Review Article

Autophagy in endometriosis

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Abstract: Endometriosis (EMS) is a common gynecologic disease that causes chronic pelvic pain, dysmenorrhea, and infertility in women. The doctrine of menstruation back flow planting and defects in the immune system are well known and widely accepted. In recent years, increasing studies have been focused on the role of autophagy in EMS, and have shown that autophagy plays a vital role in EMS. Autophagy, which is known as the non-apoptotic form of programmed cell death induced by a large number of intracellular/extracellular stimuli, is the major cellular pathway for the degradation of long-lived proteins and cytoplasmic organelles in eukaryotic cells. Autophagy commonly refers to macroautophagy, which is characterized by autophagosomes (double-membrane vesicles). In normal endometrial tissues, autophagy is induced in glandular epithelial and stromal cells throughout the menstrual cycle. However, aberrant autophagy occurs in the eutopic endometrium and ectopic endometriotic foci, which contributes to the pathogenesis of EMS by promoting the hyperplasia of endometriotic tissues and stromal cells, restricting apoptosis, and inducing abnormal immune responses. Consistent with changes in autophagy levels between normal endometria, eutopic and ectopic endometria from patients with EMS, the altered expression of autophagy-related genes (ATGs) is also observed. Currently, many factors are involved in the aberrant autophagy of endometriotic tissues, including female hormones, certain drugs, hypoxia, and oxidative stress. Therefore, studies focusing on autophagy may uncover a new potential treatment for EMS. The aim of this review is to discuss the role of aberrant autophagy in EMS and to explore the potential value of autophagy as a target for EMS therapy.

Keywords: Autophagy, endometriosis, autophagy-related genes, endometrial stromal cells

Introduction

Endometriosis (EMS) is a common gynecologic disease affecting approximately 5-15% of all women of reproductive age and 20-50% of all infertile women [1, 2], and it is one of the most common causes of chronic pelvic pain, dysmenorrhea and infertility [3, 4]. EMS is characterized by the presence, transfer, invasion, and cultivation of growing endometrial tissue outside of the uterine cavity [5]. Some hypotheses have been proposed to explain the migration, implantation and survival of the ectopic endometrial tissue and stroma, such as retrograde menstrual reflux [6], ectopic presence of endometrial stem cells [7] and defects in the immune system [8].

As shown in the recent study by Choi et al. [9], the induction of autophagy exerts a pro-apoptotic effect on normal human endometrial cells. EMS-derived endometrial tissues are characterized by reduced autophagy compared with the normal endometrium [10]. Autophagy is dysregulated in the uterine horns and eutopic endometria of mice with induced EMS and autophagic markers are differentially expressed compared with control mice [11]. Based on accumulating evidences, the level of autophagy is most likely associated with the pathogenesis of EMS.

Therefore, this paper is the first to systematically review the accumulating evidence and mechanisms reported in human and experi-
mental animal studies supporting the hypotheses regarding the origin and roles of aberrant autophagy in EMS.

**Autophagy**

The word “autophagy” is derived from the Greek and means to eat (“phagy”) oneself (“auto”). As a non-apoptotic form of programmed cell death, autophagy is the major cellular pathway for the degradation of long-lived proteins and cytoplasmic organelles in eukaryotic cells [12, 13]. It is a constitutive catabolic pathway that mediates both nonspecific and targeted sequestration of cellular organelles and other macromolecules, permits the degradation of cellular components in lysosomes, and promotes the recycling of bioenergetic metabolites [14]. Extensive activation of autophagy is detrimental to the cell and results in autophagic cell death; conversely, a moderate autophagic response acts as a housekeeping and survival mechanism that contributes to maintaining cellular homeostasis under normal conditions or to overcoming stress-induced conditions caused by a large number of intracellular/extracellular stimuli, including hypoxia, a limited nutrient supply (e.g., amino acid starvation), oxidative stress, the invasion of microorganisms [15, 16], and certain forms of therapeutic stress (e.g., cytotoxic chemotherapy) [17]. For instance, autophagy has been shown to play an important role in promoting cell death by inducing caspases-dependent apoptosis in some normal cells and cancer cells [18-22]. Consequently, autophagy plays important roles in the process of cell growth, differentiation, tissue remodeling, cell immunity, environmental adaptation, and death [23, 24].

