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Insights into endometriosis-associated endometrial dysfunctions: a review

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1. ABSTRACT

Endometriosis is defined as the presence of ectopic endometrial-like tissue outside the uterus cavity. This disease, afflicting women during their reproductive age, is mainly associated with pelvic pain and infertility. Sampson's theory which supports the ability of endometrial fragments from retrograde menstruations to slough through fallopian tubes and reach peritoneal environment has been recognized as the most plausible explanation for endometriosis during many years. However, further studies provided evidence that fundamental abnormal changes may occur within the eutopic endometrium of women with endometriosis compared to that of women without endometriosis. These dysfunctions included genetic predisposition, genes aberrantly expressed such as matrix metalloproteinases, Hox genes, integrins, anti-apoptotic genes Bcl-2, but also steroid hormones, immuno-inflammatory factors and angiogenesis. This review aims at summarizing and emphasizing a non exhaustive panel of biochemical and molecular factors abnormally expressed in the eutopic endometrium and related to the pathogenesis of endometriosis.

2. INTRODUCTION

Endometriosis is a common gynecological disorder characterized by the presence of ectopic endometrial-like tissue (glands and stroma epithelial cells) outside the uterus. This disease which is being considered as enigmatic afflicts women almost exclusively during their childbearing period with a prevalence of 10%-15% (1). More often, symptoms associated with endometriosis include severe dysmenorrhea, dyspareunia, dyschezia, and chronic pelvic pain. Recent data estimate that 5%-21% of women with pelvic pain suffer from endometriosis (2) and almost half of women with endometriosis are infertile (3). Despite decades of extensive research, the pathogenesis of endometriosis remains not only elusive (poorly understood) but controversial. Sampson's theory of retrograde menstruation (4) is the most widely accepted theory to explain the peritoneal endometriosis. In fact, this theory suggests that the disease results from ectopic implantation and growth of endometrial fragments which can slough through fallopian tubes, reach the peritoneal cavity where they can attach to the epithelium of the peritoneum, invade into the host tissue and establish a new blood supply.

However, a compelling number of studies have provided evidence that no correlation could be established between the prevalence of the retrograde menstruations and endometriosis because (a) retrograde menstruation, often associated to the reflux of the menstrual fluid, is a regular phenomenon observed in 90% of women with patent fallopian tubes (5) and (b) endometriosis is observed only in 10-15% of women during their reproductive age (1).

Now, tremendous accumulating evidence suggests that the eutopic endometrium of women with endometriosis displays fundamental changes compared to that of women without endometriosis. In women with endometriosis, the fact that eutopic endometrium shares some identical alterations with the ectopic tissue, but not with eutopic endometrium of healthy women, raised the question about the presence of dysfunctions inside the endometrium of endometriosis women. Nowadays, molecular dysfunctions in the eutopic endometrium such as gene aberrations, hormonal, immune, endocrine and environmental factors and direct effects of the peritoneal fluid and its contents should be taken into account in the mechanisms underlying endometriosis pathogenesis. This review attempted to highlight the involvement of gene aberrations, hormonal dysfunctions, immuno-inflammatory changes and tissue remodeling factors found in the eutopic endometrium of women with endometriosis.

3. SUMMARY GENETIC BASIS OF ENDOMETRIOSIS

The genetic predisposition of endometriosis in families was first recognized 26 years ago by Simpson *et al.* (6), who established that the risk for first-degree relatives of women with severe endometriosis was reported to be six times higher than that for relatives of unaffected women. In 2002, the international Endogen Study Group has published a linkage study, using genome-wide scanning of polymorphic microsatellite markers to clearly identify regions of significant sharing in affected siblings (7). Then, by using the linkage analysis and affected sibling pairs, various groups have reported candidate genes that have potential susceptibility. These abnormalities include estrogen and progesterone receptors, androgen receptor, detoxification enzyme cytochrome P450 1A1, p53, N-acetyl transferase 2 and genes often associated with malignant transformation such as tumor suppressor genes (8).

4. HORMONAL DYSFUNCTIONS IN EUTOPIC ENDOMETRIUM OF WOMEN WITH ENDOMETRIOSIS

Periodic changes of the endometrium that occur during the menstrual cycle, i.e. tissue remodeling, endometrial receptivity and embryonic implantation and development during gestation depend on the coordinated action and inter-connection of many regulatory systems and factors such as steroid hormones, cytokines, integrins, growth factors (9-13). Mainly described in the growth, metabolism and development of female reproductive system, the well known steroid hormones estrogens and progesterone (P4) represent the key systemic factors that drive the endometrium through the sequential phases of menstrual cycle and are strongly require to prepare the endometrium for a successfully implantation and gestation phases (12, 13). Estrogen is indispensable for proliferation of the uterine epithelium, whereas progesterone functions have been extensively reported in proliferation, differentiation and maintenance of the endometrium and the uterine stroma. For instance, stromal cells differentiate into decidual cells in response to progesterone during the decidualization phase, characterized by morphological changes and secretion of prolactin (14). Progesterone is also required for maintenance of the pregnancy by stimulating and maintaining uterine functions necessary for early embryonic development, implantation, placentation and fetal development. Hormones such as human chorionic gonadotropin (hCG) secreted by syncytiotrophoblastic cells, maintain progesterone production during early pregnancy (15). Biological effects of estrogen and progesterone require the presence and activation of their cognate receptors.

