Molecular profile of eutopic and ectopic endometrium in endometriosis

Ciprian Andrei Coroleucă[1,2,3], Elvira Brătilă[1,2], Cătălin Bogdan Coroleucă[1,2,3], Mihai Mitran[1,2], Diana Comandașu[1,2]

1. "Prof. Dr. Panait Sîrbu" Clinical Hospital of Obstetrics and Gynecology, Bucharest, Romania

2. "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

3. PhD Student, "Carol Davila" University of Medicine and Pharmacy Doctoral School, Bucharest, Romania

Corresponding author:
Ciprian Andrei Coroleucă
E-mail: cip_coroleuca@yahoo.com

Abstract

Endometriosis represents a chronic inflammatory disease defined by the appearance of endometrial tissue outside the uterine cavity, affecting mostly reproductive-aged women. Although some patients may be asymptomatic, the clinical features of endometriosis are frequently dominated by pelvic pain associated with infertility. Nonspecific symptomatology and the lack of biological screening markers with increased sensitivity and specificity lead to a slow diagnostic process. The hormonal profile of the eutopic and ectopic endometrium, molecular abnormalities and enzymatic mechanisms play a key role in the etiopathogenesis of endometriosis. Endometriotic implants are characterized by elevated levels of aromatase and 17β-HSD1 associated with low levels of 17β-HSD2 in response to low progesterone receptors, causing an increased level of estradiol. It is characteristic a status of progesterone resistance, described by the inability of endometrial tissue to respond adequately to progesterone, the activation failure of progesterone receptors and the use of available progesterone. Endometriosis is defined by abnormal cell proliferation of ectopic endometrial tissue associated with apoptosis mechanisms disorders. The level of apoptotic cells is reduced in the epithelium and stroma of the eutopic endometrium and does not increase at the end of the secretory phase, compared to the normal endometrium. Peritoneal endometriosis lesions associate elevated Bcl-2 levels, while ovarian endometriosis shows discordant results. The correlation between Ki-67 proliferation antigen level and the disease's stage of evolution and aggressiveness was confirmed by numerous studies. The analysis of stem cells in the eutopic endometrium of patients with endometriosis showed morphological changes, altered expression of immunomodulatory molecules and an increased potential for invasion and proliferation. We believe that the characterization of the biochemical profile of the eutopic and ectopic endometrium is a current topic of interest in the understanding of endometriosis, its etiopathogeny and therapeutic approach. The main molecular features of ectopic endometrium present in endometriosis lesions are characterized by hyperestrogenemia, progesterone resistance, a reduced apoptosis capacity, an increased proliferation index, and the presence of abnormal multipotent stem cells.

Keywords: *endometriosis, molecular profile, eutopic endometrium, ectopic endometrium, Bcl-2, Ki-67, stem cells*

Rezumat

Endometrioza reprezintă o boală inflamatorie cronică definită prin apariția țesutului endometrial în afara cavității uterine, care afectează mai ales femeile de vârstă reproductivă. Deși unele paciente pot fi asimptomatice, caracteristicile clinice ale endometriozei sunt frecvent dominate de durere pelviană asociată cu infertilitatea. Simptomatologia nespecifică și absența biomarkerilor de screening cu sensibilitate și specificitate crescută conduc la un proces de diagnostic lent. Profilul hormonal al endometrului eutopic și ectopic, anomaliile moleculare și mecanismele enzimatice joacă un rol-cheie în etiopatogeneza endometriozei. Implanturile endometriozice sunt caracterizate de niveluri ridicate de aromatază și 17β-HSD1, asociate cu niveluri scăzute de 17β-HSD2, ca răspuns la nivelul scăzut de receptori de progesteron, provocând creșterea estradiolului. Este caracteristică o stare de rezistență la progesteron, descrisă prin incapacitatea țesutului endometrial de a răspunde adecvat la progesteron, eșecul de activare al receptorilor progesteronici și utilizarea progesteronului disponibil. Endometrioza este definită prin proliferarea anormală a celulelor endometriale ectopice asociată cu tulburarea mecanismelor de apoptoză. Nivelul de celule apoptotice este redus în epiteliu și stroma endometrului eutopic și nu crește la sfârșitul fazei secretorii, în comparație cu endometrul normal. Leziunile de endometrioză peritoneală asociază niveluri ridicate de Bcl-2, în timp ce în endometrioza ovariană nivelul Bcl-2 prezintă rezultate discordante. Corelația dintre nivelul antigenului de proliferare Ki-67 și stadiul de evoluție și agresivitate al bolii a fost confirmată de numeroase studii. Analiza celulelor stem din endometrul eutopic la pacientele cu endometrioză a arătat modificări morfologice, expresie modificată a moleculelor imunomodulatoare și un potențial crescut de invazie și proliferare. Considerăm că descrierea profilului biochimic al endometrului eutopic și ectopic este un subiect actual de interes în înțelegerea endometriozei, a etiopatogeniei sale și a abordării terapeutice. Principalele caracteristici moleculare ale endometrului ectopic prezent în leziunile endometriozei sunt caracterizate de hiperestrogenemie, rezistență la progesteron, o capacitate redusă de apoptoză, un indice de proliferare crescut și prezența celulelor stem multipotente anormale.