Autophagy is a ubiquitous physiological process that occurs in all eukaryotic cells [16]. Three primary types of autophagy have been reported: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) [25]. Autophagy commonly refers to macroau-
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tophagy, because it is the most prevalent form of autophagy. Macroautophagy is a physiologically controlled, catabolic process by which cytoplasmic organelles and macromolecules are sequestered in autophagosomes (double-membrane vesicles that are derived from autophagosome precursors) and subsequently degraded after lysosomal fusion (autophagolysosomes that are derived from autophagosomes). The basic components resulting from lysosomal digestion are then reutilized for anabolic processes [13]. Four major stages of autophagosome formation have been characterized in both yeast and mammalian cells (Figure 1): initiation, expansion, closure, and fusion with the endolysosomal system [26]. The formation of a mature autophagosome plays a decisive role in the process of autophagy, which is regulated by a system of autophagy-related gene (ATG) products. The ATG proteins, which form six major groups, are recruited in a hierarchical manner to the pre-autophagosomal structure (PAS) in yeast or the omegasome in mammals. A double-track membrane, called the phagophore or isolation membrane, extends from the PAS to engulf cytoplasmic materials and organelles. The isolation membrane expands and then seals to form an autophagosome before it fuses with the vacuole in yeast or lysosome in mammals to release its contents for degradation [27].

The autophagy process is associated with numerous upstream signaling pathways and six major groups of ATG proteins. The most important signaling pathway is the class I Phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway [28]. The most upstream complex is the ATG1/ULK1 initiating complex, which contains the serine/threonine kinase ATG1/ULK1. In yeast, ATG1 forms a complex with ATG13, ATG17, ATG29, and ATG31 [29], whereas mammalian ULK1 complexes with ATG13, FIP200 (mammalian ATG17), and ATG101 [30]. Different stimuli activate AMP-dependent protein kinase (AMPK) and prevent PI3K from phosphorylating its downstream target Akt, thus inhibiting mTOR and subsequently reducing the phosphorylation of ATG13. ATG13 interacts with unc-51-like kinase 1 (ULK1), FIP 200 and ATG101 to form a ULK1 complex, which facilitates the induction of autophagy. When autophagy is induced, the initiating complex activates ATG1/ULK1 kinase activity and recruits downstream ATG complexes, including the multispansing transmembrane protein ATG9, followed by the autophagy-specific class III phosphatidylinositol 3-kinase (PI3K) complex [31]. Activation of the ULK1 complex phosphorylates Beclin1-regulated autophagy 1 (Ambr1a), thereby enhancing the activity of Beclin1-ATG14-VPS34-VPS15 class III PI3K core complexes to promote autophagosome nucleation. The PI3K complex phosphorylates phosphatidylinositol (PI) at the hydroxyl group in the 3-position to generate phosphatidylinositol 3-phosphate (PI3P) at the PAS. PI3P is required for the recruitment of the PI3P-binding protein ATG18 (WIPI2 in mammals) and its partner ATG2, which are involved in ATG9 recycling [32]. Then, the subsequent expansion of the phagophore and the formation of the autophagosome require two complexes: the ATG16L1 complex (ATG12-ATG5-ATG16L1) and LC3-phosphatidyl ethanolamine (PE). The ATG12 system results in the formation of the ATG16/ATG12/ATG5 complex, which acts as an E3 ligase for the conjugation of ATG8 (LC3 in mammals) to phosphatidylethanolamine (PE) [33]. Although one ATG8 family member has been identified in yeast, mammals contain several homologues that form three subfamilies, including LC3, GABARAP, and GATE-16 [34]. Lipidation of LC3 is an important ubiquitin-like conjugation pathway. LC3 also recruits adaptor proteins such as p62 to autophagosomes, mediating the selective autophagy of cellular structures, protein aggregates and microorganisms. ATG4B cleaves the C-terminal 22 residues of the LC3 precursor (proLC3) to produce LC3-I. Following the interaction of ATG3 (E2-like enzyme) with the ATG16L1 complex (E3-ligase) and ATG7, LC3 is then conjugated to PE to produce LC3-PE (also called LC3-II). A mature autophagosome directly fuses to a lysosome or first fuses with an endosome before trafficking to the lysosome, forming an autolysosome. LC3-II specifically localizes to both the inner and outer autophagosomal membranes and remains on mature autophagosomes until they fuse with lysosomes to generate autolysosomes, after which the contents are then degraded by proteases, lipases, nucleases and glycosidases [35, 36].

