent mechanisms and verification of its effectiveness and viability in clinical condition is lacking.

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4.3.4 Adjusting phenotypes of macrophages

LPS^{low}-trained macrophages

The paradigm of modifying innate immune cells' capacity to respond to a second stimulation based on metabolic changes and epigenetic reprogramming is called "innate immune memory" or "trained immunity"[139-141]. Mohamed et al. treated peritoneal macrophages with low doses of lipopolysaccharide (LPS^{low}) for *in vivo* immune training. In a mice model of endometriosis, intraperitoneal injection of LPS^{low}-trained macrophages leads to a decrease in the expression of CXCR4, CCR2, and CD206, an increase in IL-10 expression, and the inhibition of endometriotic lesion growth[142]. This study presents a novel therapeutic approach for endometriosis, involving the manipulation of innate immune memory to target immune dysfunction.

Niclosamide

Niclosamide is originally an effective anthelmintic drug against human tapeworm (cestode) infection. However, the following studies unraveled its efficacy in multiple diseases including metabolic syndrome, bacterial infection and cancer[143]. In the past decade, the outstanding efficacy of Niclosamide in EMS treatment without disrupting reproductive abilities promoted further exploration on its mechanisms, in which the correlation between Niclosamide and macrophages was discovered[144-146]. Niclosamide alleviates macrophage-induced cell viability and inflammatory factors secretion in human ESCs and endometriotic epithelial cells[145, 146]. During the progression of EMS, EMS-like lesions could ablate embryo-derived Tim4^{+} macrophages in peritoneal fluid and were replenished by CCR2^{+} monocyte-derived macrophages, which show strikingly different functions and disrupt the homeostasis of local immune environment[147]. Zhao et al. further revealed that Niclosamide could stabilize the composition of peritoneal macrophages from different origins, partly reverse the gene expression of peritoneal macrophages dysregulated by EMS-like lesions and reconstitute the interaction between macrophages and B cells[147]. Another study found that Niclosamide was capable of reducing pro-inflammatory GATA6^{+} LpMs, thereby suppressed aberrant inflammation in the peritoneal fluid, EMS-like lesions, pelvic organs and dorsal root ganglion[148]. Nevertheless, current research has only been conducted in rodents.

4.3.5 Other

Extracellular ATP

The presence of elevated levels of extracellular adenosine triphosphate (eATP) has been shown to induce the processes of apoptosis and pyroptosis in endometriotic epithelial cells, with this effect being mediated through the MAPK/JNK/Akt pathway, thereby impeding their progression. The administration of eATP has been observed to result in a substantial reduction in the size of endometriosis lesions, accompanied by a notable increase in macrophage infiltration and the resolution of functional defects in macrophages. Furthermore, the presence of eATP in conjunction with macrophages fosters an inflammatory milieu, thereby inciting pyrodeath and the subsequent apoptosis of EM epithelial cells. Consequently, eATP treatment emerges as a promising candidate for non-hormonal treatment of endometriosis[149].

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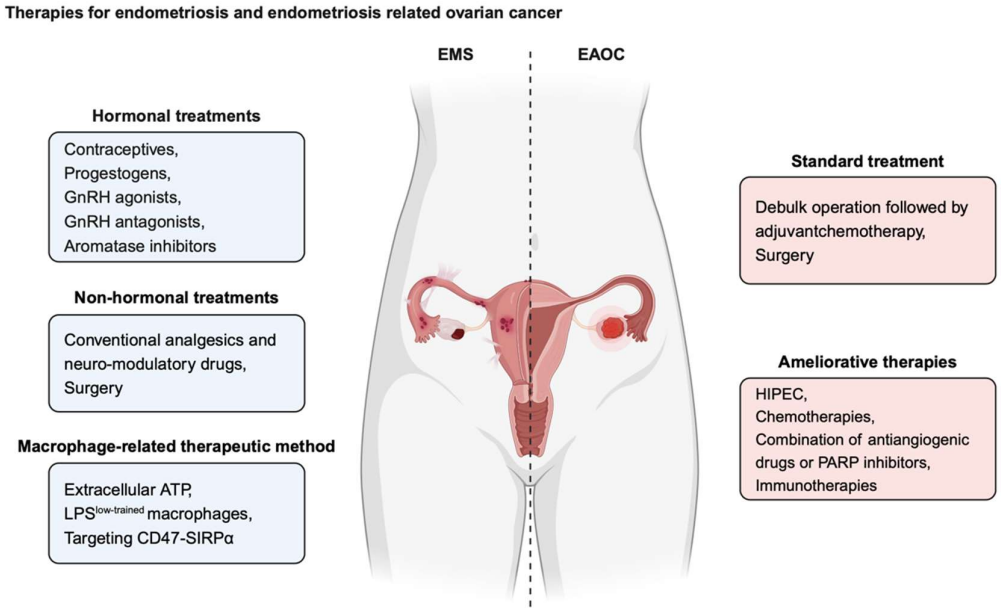

Figure 5 Therapies for endometriosis and endometriosis related ovarian cancer.
Prevalently applied therapies of EMS include hormonal treatments, non-hormonal treatments and surgery. Hormonal treatment is a long-term remedy using contraceptives, progestogens, GnRH agonists, GnRH antagonists and aromatase inhibitors. Non-hormonal treatments consist of conventional analgesics, neuro-modulatory drugs and surgery. Macrophage-related therapeutic strategies in EMS includes extracellular ATP, $LPS^{low\text{-}trained}$ macrophages and targeting CD47-SIRPα. Standard treatment of EAOC is debulk operation followed by adjuvant chemotherapy with paclitaxel and carboplatin or neoadjuvant chemotherapy with interval debulking, and surgery. The ameliorative therapies of EAOC includes HIPEC, adjustment in the cycle and frequency of chemotherapies, combine use of antiangiogenic drugs or PARP inhibitors and immunotherapies represented by checkpoint inhibiting. EMS, endometriosis; EAOC, endometriosis-associated ovarian cancer; GnRH, Gonadotropin releasing Hormone; ATP, adenosine triphosphate; LPS, lipopolysaccharide; SIRPα, signal regulatory protein α; HIPEC, hyperthermic intraperitoneal chemotherapy; PARP, poly-adenosine diphosphate ribose polymerase. (Created by BioRender).

5. Concluding remarks

Since menarche, women experience monthly menstruation until menopause. During this time, incomplete removal of menstrual reflux and endometrial debris may lead to the formation of EMS. Dysfunctions in phagocytosis by peritoneal macrophages and phenotypic switching to M2 macrophages promote the development of endometriotic lesions, angiogenesis, and neurogenesis. Additionally, M2 macrophages contribute to the malignant transformation of EMS. Analyzing the role of macrophages in EMS is crucial for understanding the pathogenesis of EMS, developing targeted treatments involving macrophages, and reducing the incidence of EMS-associated tumors.

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Author's Contributions

MQL, CRS, LHK and LYS drafted the manuscript. MQL and CRS drew the figures. HZZ, HXR and ZQB revised draft. HW, ZLY and YZY made forms. MQL, LHK, LYS, CRS, HW, ZLY, YZY, LYR and DZH searched the literature. WS AND ZW discussed the concepts of the manuscript and approved the version to be submitted.

Conflict of Interest

The authors declare no competing interests.

Ethical Standards

The authors assert that this work does not involve human or animal experimental procedures.

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