Cuvinte-cheie: *endometrioză, profil molecular, endometru eutopic, endometru ectopic, Bcl-2, Ki-67, celule stem*

Submission date: 16.04.2020
Acceptance date: 5.05.2020

Profilul molecular al endometrului eutopic și ectopic în endometrioză

Suggested citation for this article: Coroleucă CA, Brătilă E, Coroleucă CB, Mitran M, Comandașu D. Molecular profile of eutopic and ectopic endometrium in endometriosis. Ginecologia.ro. 2020;28(2):29-35.

Endometriosis represents a chronic inflammatory disease defined by the appearance of endometrial tissue (endometrial glands and stroma) outside the uterine cavity[1]. The ectopic endometrial tissue is estrogen-dependent[2], generally affecting reproductive-aged women, but is also found to have a lower incidence in adolescents or postmenopausal women[3]. The symptomatology generated by endometriosis is often nonspecific. Although some patients may be asymptomatic, especially in the early stages, the clinical features of endometriosis are frequently dominated by pelvic pain (chronic pelvic pain, dysmenorrhea, dyspareunia, dysuria, dyschezia) associated with infertility[4,5]. Despite numerous attempts of the scientific community, materialized in extensive studies, the etiology and pathophysiological mechanisms of endometriosis are still incompletely elucidated to date, being called the "disease of theories"[6].

Nonspecific symptomatology and the lack of biological screening markers with increased sensitivity and specificity lead to a burdened diagnostic process, causing an average delay in the diagnosis of endometriosis between 4 and 11 years[7]. Consequently, endometriosis markedly affects the long-term quality of life of patients, causing psychological and socioeconomic damages[8]. The medical and surgical treatment of endometriosis is insufficiently standardized[1]. Thus, the overall recurrence rate varies within wide limits (between 6% and 67%), depending on the parameters taken into account by various authors[9]. The recurrence rate of endometriosis after surgery is estimated at 21.5% after 2 years, and at 40-50% after 5 years[10].

The multifactorial origin, the complexity of pathophysiological mechanisms and the lack of a consensus on the diagnosis and treatment of endometriosis transform this disease into an important public health issue[7]. The economic resources attributed to endometriosis are similar to those for diabetes, Crohn's disease and rheumatoid arthritis[11]. Recent research stress the importance of the biochemical and molecular profile of the endometrium in the etiology and evolution of the disease[12]. The goal is identifying biological markers that can be used both as screening tools and disease-progression factors[13].

This paper is a literature review and aims to characterize the molecular profile of the eutopic and ectopic endometrium in endometriosis, from the perspective of hormonal profile, apoptosis capacity, cell proliferation potential and the role of stem cells.

Hormonal profile – estrogen and progesterone

All data suggest that endometriosis is an estrogen-dependent disease, estrogen playing a key role in its pathophysiological mechanisms[1]. Alterations in the hormonal profile have the ability to influence endometrial tissue, acquiring increased properties of adhesion, proliferation and evasion of the immune system[14]. Endometriosis-specific ectopic endometrial tissue reacts to ovarian hormones differently, compared to the normal eutopic endometrium[15]. Under physiological conditions, the eutopic endometrium undergoes a cyclic transformation under the influence of hormones, causing the superficial layer to undergo successively the proliferative phase, the differentiation phase and, finally, the elimination through menstruation if implantation does not occur[15]. Estrogen stimulates epithelial proliferation, causing thickening of the endometrium during the proliferative phase of the menstrual cycle. During the secretory phase, progesterone inhibits estrogen-induced proliferation and promotes decidualization of stromal cells[16].

Estradiol plays a key role in the reconstruction phase of the endometrium after menstruation, through the estrogen receptors alpha (RE-α/ESR1) and beta (RE-β/ESR2)[15,16]. Although the main source of estrogen are the ovaries, it can also be synthesized by other tissues, such as adipose tissue and adrenal glands[15]. RE-α/ESR1 stimulates epithelial cell proliferation[16]. RE-β/ESR2 ablation by genetic engineering in female mice caused infertility due to ovulatory failure without uterine defects[16]. RE-α/ESR1 ablation in female mice resulted in hypoplastic uterus, ovarian and uterine infertility, lack of response to estrogen treatment, and decreased progesterone receptor (PR) expression[16]. Studies analyzing embryo insemination with optimal hormonal stimulation showed that the uterus with ESR1 depletion cannot support implantation[16]. However, epithelial ESR1 ablation determines estrogen-induced epithelial proliferation, thus stromal ESR1 stimulates estrogen-induced epithelial proliferation by regulating reactions between the stromal and epithelial components[16]. The decidualization in response to hormonal stimulation requires the presence of ESR1 at both the epithelial and stromal levels[16]. Aromatase stimulates the conversion of ovarian androstenedione to estrone, and 17β-hydroxysteroid dehydrogenase 1 (17β-HSD1) stimulates the conversion of estrone to estradiol[15]. 17β-hydroxysteroid dehydrogenase 2 (17β-HSD2), stimulated by progesterone, converts estradiol into estrone in the luteal phase[15].