Aberrant autophagy in EMS

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Table 1. Autophagy levels in EECs and ESCs of normal endometrial tissues, eutopic endometrial tissues in patients with EMS and ectopic endometriotic tissues during the menstrual cycle

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+++++: The autophagy level in normal endometrial EECs in the late secretory phase.

and up-regulated during autophagy induction [37, 38], is expressed in endometrial glandular epithelial cells (EECs) and endometrial stromal cells (ESCs) throughout the menstrual cycle and is localized within the cytoplasm. In the early and late proliferative phases, MAP1LC3A staining in EECs and ESCs is negative or very weakly positive [9]. LC3-II expression increases during the late proliferative phase compared with the early proliferative phase, although the difference is not significant [39]. The ability of cells to undergo autophagy is reduced in the ectopic and eutopic endometrium of patients with EMS, and autophagy has been shown to be related to the pathogenesis and progression of EMS [40]. In eutopic EMS foci, a slightly decreased level of autophagy is identified in both proliferative EECs and ESCs compared with the endometrium from controls [10, 11]. In eutopic EMS tissues obtained from the secretory phase, autophagy in ESCs is still down-regulated compared to ESCs in the normal endometrium during the same phase. Regarding ectopic endometriotic tissues, Ruiz et al. [11] have identified a decrease in autophagy levels in EECs and ESCs; however, differences were observed among distinct endometriotic lesions (from ovaries, fallopian tubes, peritoneal, gastrointestinal, and skin). Ectopic EECs and ESCs in either proliferative or secretory phase are characterized by reduced autophagy compared with the normal endometrium; autophagy is very slightly reduced in the former (proliferative phase) and more significantly reduced in the latter (secretory phase). Moreover, the autophagy level in the secretory phase is further reduced in ectopic ESCs compared with eutopic ESCs [10].

As shown in Table 1, autophagy is primarily induced in human EECs during the secretory phase of the menstrual cycle [9, 11]. MAP1LC3A expression peaks in EECs during the late secretory phase [9]. Both the autophagosome number and LC3B expression are increased in secretory ESCs compared with proliferative ESCs, suggesting that the autophagy level is higher in secretory ESCs than in proliferative ESCs [10]. Accordingly, the induction of autophagy in endometrial cells treated with estrogen alone (as in the proliferative phase) increase with the addition of progesterone (as in the secretory phase) and simultaneously diversification is observed with the removal of estrogen and progesterone (as in the menstrual phase) [39]. In contrast, another study has recently found that the autophagy level (detecting LC3B) is reduced in the secretory phase compared with the proliferative phase in both human EECs and ESCs of controls, possibly because of the small sample size or the limitations of the immunohistochemical staining technique, which require further study [11]. Similar to normal ESCs, the autophagy level is higher in eutopic ESCs in the secretory phase than in eutopic ESCs in the proliferative phase of the menstrual cycle from patients with EMS [10]. However, a similar change in the endometriotic tissue-derived ESCs is not observed during the menstrual cycle, which maintains a nearly constant autophagy level throughout the menstrual cycle [10]. Similarly, cycle-dependent
induction of endometrial cell autophagy in the ectopic endometrium of patients with EMS is described by Choi et al. [39]. A constant level of autophagy induction is detected throughout the menstrual cycle in ovarian endometriotic cysts, which is mediated by the disinhibition of mTOR activity and is related to decreased apoptosis. Based on these findings, the low level of autophagy observed in secretory phase ESCs is involved in the pathogenesis of EMS. Accordingly, ESCs are primarily involved in the interaction between the endometrial tissue and the mesothelial cell lining of the peritoneum and play a fundamental role in the pathogenesis of EMS [41].

Generally, the level of autophagy in ectopic foci (actually in both the ectopic and eutopic endometrium of patients with EMS) is decreased (Table 1). Nevertheless, Allavena et al. have reported a significant up-regulation of autophagy in ovarian endometriomas compared with the eutopic endometrium of patients with EMS or healthy women [42]. Moreover, a significant increase in LC3B expression in the epithelium of fallopian tube and ovarian endometriotic lesions has recently been observed compared with the epithelium from the secretory endometrium of controls [11]. On one hand, endometriotic cells inside the ovarian endometriotic cysts experience a persistent condition of oxidative stress with high levels of free redox-active iron that retrospectively act as an autophagic stimulus, which has been confirmed by the notably up-regulated expression level of H0-1 [42], the rate-limiting enzyme in heme degradation that is induced by high levels of oxidative stress and inflammation [43]. On the other hand, a dramatic loss of the master inducer of apoptosis and negative regulator of cell proliferation, p53 [44], has been observed in ovarian endometriotic cyst tissues, which in addition to suggesting that apoptosis is impaired, may also be responsible for stimulating autophagy [42]. Low cytosolic levels of p53 obtained through pharmacologic inhibition or genetic depletion/deletion trigger autophagy [45], likely by inducing the derepression of the autophagy-initiating ULK1 complex [46]. According to a more recent study, the expression of HIF-1α (hypoxia-inducible factor-1α), a heterodimeric transcriptional factor mediating the cellular response to hypoxia, is increased in ovarian endometriotic lesions and enhances the migration and invasion of HESCs by upregulating autophagy in a hypoxic environment. Thus, autophagy is also induced through HIF-dependent pathways, which are involved in the high autophagy level detected in ovarian endometriotic lesions [47].

**Autophagy-related genes (ATGs) in EMS**

Autophagy is a catabolic process with complex regulatory mechanisms that has been highly conserved throughout biological evolution. Currently, more than 30 species of autophagy-related genes (ATGs) and multiple cellular pathways have been shown to be involved in autophagy [48]. The formation of autophagosomes requires a number of components called autophagy-related proteins, which are regulated through the autophagy pathway [49]. Gene expression is altered in eutopic endometria from patients with EMS compared with controls [50]. Retrospectively, autophagy is down-regulated in both the ectopic (lower) and eutopic endometrium of patients with EMS, with the exception of the ovarian endometriotic tissues. Accordingly, differences in ATG expression have been detected between eutopic and ectopic ESCs as well as between these cells and control ESCs [10]. Furthermore, the mRNA and protein levels of markers of autophagy are further down-regulated in the ectopic murine endometrium compared with the eutopic murine endometrium [11].

As shown in our previous work, the genes involved in autophagic vacuole formation or regulators of autophagy and apoptosis, such as SNCA, RGS19, IGF1, ATG9B, ATG12, ATG10, IFNG, PIK3CG, and DAPK1, are also decreased in eutopic secretory human ESCs compared with normal ESCs [10]. The expression of genes associated with autophagy initiation and regulation, such as p62, CXCR4, ESR1, and mTOR, is up-regulated and LC3-II and BECN1 expression are down-regulated in eutopic ESCs compared with eutopic ESCs [10]. More recently, Ruiz et al. have established a mouse model of EMS and obtained the similar results. Upon the induction of EMS in the murine eutopic endometrium, the RNA levels of 13 markers of autophagy in uterine horns (the phase in the menstrual cycle was not specified) are dysregulated, including 12 markers (ATG4C, ATG9B, BNIP3, IRGM1, EIF2AK3, FAS, LC3A, LC3B, GABARAPL1, PTEN, SQSTM1, and PRKAA1) with significantly decreased expression and a remarkable increase in IGF1 expression, com-
pared with controls (non-induced) [11]. RNA levels of markers of autophagy, including ATG5, ATG4B, ATG2B, ATG7, BECN1, p62, GABARAPL1-I, LC3B-I, LC3B-II, LC3A-I, and LC3A-II, are significantly decreased in endometriotic lesions compared with the uterine horns of PBS-treated mice. In addition, the authors have noted a significant increase in the expression of the p21 protein, a cyclin-dependent kinase inhibitor involved in cell cycle arrest that attenuates the viability of cells transfected with the ATG7 siRNA. Thus, the reduction in cell viability is proposed to have occurred through a similar noncanonical autophagy mechanism or independently of autophagy; however, further studies are required to elucidate the mechanism [11]. Specifically, the differences in the examined species, specimens and phases of menstrual cycle in these two studies may have contributed to the distinct changes in IRGM1 expression between eutopic EMS tissues and the normal endometrium. Therefore, further study is warranted.

In particular, an increasing number of studies have recently focused on several representative ATGs (Table 2). BECN1, the product of autophagy promoting gene (ATG6), is required for vesicle nucleation during autophagosome formation, forms a complex with UVRAG to regulate PIK3C3, and is an important convergence point between autophagy and apoptosis because it interacts with the anti-apoptotic and anti-autophagic protein Bcl-2 [51, 52]. Autophagy defects caused by loss of BECN1 may be associated with the malignant phenotype and a poor prognosis for patients with ovarian clear cell carcinomas [53]. BECN1 mRNA and Beclin-1 protein expression are significantly decreased in both eutopic and ectopic endometriotic tissues and are negatively correlated with serum CA125 levels and pelvic pain, which may facilitate the invasion of the endometrial stroma and glands into the myometrium and the diffusive process of adenomyosis in the myometrium, subsequently contributing to the pathogenesis and progression of EMS [40, 54].