Progesterone is secreted mainly during the secretory phase of the menstrual cycle and binds to progesterone A and B receptors (RP-A and RP-B), showing duality: it inhibits the estrogen's action and prepares the endometrium for implantation[15]. Progesterone exerts an antiestrogenic effect on the endometrium[17]. PR expression is stimulated by estrogen through ESR1, and consequently progesterone receptors inhibit ESR1 expression, thus creating a feedback mechanism, maintaining hormonal homeostasis[16]. In the eutopic endometrium of healthy women, the level of progesterone receptors rises in the proliferative phase, reaches its maximum before ovulation and decreases thereafter, a variation regulated by the value of estradiol[18]. The ablation of both forms of receptors in female mice leads to sterility, through

several mechanisms: reduction/absence of ovulation, uterine hyperplasia, absence of decidualization, limited development of the mammary gland and inability to express sexual behavior[16]. RP-A ablation showed that it is the main promoter of uterine progesterone receptor function and that it plays a key role in fertility[16]. RP-B deletion highlighted its importance in the development of the mammary gland during pregnancy[16]. RP-B stimulates uterine epithelial proliferation when not suppressed by RP-A, and increased RP-A levels lead to endometrial hyperplasia and infertility, emphasizing the importance of the RP-A/RP-B ratio for optimal physiological response of progesterone[16]. Both the epithelial and stromal components of progesterone receptors are involved in inhibiting epithelial proliferation[16].

Normal endometrium in healthy women has undetectable aromatase activity and elevated 17β-HSD2 levels, consequently estrogenic effects are counteracted by progesterone during the luteal phase of the menstrual cycle[15,19]. The hormonal profile of the eutopic and ectopic endometrium, molecular abnormalities and enzymatic mechanisms play a key role in the etiopathogenesis of endometriosis. The disorders in the mechanism of action of progesterone and estrogen and implicitly the relationship between epithelial cells and endometrial stroma cause the appearance of progesterone resistance and increased estrogenic effects, leading to endometriosis[16]. The impairment of ovarian hormone function, regulation and signaling mechanisms, generating hyperestrogenemia-induced inflammatory process and progesterone resistance, decisively influences both the development of endometriotic lesions and the functioning of the eutopic endometrium[16]. High concentrations of estrogen stimulate cell proliferation and inflammation, thus causing an increase in endometriotic lesions and alteration of the receptivity of the endometrium[16]. In patients with endometriosis, the level of aromatase is increased both in the eutopic endometrium and in the ectopic endometrium[15,16].

In patients with endometriosis, the level of ESR1 in the secretory phase of eutopic endometrium is increased 9 times compared to the eutopic endometrium of healthy women, which may increase estrogenic and proliferative activity by altering normal uterine function[16,17]. The ESR2 level in the eutopic endometrium of patients with endometriosis remains unchanged, although some researchers indicate an increase[16]. The ectopic endometrium in endometriotic lesions is characterized by an increase in ESR2 (approximately 140-fold compared to the eutopic endometrium of healthy patients) and a decrease in ESR1 levels. ESR2 in endometriotic stromal cells saturates the implantation sites of ESR1 precursors and causes the suppression of ESR1, with consequent increase of the ESR2/ESR1 ratio[16,17]. Given that estrogen increases the expression of progesterone receptors through ESR1, the low level of ESR1 in endometriotic lesions might explain the low level of progesterone receptors in the ectopic endometrium[16].

Endometriotic implants are characterized by elevated levels of aromatase and 17β-HSD1 associated with low levels of 17β-HSD2 (in response to low progesterone receptors), therefore an increased level of estradiol is observed in patients with endometriosis, both in the eutopic as well as ectopic endometrium[14,15,17]. Cyclooxygenase-2 (COX-2) stimulates the synthesis of prostaglandin E2 (PGE2) from arachidonic acid, inducing aromatase production in stromal cells in ectopic endometrium[15]. In patients with endometriosis, estrogen, through ESR2, stimulates COX-2, generating increased synthesis of PGE2 and thus the production of aromatase (with consequent increase in estrogen production)[15,16,20]. This causes a positive feedback mechanism with decisive action in creating a hyperestrogenic status, promoting inflammation and supporting the proliferation of endometriotic lesions[15,16,20].

Endometriosis is defined by a status of progesterone resistance[1,15]. Progesterone resistance is characterized by the inability of endometrial tissue to respond adequately to progesterone, manifested in endometriosis by activation failure of progesterone receptors and the use of available progesterone[16]. Progesterone resistance in endometriosis patients is specific to both the ectopic endometrium in endometriotic lesions and the eutopic endometrium[16]. The status of progesterone resistance is linked both to the proliferation of endometriotic lesions (by absence of estrogen's antagonist effect) and to the reduction of endometrial receptivity[16]. The eutopic endometrium of patients with endometriosis associates a low level of progesterone receptors[16]. The ectopic endometrium in endometriotic implants manifests low progesterone receptors due to the absence of RP-B and a very low level of RP-A compared to the normal endometrium[18]. Moreover, the alteration of mediators and PR regulatory molecules adds to progesterone resistance in endometriosis[16]. Resistance to progesterone in endometriosis was also confirmed by a significantly low level of prolactin (whose expression is stimulated by progesterone) after treating endometriotic tissue with synthetic progesterone, compared to normal endometrium[21]. Low levels of PR decrease the concentration of 17β-HSD2 and thus reduce the inhibitory action of estrogen (by decreasing the ability of 17β-HSD2 to convert estradiol to estrone, a less potent form), the result being a high level of estradiol in patients with endometriosis[15].

Apoptosis – B-cell lymphoma 2 protein

The apoptosis or programmed cell death represents a process with a fundamental role in the body's physiology, tissue homeostasis and the elimination of compromised or infected cells[22]. The alteration of its normal process is one of the determining causes, leading to a range of pathologies[22]. Many neoplasms associate

an imbalance between proapoptotic and antiapoptotic proteins, causing abnormal cell proliferation and the organism's inability to respond to apoptotic stimuli, thus having a low response to cellular apotosis inducing therapies[22]. Inappropriate apoptosis can cause persistence of infections through the inability to eradicate infected cells[22]. Unbalanced apoptosis is also involved in neurodegenerative diseases, such as Huntington's, Parkinson's or Alzheimer's diseases, defined by a premature destruction of neurons[22]. Excessive apoptotic mechanism causing tissue damage is found, on the other hand, in myocardial infarction, stroke or inflammatory processes[22]. Apoptosis is controlled by regulatory proteins with the role of maintaining homeostasis by adjusting the proapoptotic and antiapoptotic effects at tissue level[22].