During the induction of autophagy, microtubule-associated protein light chain 3 (LC3) is converted from LC3-I to LC3-II, and then LC3-II localizes to isolated membranes and autophagosomes [49, 55]. Accordingly, LC3A-I, LC3A-II, LC3B-I and LC3B-II tend to be down-regulated in eutopic and ectopic endometriotic tissues. The adaptor molecule p62 (which binds ubiquitinated cargo for degradation) enables the autophagy pathway to selectively target designated cargo and facilitates the killing of the ingested organism. The p62 protein is up-regulated in cells with autophagy defects [56]. As shown in our previous studies, secretory phase eutopic and endometriotic tissues and ESCs are characterized by the down-regulation of autophagosomes, decreased conversion of LC3-I to II, reduced BECN1 expression and elevated p62 and CXCR4 expression compared with the normal endometrium and ESCs. Additionally, the change in ectopic ESCs is even more intense. We have also defined CXCR4 as a candidate ATG in the EMS-derived ESCs [10]. In contrast,
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The expression of the LC3-II protein is increased and the expression of the p62 protein is decreased in patients with ovarian endometriomas compared with patients with eutopic endometria or disease-free participants [42]. Interestingly, an independent report has postulated that the levels of LC3B, which is predominantly expressed in and localized to the epithelium compared to the stromal components, are increased in ectopic endometria of EMS-induced mice compared with eutopic endometria of controls, which may be associated with an accumulation of lipid droplets in the epithelial cells, but further work is necessary to understand the clinical implications of this finding [11].

The role of autophagy in EMS

Autophagy and the regulation of ESC proliferation
EMS is increasingly being recognized as a condition in which ectopic endometrial cells exhibit abnormal regulation of proliferation and apoptosis in response to appropriate stimuli [57]. The PI3K/Akt/mTOR signal transduction pathway, the core regulatory pathway related to autophagy, has been widely studied in breast cancer, endometrial cancer, bladder cancer, and other malignant tumors [58]. Akt up-regulation, along with mTOR up-regulation, is also observed in EMS and is expected to impair the autophagic response in endometriotic tissues [59, 60] and promote the survival of endometriotic cells [61]. According to the study by Leconte et al., the rate of endometrial cell proliferation is significantly decreased in mice with EMS that are treated with inhibitors of the PI3K/Akt/mTOR pathway that act on the deep infiltrating EMS uterus [62]. Moreover, suppression of autophagy by CXCL12 promotes the growth and proliferation of ESCs in vitro [10].

Autophagy and the regulation of ESC apoptosis
As reported by Chang et al., EMS is associated with p53, a tumor suppressor protein that negatively regulates cell proliferation and induces apoptotic cell death [44, 63]. In addition, the levels of Bcl-2, an oncoprotein that inhibits apoptotic cell death [64], differ in different phases of the menstrual cycle and in endometriotic lesions at different sites [57]. Based on the findings from these studies, apoptosis plays a major role in the pathophysiology of EMS. Rather than being two independent events, autophagy and apoptosis are two cross-talking mechanisms [65], and autophagy facilitates the engulfment and lysosomal degradation of apoptotic bodies [66]. Autophagy has also been shown to exert a proapoptotic effect on human endometrial cells because the accumulation of autophagosomes promotes apoptosis through an increase in the Bax: Bcl-2 ratio, followed by caspase activation in endometrial Ishikawa cells; the induction of autophagy also plays a key role in regulating endometrial cell apoptosis during the human endometrial cycle [9]. According to several subsequent studies, alterations in the induction of autophagy induced by aberrant mTOR activity may contribute to abnormal apoptosis in EMS, and the induction of autophagy induced by mTOR inhibition is closely related to the induction of apoptosis in both endometrial and endometriotic cells [39]. Moreover, a recent study has concluded that a decrease in autophagic activity in ectopic and eutopic endometrial cells leads to a reduction in autophagy-dependent degradation of proteins and programmed cell death [67].

Autophagy and the crosstalk between ESCs and NK cells
Autophagy has been shown to play a role in antigen presentation [68], and the inhibition of autophagy may allow endometrial cells to escape from immune surveillance and facilitate intramyometrial implantation [54] and EMS. Similarly, autophagy is associated with IL-15 and possibly indirectly influences NK cell differentiation. As shown in our recent study, abnormally high levels of IL-15 in the ectopic endometrium not only directly stimulate the proliferation and invasion and restrict the apoptosis in ESCs in an autocrine manner but also decrease the killing activity of the NK cells in a paracrine manner, which may further contribute to the immune escape of ESCs, ultimately promoting the ectopic growth and implantation of ESCs within the peritoneal cavity [67]. The decrease in ESC autophagy in subjects with EMS may enhance the reactivity of ESCs to IL-15 by increasing the expression of IL-15 receptors and amplifying the role of IL-15 in the dialogue between ESCs and NK cells [67]. Moreover, ESCs restrict NK cell differentiation within the abdominal cavity and may partici-
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Autophagy may play a role in the induction and maintenance of phenotypes and functions of the NK cells and influence the level of inflammation in the endometriotic milieu. The phenotypes and functions of the NK cells in the endometriotic milieu may be involved in the dysregulated autophagy of ESCs, which requires additional research [67].

Moreover, autophagy may be involved in EMS through the CXCR4-CXCL12 axis, which has also been shown to have both immune (lymphocyte chemotaxis) and non-immune functions. The CXCR4-CXCL12 axis has roles in tissue repair, angiogenesis, invasion and migration in EMS, inhibits sex hormone-regulated autophagy, and leads to the anomalous growth of endometrial cells at the ectopic sites in EMS [10, 69]. CXCL12/CXCR4 signaling at the maternal-fetal interface is involved in recruiting NK cells from peripheral blood (pNK) to the decidua during early pregnancy, further inducing pNK to differentiate into decidual NK cells, which promotes the formation of the maternal-fetal interface and the establishment and maintenance of a normal pregnancy [70-72]. Therefore, estrogen-CXCL12/CXCR4 signaling not only directly inhibits ESC autophagy but also recruits more pNK cells to the microenvironment of an ectopic lesion, regulating the function of these NK cells, promoting the immune escape of ESCs, and ultimately accelerating the development of EMS.

Factors involved in regulating the autophagy level in ectopic foci

Hormones

In the human endometrium, two central balancing factors, estrogen and progesterone, control autophagy in endometrial Ishikawa cells during the menstrual cycle. Ishikawa cells are typically cultured in the presence of both hormones, and an increased degree of autophagy and a higher incidence of apoptotic cell death is observed upon the withdrawal of one or both hormones [9, 39]. Hormone deprivation and acute inflammation are identified as two potent inducers of autophagy in the mouse uterus. As the uterus exhibits an acute inflammatory response to incoming semen, the activation of autophagy in the uterine stroma in the first days of pregnancy is attributed to the effects of inflammation. The mouse ovariectomy (OVX) model is used to monitor the effects of individual steroid hormones on autophagy. In response to hormone deprivation after OVX, the uterus shows the highest levels of autophagy, as indicated by the higher expression levels of LC3-II and ATG5 proteins. After OVX, autophagy is activated in all major uterine cell populations, which differs from the more localized autophagy pattern observed on day 1 of pregnancy. When either 17β estradiol (E2) or progesterone (P4) is administered, the levels of LC3-II and ATG5 decrease as early as 2 h after hormone administration. Beclin1 represents a distinct expression profile, suggesting that it is regulated by a different mechanism [74]. In the uterus, mammalian target of rapamycin (mTOR) is currently considered a key player in mediating the effects of hormones on autophagy. mTOR itself is an estrogen-responsive factor that strongly inhibits autophagy [75].

The typical characteristics of EMS are increased production of estradiol, which stimulates the proliferation of endometriotic tissues, and perturbations in the progesterone response, which is known as progesterone resistance [76-78].
Estrogen and progestogen modulate apoptosis in human endometrium and endometriotic cells and tissues and further contribute to the incidence and development of EMS [79]. Endometriotic cells have been shown to respond abnormally to ovarian steroids, which contributes to the dysregulation of autophagy in these cells [39]. Cornillie et al. [80] have described an increase in lysosomal autophagy after endometriotic implants are administered an antiprogestosterone treatment. However, low progesterone has recently been suggested to be associated with decreased autophagy in the ectopic foci of patients with EMS, as dienogest treatment of endometriotic cells suppresses AKT and ERK1/2 activity, thereby inhibiting mTOR, inducing autophagy, and promoting apoptosis, possibly through progestogenic actions [81]. EMS appears to contain an altered complement of steroid hormone receptors compared with the normal endometrium. Estrogen receptor α (ERα) is significantly up-regulated, whereas progesterone receptor (PR) is down-regulated in eutopic and ectopic (more significantly) ESCs. ERα up-regulation and an enhancement of estrogen function repress ESC autophagy by up-regulating CXCL12/CXCR4 signaling to further promote ESC growth [10]. In contrast, progestogen inhibits the effect of estrogen on ESC autophagy [10]. Thus, estrogen seems to negatively regulate autophagy in the human endometrium through a novel chemokine-mediated mechanism. However, the regulatory axis that involves CXCL12 seems to be mTOR-independent [82]. Moreover, the production of IL-15, which regulates the proliferation, apoptosis, and invasion of ESCs in vitro, is regulated by ovarian steroid hormones in normal human endometrial cells. A decrease in ESC autophagy promotes the expression of IL-15 receptors, increases the sensitivity of ESCs to IL-15, and improves the stimulatory effect of IL-15 on ESCs [67, 83]. Therefore, ovarian steroid hormones may regulate IL-15 production through effects on ESC autophagy and the subsequent growth and invasion of ESCs.