B-cell lymphoma 2 protein family (Bcl-2), containing more than 25 members, has a major role in the process of programmed cell death[22]. The Bcl-2 protein family is divided into two categories, depending on their action: proapoptotic proteins (Bax, Bak, Bok, Bad, Bid), and antiapoptotic proteins (Bcl-2, Bcl-XL, Bcl-w, Ced-9, Mcl-1, BHRF1, KSHV)[22-24]. The members of the Bcl-2 protein family have the ability to form homodimers and heterodimers with each other[25].

The dimerization process occurring between molecules leads to the predominance of one type of protein and implicitly to the prevalence of proapoptotic or antiapoptotic action in the tissues[25]. In addition to the heterodimerization process, apoptosis is also regulated independently by the intrinsic action of each protein in the Bcl-2 family[25].

The Bcl-2 protein is an important element of the Bcl-2 protein family, ensuring cell survival through its antiapoptotic role[24,25,26]. Bcl-2 controls the permeability of the mitochondrial membrane and prevents cell death without promoting cell proliferation[24]. The Bcl-2 protein inhibits the release of cytochrome C and other apoptosis inducing factors from the mitochondrial intermembrane space to the cytoplasm[25]. Its action depends on both concentration and interaction with proapoptotic proteins (Bax)[24]. Bax protein is part of the Bcl-2 family of proteins, characterized by the ability to heterodimerize with Bcl-2 and to homodimerize with itself[24]. When Bcl-2 levels are increased, Bcl-2 heterodimerizes with Bax and suppresses cell death – thus, the balance between Bcl-2 and Bax is important for regulating the apoptosis process[24].

Apoptosis is involved in the homeostasis of eutopic endometrium in healthy women by removing tissues from the functional layer of the endometrium at the end of the secretory phase and the menstrual phase[24]. The level of Bcl-2 protein in the eutopic endometrium varies during the menstrual cycle and appears to be influenced by steroid hormones[27]. In the normal endometrium, Bcl-2 protein in the glandular epithelium has the maximum value at the end of the proliferative phase and is absent in the secretory phase, while Bcl-2 in the stroma registers the maximum value at the end of the secretory phase[27]. Endometriosis is defined by abnormal cell proliferation of ectopic endometrial tissue associated with apoptosis mechanisms disorders[24]. In patients with endometriosis, the level of apoptotic cells is reduced in the epithelium and stroma of the eutopic endometrium and does not increase at the end of the secretory phase, compared to the normal endometrium[24]. This fact has been confirmed in ectopic endometrium in peritoneal endometriotic lesions, as well as in ovarian endometriomas[24,27].

Also, the proteins involved in apoptosis regulation have an altered expression in the eutopic and ectopic endometrium of patients with endometriosis[24]. A number of researches identified Bcl-2 expression in the glandular epithelium and ectopic endometrial stroma of endometriotic implants, as well as in the eutopic endometrium of women with or without endometriosis, with the peak concentration in the proliferative phase[24]. In case of the eutopic endometrium of patients with endometriosis, Bcl-2 expression was maximal at the end of the proliferative phase and almost absent at the end of the secretory phase[28]. Peritoneal endometriosis lesions associate elevated Bcl-2 levels[24]. In ovarian endometriosis, the level of Bcl-2 protein shows discordant results, while in older studies Bcl-2 was low or absent; in recent ones, Bcl-2 is increased in the stroma of ovarian endometriotic lesions compared to the eutopic endometrial stroma of patients with or without endometriosis[24]. Some researchers showed that Bcl-2 expression is lower in cystic endometriotic compared to non-cystic lesions and suggested a variation in Bcl-2 levels depending on the location of endometriotic implants[24]. Peritoneal macrophages in patients with endometriosis show an increased level of Bcl-2 protein[24]. The high concentration of estrogen receptors in endometriosis might inhibit apoptosis by stimulating the expression of Bcl-2 protein[24].

Cell proliferation – Ki-67 antigen

Ki-67 antigen represents a nuclear protein strictly associated with cell proliferation[29]. Ki-67 antigen is formed from two molecules[30], being detected at the interface of the nucleus and on the chromosome surface during mitosis[31]. Ki-67 antigen is present in all active phases of the cell cycle, both in the interphase, being closely correlated with cell proliferation and active phases of the cell cycle (G1, S, G2), and in the period of cell division associated with mitosis, being absent in the inactive G0 phase[29]. This pattern of expression during the cell cycle phases (in both normal and tumor cells) underlies the use of Ki-67 as a proliferation marker of a specific cell population and is frequently used to calculate the cell and tumor proliferation index[29,30]. The identification of Ki-67 antigen by immunohistochemistry techniques can be performed on various cellular or histopathological

preparations, both in case of benign and malignant tumors[29,32]. The percentage of positive Ki-67 cells represents the proliferation index, which is high in aggressive tumors[30], being used to estimate prognosis, survival and recurrence. An increased Ki-67 proliferation index is associated with a high aggressiveness tumor and metastasis[33].