Drugs

Hydroxychloroquine (HCQ), an autophagic flux inhibitor used to treat malaria and inflammatory and autoimmune diseases [84], is considered a lysosomotropic agent because it increases the pH of acidic compartments and inhibits the fusion of the autophagosome with the lysosome [85-87]. As shown in the study by Ruiz et al., the levels of autophagic markers in human endometriotic cells and human ESCs increase following HCQ treatment, suggesting that the use of HCQ (or an inhibitor targeting specific autophagic mediator) may be detrimental to both human endometrial and endometriotic cell survival in vitro. The drug also appears to have an effect on lesion histopathology (the absence of glandular components) and lesion numbers, but not on lesion size. Additionally, HCQ increases the number of macrophages and the levels of the IP-10 chemokine within the peritoneal cavity of a mouse model of EMS [11].

Rapamycin, a specific mTOR inhibitor [88], induces autophagy in all mammalian cell types tested to date [89]. According to Choi et al., rapamycin induces autophagy and promotes apoptosis. However, the pro-apoptotic effect of rapamycin is reversed by an autophagy inhibitor, 3-MA. Thus, mTOR inhibition promotes endometriotic cell apoptosis by inducing autophagy [39]. Moreover, rapamycin significantly inhibits the expression of the IL-15 receptor, an autophagy inhibitor [67].

Mullerian inhibiting substance (MIS), a 140-kDa homodimer glycoprotein and a member of the TGF-β superfamily of biological response modifiers, causes Mullerian ducts to regress in developing male embryos [90]. According to the study by Renaud et al., both the normal human endometrium and endometrial cancers express the MIS receptor, and MIS inhibits the proliferation of a number of human endometrial cancer cell lines [91]. As shown in the study by Borahay et al. [92], MIS treatment induces autophagy in endometriotic cells by inducing Beclin-1 and ERK activity. The MIS treatment also inhibits the proliferation and induces the apoptosis of ectopic endometrial cell lines.

The GnRH-II antagonist triptorelix-1 has been shown to induce autophagy in prostate cancer cells [93]. A GnRH-II antagonist has recently been shown to exert a significantly stronger anti-proliferative effect on breast, ovarian, and endometrial cancer cells than the GnRH-I agonist triptorelin [94]. Therefore, Ren et al. propose that GnRH-II antagonists might hold promise in the treatment of adenomyosis as autophagy inducers [53], as well as in the treatment in EMS, which requires further study.
Bafilomycin A1 (Baf A1), a chemical inhibitor of V-ATPase, is commonly used to block autophagosome-lysosome fusion in mammalian cell culture studies, causing an accumulation of autophagosomes upon autophagy induction that is independent of its effect on lysosomal pH, possibly through a Ca²⁺-dependent mechanism [95-97]. A study has concluded that the rates of cell death and apoptosis in Baf A1-treated Ishikawa cells are significantly higher than in Ishikawa cells treated with 3-MA, suggesting that a certain level of autophagosome accumulation may be needed to promote endometrial cell death and apoptosis [9]. Similarly, the Baf A1 treatment may influence the autophagy level of eutopic and ectopic foci in EMS by causing an accumulation of autophagosomes and subsequently promoting cell death and apoptosis, which still requires further study.

**Hypoxia and oxidative stress**

Autophagy acts as a spontaneous pro-survival mechanism in cells under hypoxia and oxidative stress [13]. Hypoxia is a well-known inducer of autophagy [98]. Based on accumulating evidence, hypoxia may play a role in the survival and angiogenesis of ectopic endometrial cells [99-101], which is likely associated with hypoxia-responsive miRNAs, such as miR-20a and miR-199a [102, 103]. Xu et al. identified higher levels of miR-210 expression in endometriotic cells grown in a hypoxic environment, which may contribute to the pathological development of EMS by reducing the apoptosis of endometriotic cells, enhancing cell survival and promoting autophagy through a Bcl-2/Beclin-1 pathway [104]. Autophagy induction in ectopic foci would be facilitated by regional oxidative stress. Because of the cyclic bleeding in endometriotic tissues, the hemoglobin released during hemolysis leads to the accumulation of high levels of heme. Heme undergoes heme oxygenase-catalyzed degradation into biliverdin, carbon monoxide, and iron [105]. Oxidative stress has been reported to promote the induction of autophagy in patients with ovarian EMS compared with the eutopic endometria of affected or healthy women, because a significant increase in the levels of the heme oxygenase-1 (HO-1) protein has been detected in endometriomas [42].

**Other related factors**

In many cell types, autophagy is negatively regulated by the PI3K/AKT and MEK1/2-ERK1/2 pathways, both of which activate mTOR, the major negative regulator of autophagy [89, 106]. Interestingly, endometriotic lesions have been shown to exhibit enhanced activation of AKT, ERK1/2, and mTOR compared with the normal endometrium, suggesting that inappropriate activation of AKT and ERK1/2 may lead to increased mTOR activity and the subsequent inhibition of autophagy in endometriotic tissue [107-109]. Accordingly, mTOR activity is abnormally increased in endometriotic lesions compared with the normal eutopic endometrium [59, 110]. Moreover, aberrant mTOR activity in ovarian endometriotic cysts leads to alterations in endometrial cell autophagy, which are associated with abnormal apoptosis, and mTOR inhibition promotes endometriotic cell apoptosis by inducing autophagy [39]. CXCL12 mainly inhibits autophagy by down-regulating autophagosomes and the conversion of LC3B-I to LC3B-II, reducing Beclin-1 expression and increasing p62 levels; these activities of CXCL12 are partially dependent on the NF-κB signaling pathway. The abnormally high level of CXCL12/CXCR4 expression may promote the survival and growth of ESCs in the endometriotic milieu by restricting secretory phase ESC autophagy [10]. Retrospectively, in endometriotic cells, miR-210 promotes autophagy in response to hypoxia, contributing to the enhanced survival of hypoxic endometriotic cells [104]. An HCQ treatment increases the levels of IP-10 in the peritoneal cavity in a mouse model of EMS, which may have created an unfavorable environment for lesion development. However, further studies are required to determine whether IP-10 modulates the autophagic pathway [11].

**Summary**

Autophagy is accepted to play important roles in the development and treatment of cancers. In recent years, accumulating studies have focused on the effect of altered autophagy on EMS. As shown in **Figure 2** and **Figure 3**, the level of autophagy in both ectopic stromal and epithelial cells decreases in ectopic foci from patients with EMS, particularly during the secretory phase of the menstrual cycle, which is regulated by hormones, hypoxia, oxidative stress, and many other related factors, leading...
Autophagy in endometriosis

Figure 2. Factors involved in regulating the autophagy level in ectopic foci. Female hormones (estrogen and progesterone), several drugs (hydroxychloroquine, rapamycin, mullerian inhibiting substance, GnRH-II antagonist, and bafilomycin A1), hypoxia and oxidative stress are reported to affect the dysregulated autophagy level in EMS via different mechanisms or pathways.

Figure 3. The role of autophagy in the progression of endometriosis. Hormones, hypoxia, oxidative stress, and many other related factors significantly decrease autophagy in the ectopic endometrium, resulting in increased proliferation, invasion and angiogenesis, and decreased apoptosis and NK activity in ectopic foci that ultimately contributes to the occurrence and development of EMS.

to increased proliferation and decreased apoptosis of ectopic foci through downstream molecules and finally contributing to the occurrence and development of EMS. Based on these results, autophagy is decreased in endometriotic cells, which is probably a significant mechanism in EMS. The mRNA and protein levels of the currently known markers of autophagy are further down-regulated and important signal transduction pathways involved in autophagy are altered in ectopic tissues. Thus, fundamental and clinical studies are now focusing on new therapeutic strategies for EMS aimed at autophagic markers or pathways. Additionally, some drugs (Figure 2), such as dienogest and HCQ, have been verified to exert a therapeutic effect on EMS by promoting the autophagy of ectopic tissues. Therefore, strategies that adjust the level of autophagy in ectopic foci may be a potential target in the clinical treatment of EMS, and fur-
ther studies are necessary to identify and evaluate this new, latent therapy.

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Disclosure of conflict of interest

None.

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