Although endometriosis is a benign disease, its proliferative character associated with molecular, histological and genetic similarities to malignant tumors leads to the recommendation to evaluate it similarly to a neoplastic process, especially regarding proliferation and aggression[30]. The proliferation of endometriosis is directly linked to the ability to progress, involving the appearance of complex lesions and the property of endometriotic lesions to invade adjacent tissues[31]. Given these aspects, multiple researchers evaluated the disease by using the relationship between Ki-67 antigen and the endometriotic lesions[30].

Different studies, including patients diagnosed with stage III or IV endometriosis, showed a significant correlation between Ki-67 antigen levels and the size of endometriotic ovarian cyst and the stage of endometriosis, Ki-67 protein expression increasing proportionally to the two parameters analyzed[31,33]. A comparative analysis between the Ki-67 proliferation index in the eutopic and ectopic endometrium showed an increase in the Ki-67 proliferation index proportionally to the increase in endometriosis stage, proliferative activity and the degree of disease severity[32]. It also demonstrated that cells with high proliferative potential develop autonomy (similar to malignancy), leading to lesion progression to invade adjacent tissues, form adhesions and alter the normal anatomy[32]. Another study searched the expression of the Ki-67 proliferation index in patients who underwent surgery for ovarian endometrioma, half of which had recurrent disease[30]. The results of the study showed that Ki-67 proliferation index was significantly increased in patients with recurrence, the increase in Ki-67 being associated with advanced stages of endometriosis[30]. The research concluded that Ki-67 proliferation index is a useful tool for estimating the prognosis and the risk of recurrence[30].

Stem cells

Stem cells represent undifferentiated cells characterized by high proliferation, self-regeneration and differentiation into different specialized cells properties[34]. Stem cells can be embryonic, identified in the inner cell mass of the blastocyst and adult stem cells, derived from post-embryonic cells, found in a multitude of human tissues, but extensively studied in the hematopoietic system, expressing two common features: the ability to multidifferentiate and regenerate[34]. Cell differentiation represents the phenotypic change due to gene expression associated with a particular cell function, while potency is the ability to differentiate[35]. Trans-differentiation or plasticity characteristic of stem cells is the ability of a particular cell line to lose its specific markers and function and differentiate into a new cell line with new specificity and function[36]. Depending on their ability to differentiate, stem cells are classified into totipotent, pluripotent, multipotent and unipotent cells[34]. Totipotent stem cells (e.g., zygote) are completely undifferentiated cells that have the ability to generate all embryonic tissues (ectoderm, mesoderm, and endoderm), as well as extraembryonic cell structures (extraembryonic membranes, placenta, and trophoblast)[34]. Pluripotent stem cells, represented by embryonic stem cells, can differentiate into all embryonic tissues, except extraembryonic tissues[34]. As the cell differentiation cycle progresses, stem cells lose their potency and become multipotent cells (with ability to differentiate into multiple cell types belonging to a single germ line) or unipotent cells (with ability to differentiate into a single cell line)[37].

Embryonic stem cells are characterized by an increased potential for long-term proliferation and self-regeneration and have the ability to transform (*in vivo* or *in vitro*) into any cell[36]. The use of adult stem cells in current medical practice is considered safer compared to embryonic stem cells, which associate a number of limitations: complicated culture techniques, more difficult to control differentiation pattern that can lead to the development of teratomas and ethical considerations[36,38]. Adult stem cells are found in many specific tissues and organs: skin, adipose tissue, muscle, bone marrow, synovial membrane, nervous system, thymus, lung, liver, intestine, heart, blood, dental pulp, amniotic fluid, umbilical cord blood and endometrium[36,38]. Under normal conditions, adult stem cells are found in a dormant state, their proliferation and differentiation being triggered by various pathologies and external stimuli[36,39].

Given the therapeutic capacity of stem cells, multiple researches attempted to identify alternative sources, and the endometrium is an excellent source[36]. The endometrium is a generable tissue with a growth rate of about 7 mm at each menstrual cycle[37]. Some hypotheses claim that endometrial stem cells are involved in the cyclic regeneration of the endometrial functional layer[34]. Stem cells were first isolated from the endometrium in 2004, while in 2007 they were isolated from menstrual blood[36,40,41]. Menstrual blood is an easily accessible, cheap and renewable resource, with the advantage of noninvasive collection and the possibility of *in vitro* processing[36]. There were obtained cultures from endometrial stem cells which put on induction medium for 4 weeks generated different cell lines (adipocytes, osteocytes, chondrocytes, myocytes)[42]. Another research collected tissue samples from endometrium, myometrium, fallopian tubes and utero-sacral ligaments, cultured them for 21 days on chondrogenic induction medium, and observed that only the endometrial cells transformed into chondrocytes, highlighting the multipotent character of

endometrial stem cells[43]. The origin of endometrial stem cells seems to be fetal stem cells and bone marrow[34,44]. Fetal stem cells persist in the adult uterus and contribute to endometrial tissue replacement at each menstruation[44]. Some hypotheses consider that stem cells migrate to damaged tissues, where they act by angiogenesis and plasticity to form a new tissue[34]. A study demonstrated the differentiation of bone marrow stem cells of a male donor into endometrial glands at the female receptor[45].

Considering the theory of retrograde menstruation, endometrial stem cells can have an additional role in the etiopathogenesis of endometriosis, by inoculation into the peritoneal cavity where they can cause the appearance of endometriotic implants[44]. Neonatal retrograde uterine bleeding is a rare event transporting stem cells from the neonatal endometrium and may be responsible for early-onset endometriosis[46]. Endometrial fragments develop successfully in ectopic locations in numerous experimental models – human endometrial tissue transplanted in mice generated the appearance of endometriotic lesions[46]. Similarly, traumatic dislocation of endometrial stem cells into the myometrium associated with alterations of the regulatory mechanism of adjacent endometrium may explain the etiopathogenesis of adenomyosis[46]. In addition to endometrial stem cells, extrauterine stem cells transported through the vasculo-lymphatic system can cause endometriotic lesions by transdifferentiation to endometrial cells[46,47]. Abnormal cell migration during organogenesis associated with certain genetic changes (aberrant expression of Wnt and Hox genes) is a form of primordial stem cell migration which may be involved in the etiopathogenesis of reproductive endometriotic lesions[46,47]. An experimental study documented the ability of stem cells harvested from bone marrow to develop endometrial lesions after transplantation in mice, the results showing a low level of stem cells in the eutopic endometrium[47].

The genetic and phenotypic profile of stem cells present in endometriosis is different compared to physiological stem cells, differences observed both between ectopic endometriotic cells compared to eutopic endometrial cells in patients with endometriosis and in the eutopic endometrial profile of patients with endometriosis compared to healthy women[46]. The regulatory mechanism controlling the function and rate of stem cell regeneration is altered in endometriosis[46]. A study confirmed the increased level of stem cell-associated genes (SALL4, TCL1A, ZFP42, UTF1) in endometriotic lesions and increased expression of the GDF3 differentiation marker in the eutopic endometrium[48]. The aberrant functionality of stem cells expressed by phenotype alteration caused by certain chromosomal aberrations (micro-RNA expression imbalance, altered DNA methylation, histone modification) facilitates the appearance of endometriosis[46,49]. Micro-RNA expression alteration causes endometriosis progression by stimulating the invasiveness and increasing the angiogenic capacity of stem cells[46]. Altering the endometriotic stem cell phenotype contributes to major functional aberrations: imbalance of immunological modulators, uncontrolled cell proliferation, overexpression of proangiogenetic factors, increased migration capacity and cell adhesion[46,49].

Mesenchymal stem cells in the ectopic endometrium have an increased angiogenic, proliferative and migratory potential compared to mesenchymal stem cells in the eutopic endometrium of patients with endometriosis or in healthy women, supported by the presence of elevated levels of IL-1β and COX-2 in ectopic mesenchymal stem cells[46,50,51]. COX-2 inhibitor therapy stimulates apoptosis, reducing the ability of stem cells to migrate and invade from adenomyosis lesions, while eutopic endometrial stem cells do not express the same effects[52]. The phenotype evaluation of mesenchymal stem cells in the eutopic and ectopic endometrium shows an increase in proinflammatory cytokines (IFN-γ, IL-6), VEGF angiogenetic factor and migration factors (MMP-2, MMP-3, MMP-9) associated with reduced expression of antiinflammatory cytokines TGF-β (transforming growth β-factor) in the ectopic endometrium compared to the eutopic one[53]. The analysis of stromal stem cells in the eutopic endometrium of patients with endometriosis, collected from menstrual blood, showed morphological changes, altered expression of immunomodulatory molecules and an increased potential for invasion and proliferation compared to stromal stem cells in the eutopic endometrium[54]. Some researchers affirm that the severity of endometriosis increases proportionally to the angiogenic potential, altered immune response and cell invasion, features which are apparently different depending on the type of stem cells involved in the occurence of endometriosis[46]. They advocate that endometrial stem cells underlie the development of severe endometriotic lesions, while self-limiting lesions originate in mature cells[55].

Conclusions

Given these facts, we believe that the characterization of the biochemical profile of the eutopic and ectopic endometrium is a topic of interest in the understanding of endometriosis, its etiopathogeny and therapeutic approach. The main molecular features of the ectopic endometrium present in endometriosis lesions are characterized by hyperestrogenemia, progesterone resistance, a reduced apoptosis capacity, an increased proliferation index and the presence of abnormal multipotent stem cells. This paper could represent a starting point in the perspective of embracing new directions in the diagnosis and personalized management of patients with endometriosis. ■

Conflict of interests: The authors declare no conflict of interests.

References

1. Giudice LC. Clinical practice: endometriosis. *N Engl J Med*. 2010; 362(25):2389–98.
2. Pabona JM, Simmen FA, Nikiforov MA, Zhuang D, Shankar K, Velarde MC, Zelenko Z, Giudice LC, Simmen RC. Krüppel-like factor 9 and progesterone receptor coregulation of decidualizing endometrial stromal cells: implications for the pathogenesis of endometriosis. *J Clin Endocrinol Metab*. 2012; 97(3):E376–92.
3. Brosens I, Puttemans P, Benagiano G. Endometriosis: A life cycle approach? *Am J Obstet Gynecol*. 2013; 209(4):307-16.
4. Kennedy S, Bergqvist A, Chapron C, D'Hooghe T, Dunselman G, Greb R, Hummelshoj L, Prentice A, Saridogan E, ESHRE Special Interest Group for Endometriosis and Endometrium Guideline Development Group. ESHRE guideline for the diagnosis and treatment of endometriosis. *Hum Reprod*. 2005; 20(10):2698–704.
5. Lessey DA. Assessment of endometrial receptivity. *Fertil Steril*. 2011; 96(3):522–9.
6. Sourial S, Tempest N, Hapangama DK. Theories on the pathogenesis ofendometriosis. *Int J Reprod Med*. 2014; 2014:179515.
7. Agarwal SK, Chapron C, Giudice LC, et al. Clinical diagnosis of endometriosis: a call to action. *Am J Obstet Gynecol*. 2019; 220(4):354.e1-354.e12.
8. Moradi M, et al. Impact of endometriosis on women's lives: a qualitative study. *BMC Womens Health*. 2014; 14:123.
9. Bozdag G. Recurrence of endometriosis: risk factors, mechanisms and biomarkers. *Women's Health (Lond)*. 2015; 11(5):693–9.
10. Guo SW. Recurrence of endometriosis and its control. *Hum Reprod Update*. 2009; 15(4):441–61.
11. Simoens S, Dunselman G, Dirksen C, Hummelshoj L, Bokor A, Brandes I, D'Hooghe T. The burden of endometriosis: costs and quality of life of women with endometriosis and treated in referral centres. *Hum Reprod*. 2012; 27(5):1292–9.
12. Rogers PA, D'Hooghe TM, Fazleabas A, Giudice LC, Montgomery GW, Petraglia F, Taylor RN. Defining future directions for endometriosis research: workshop report from the 2011 World Congress of Endometriosis in Montpellier, France. *Reproductive Sciences*. 2013; 20(5):483–99.
13. Fassbender A, Burney RO, O DF, d'Hooghe T, Giudice L. Update on biomarkers for the detection of endometriosis. *Biomed Res Int*. 2015; 2015:1–14.
14. Burney RO, Giudice L. Pathogenesis and pathophysiology of endometriosis. *Fertil Steril*. 2012; 98(3):511–9.
15. Ahn S, Monsanto S, Miller C, Singh S, Thomas R, Tayade C. Pathophysiology and immune dysfunction in endometriosis. *Biomed Res Int*. 2015; 2015:795976.
16. Marquardt RM, Kim TH, Shin JH, Jeong JW. Progesterone and estrogen signaling in the endometrium: What goes wrong in endometriosis? *Int J Mol Sci*. 2019; 20(15). pii:E3822.
17. Qi QM, Guo SW, Liu XS. Estrogen biosynthesis and its regulation in endometriosis. *Reprod Dev Med*. 2017; 1(1):55–61.
18. Attia GR, Zeitoun K, Edwards D, Johns A, Carr BR, Bulun SE. Progesterone receptor isoform A but not B is expressed in endometriosis. *J Clin Endocrinol Metab*. 2000; 85(8):2897–902.
19. Zeitoun K, Takayama K, Sasano H, Suzuki T, Moghrabi N, Andersson S, et al. Deficient 17beta-hydroxysteroid dehydrogenase type 2 expression in endometriosis: failure to metabolize 17beta-estradiol. *J Clin Endocrinol Metab*. 1998; 83(12):4474–80.
20. Noble LS, Takayama K, Zeitoun KM. et al. Prostaglandin E2 stimulates aromatase expression in endometriosis-derived stromal cells. *J Clin Endocrinol Metab*. 1997; 82(2):600–6.
21. Bulun SE, Cheng YH, Yin P, Imir G, Utsunomiya H, Attar E, et al. Progesterone resistance in endometriosis: link to failure to metabolize estradiol. *Mol Cell Endocrinol*. 2006; 248(1-2):94–103.
22. Petros AM, Olejniczak ET, Fesik SW. Structural biology of the Bcl-2 family of proteins. *Biochim Biophys Acta*. 2004; 1644(2-3):83–94.
23. Lindsay J, Esposti MD, Gilmore AP. Bcl-2 proteins and mitochondria: specificity in membrane targeting for death. *Biochim Biophys Acta*. 2011; 1813(4): 532–9.
24. Nasu K, Nishida M, Kawano Y, Tsuno A, Abe W, Yuge A, et al. Aberrant expression of apoptosis-related molecules in endometriosis: a possible mechanism underlying the pathogenesis of endometriosis. *Reprod Sci*. 2011; 18(3):206–18.
25. Tsujimoto Y. Role of Bcl-2 family proteins in apoptosis: apoptosomes or mitochondria? *Genes Cells*. 1998; 3(11):697–707.
26. Kirkin V, Joos S, Zornig M. The role of Bcl-2 family members in tumorigenesis. *Biochim Biochim Biophys Acta*. 2004; 1644(2-3):229–49.
27. Jones RK, Searle RF, Bulmer JN. Apoptosis and bcl-2 expression in normal human endometrium, endometriosis and adenomyosis. *Hum Reprod*. 1998; 13(12):3496-502.
28. Meresman GF, Vighi S, Buquet RA, Contreras-Ortiz O, Tesone M, Rumi LS. Apoptosis and expression of Bcl-2 and Bax in eutopic endometrium from women with endometriosis. *Fertil Steril*. 2000; 74(4):760-6.
29. Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. *J Cell Physiol*. 2000; 182(3):311-22.
30. Yalcin SE, Ocal I, Yalcin Y, Selim HS, Caltekin MD, Aydogmus H, Kelekci S. Evaluation of the Ki-67 proliferation index and urocortin expression in women with ovarian endometriomas. *Eurasian J Med*. 2017; 49(2):107–12.
31. Alif M, Anwar R, Pribadi A. Ki-67 expression is correlated with cyst size and stage of endometriosis. *Indones J Obstet Gynecol*. 2013; 1(3):124–8.
32. Kahyaoglu I, Kahyaoglu S, Moraloglu O, Zergeroglu S, Sut N, Batioglu S. Comparison of Ki-67 proliferative index between eutopic and ectopic endometrium: a case control study. *Taiwan J Obstet Gynecol*. 2012; 51(3):393-6.
33. Li SF, Nakayama K, Masuzawa H, Fujii S. The number of proliferating cell nuclear antigen positive cells in endometriotic lesions differs from that in the endometrium. Analysis of PCNA positive cells during the menstrual cycle and in post-menopause. *Virchows Arch A Pathol Anat Histopathol*. 1993; 423(4):257-63.
34. Figueira PG, Abrao MS, Krikun G, Taylor HS. Stem cells in endometrium and their role in the pathogenesis of endometriosis. *Ann NY Acad Sci*. 2011; 1221(1):10-7.
35. Bach SP, Renehan AG, Potten CS. Stem cells: the intestinal stem cell as a paradigm. *Carcinogenesis*. 2000; 21(3):469–76.
36. Du X, Yuan Q, Qu Y, Zhou Y, Bei J. Endometrial mesenchymal stem cells isolated from menstrual blood by adherence. *Stem Cells Int*. 2016; 2016:3573846.
37. Trounson A. The production and directed differentiation of human embryonic stem cells. *Endocr Rev*. 2006; 27(2):208–19.
38. Verdi J, Tan A, Shoae-Hassani A, Seifalian AM. Endometrial stem cells in regenerative medicine. *J Biol Eng*. 2014; 8:20.
39. Lin J, Xiang D, Zhang JL, Allickson J, Xiang C. Plasticity of human menstrual blood stem cells derived from the endometrium. *J Zhejiang Univ Sci B*. 2011; 12(5):372–80.
40. Chan RW, Schwab KE, Gargett CE. Clonogenicity of human endometrial epithelial and stromal cells. *Biol Reprod*. 2004; 70(6):1738–50.
41. Meng X, Ichim TE, Zhong J, Rogers A, Yin Z, Jackson J, Wang H, Ge W, Bogin V, Chan KW. Endometrial regenerative cells: a novel stem cell population. *J Transl Med*. 2007; 5(1):57.
42. Schwab KE, Gargett CE. Co-expression of two perivascular cell markers isolates mesenchymal stem-like cells from human endometrium. *Hum Reprod*. 2007; 22(11):2903–11.
43. Wolff EF, Wolff AB, Hongling D, Taylor HS. Demonstration of multipotent stem cells in the adult human endometrium by in vitro chondrogenesis. *Reprod Sci*. 2007; 14(6):524–33.
44. Sasson IE, Taylor HS. Stem cells and the pathogenesis of endometriosis. *Ann N Y Acad Sci*. 2008; 1127:106–15.
45. Ikoma T, Kyo S, Maida Y, Ozaki S, Takakura M, Nakao S, Inoue M. Bone marrow-derived cells from male donors can compose endometrial glands in female transplant recipients. *Am J Obstet Gynecol*. 2009; 201(6):608.e1-8
46. Djokovic D, Calhaz-Jorge C. Somatic stem cells and their dysfunction in endometriosis. *Front Surg*. 2014; 1:51.
47. Sakr S, Naqvi H, Komm B, Taylor HS. Endometriosis impairs bone marrow-derived stem cell recruitment to the uterus whereas bazedoxifene treatment leads to endometriosis regression and improved uterine stem cell engraftment. *Endocrinology*. 2014; 155(4):1489-97.
48. Forte A, Schettino MT, Finicelli M, Cipollaro M, Colacurci N, Cobellis L, Galderisi U. Expression pattern of stemness-related genes in human endometrial and endometriotic tissues. *Mol Med*. 2009; 15(11-12):392-401.
49. Forte A, Cipollaro M, Galderisi U. Genetic, epigenetic and stem cell alterations in endometriosis: new insights and potential therapeutic perspectives. *Clin Sci (Lond)*. 2014; 126(2):123–38.
50. Kao AP, Wang KH, Chang CC, Lee JN, Long CY, Chen HS, et al. Comparative study of human eutopic and ectopic endometrial mesenchymal stem cells and the development of an in vivo endometriotic invasion model. *Fertil Steril*. 2011; 95(4):1308–15.
51. Moggio A, Pittatore G, Cassoni P, Marchino GL, Revelli A, Bussolati B. Sorafenib inhibits growth, migration, and angiogenic potential of ectopic endometrial mesenchymal stem cells derived from patients with endometriosis. *Fertil Steril*. 2012; 98(6):1521–30.
52. Chen YJ, Li HY, Chang YL, Yuan CC, Tai LK, Lu KH, Chang CM, Chiou SH. Suppression of migratory/invasive ability and induction of apoptosis in adenomyosis-derived mesenchymal stem cells by cyclooxygenase-2 inhibitors. *Fertil Steril*. 2010; 94(6):1972–9.
53. Koippallil Gopalakrishnan Nair AR, Pandit H, Warty N, Madan T. Endometriotic mesenchymal stem cells exhibit a distinct immune phenotype. *Int Immunol*. 2015; 27(4):195–204.
54. Nikoo S, Ebtekar M, Jeddi-Tehrani M, Shervin A, Bozorgmehr M, Vafaei S, et al. Menstrual blood-derived stromal stem cells from women with and without endometriosis reveal different phenotypic and functional characteristics. *Mol Hum Reprod*. 2014; 20(9):905–18.
55. Gargett CE, Masuda H. Adult stem cells in the endometrium. *Mol Hum Reprod*. 2010; 16(11):818–34.