Thus, several studies performed by molecular analysis have identified isoforms of steroid receptors for estrogen (ERα and ERβ), and progesterone (PR-A and PR-B) in the epithelial, stromal and vascular cells of human endometrium (16). Both estrogen and progesterone receptors expression in endometrial cells varies during the different phases of the menstrual cycle. For instance, it has been shown that ER levels were highly detected during the late proliferative and early secretory phase of menstrual cycle whereas maximum peak level of PR levels was observed later from the early to mid-secretory stage (17). In normal endometrium, ERα and PR-B isoforms have been characterized as being the predominant steroid receptors and abnormal variations in the ERα/ERβ or PR-A/PR-B ratio expression have been observed in tissues or cells of women with endometriosis (18, 19). The loss of PR in endometrial epithelium is critical to the establishment of uterine receptivity and abnormal persistence of PR have been associated with infertility (20). In addition, endometriotic lesions show high oestradiol (E2) biosynthesis compared with endometrium from healthy women (21, 22). The fact to observe high E2 level is adequate since endometriosis is a estrogen-driven disease and current treatments have aimed to decrease endogenous ovarian synthesis and production of E2 (23). The biosynthesis of E2 is triggered by an ovarian enzyme called aromatase, which catalyzes the conversion androstenedione and testosterone to oestrone and E2, respectively. The expression of aromatase was generally found in ovarian granulose cells, placental syncytiotrophoblasts, adipose tissue, skin fibroblasts and brain but a totally absence of its expression was shown in normal endometrium (22). Nevertheless, the same authors have discovered aberrant expression of aromatase in endometriotic lesions and, in lower levels within eutopic endometrium of women with endometriosis. A summary of hormonal factors related to eutopic endometrial dysfunctions are illustrated in the Table 1.

Table 1. Endometrial dysfunctions in eutopic endometrium from women with endometriosis *versus* healthy women

Abnormal factors (endometrium)	Description	References
Steroid hormones and receptors	- ER-α and PR-B isoforms altered expression - Increased oestradiol biosynthesis - Aberrant expression of aromatase P-450	(18, 19) (21, 22) (22)
Proteases and Inhibitors	- Increased expression and secretion (MMP-1,MMP-2, MMP-3, MMP-7, MMP-9, MMP-11). Reduced levels (TIMP-1 and TIMP-2).	(57-59, 61, 67)
COX enzymes and prostaglandins	- Elevated PGs synthesis, overexpression of COX-2 inducible enzyme.	(47, 48)
Cytokines and growth factors	- Increased secretion of MCP-1, IL-1α and IL-1β - Defective IL-1RII expression - Increased levels of VEGF, IL-6, IL-8, HGF, EGF - Marked increase in MIF expression. - Elevated HIF-1α levels in endometriotic cells	(28, 32, 33, 35) (36) (73, 78, 95, 100, 102, 136) (89) (107)
Apoptosis	- Increased expression of Bcl-2 and concomitant with an absence of Bax expression.	(108)
CA-125	- Abnormal expression.	(109, 110)
Oxidative stress	- Aberrant expression of defensive enzymes (SOD, GSH-Peroxidase, Xanthine oxidase). - Increased NO and iNOS levels.	(114-116) (111)
Endometriosis related infertility	- Excessive NO production, increased eNOS expression concomitant with a drastic decrease in αvβ3 integrin expression. - Altered expression of HOXA-10 and HOXA-11 genes. - Aberrant EMX-2 levels mediated by HOXA-10. - Reduced expression of LIF.	(124, 126) (128) (129) (135)

5. IMMUNO-INFLAMMATORY CHANGES IN EUTOPIC ENDOMETRIUM OF WOMEN WITH ENDOMETRIOSIS

One of the most common given definition attributed to endometriosis is the notion of "inflammatory disease", associated with chronic pelvic pain in 5%-21% of women (2). It is now believed that the immune system and immunological changes play a key role in the onset and development of endometriosis. Although many changes reported by several studies have brought a better understanding in the pathogenesis of the disease, the question frequently arisen is to know whether these immunological changes are a cause or an effect of the disease. However, it is admitted that endometrial cells of patients may chronically stimulate the immune system, leading to its deregulation and ultimately to pathophysiological changes in the endometrium.

5.1. Monocyte chemoattractant protein-1 (MCP-1)

MCP-1 is an activating cytokine produced by monocytes, T lymphocytes, fibroblasts, endothelial cells and endometrial cells. In human endometrium, MCP-1 expression follows a cyclical pattern during the menstrual cycle and highest level peaks has been detected peri-menstrually (24). The dysregulation of MCP-1 cytokine in patients with endometriosis was first described in our laboratory in 1995, whose study showed that incubation of endometrial epithelial cells from women with endometriosis with interleukin-1β (IL-1β) or tumor necrosis factor α (TNF-α) resulted in an increased secretion of MCP-1, compared to endometrial cells from normal patients (25). Moreover, elevated concentration of MCP-1 was found in the peritoneal fluid of women with endometriosis and these secreted levels closely varied with the stage of the disease (26, 27). Interestingly, studies from our laboratory also revealed that MCP-1 expression is increased in eutopic endometrium from women with endometriosis *versus* eutopic from healthy women, both at level of protein and mRNA expression (28).

5.2. Interleukin-1 (IL-1)

IL-1, a multifunctional cytokine is believed to play a fundamental role in inflammation, immune response and reproductive activities in normal endometrium, including sequential processes during the menstrual cycle, or during the embryo implantation and development (29). This cytokine is produced by endometrial tissue and is highly secreted with maximum level during the secretory phase of the menstrual cycle (just after ovulation) (30).

The mechanisms that modulate both expression and action of IL-1 cytokine in the endometrium involve all the family members of this cytokine, notably IL-1α, IL-1β, a receptor antagonist (IL-Ra) and two receptors (IL-1RI and IL-1RII). IL-1Ra is a natural inhibitor of IL-1 and competes with IL-1α and IL-1β for binding to IL-1RI, the functional signaling receptor. In constrast, IL-1RII appears to be dispensable for IL-1 signaling and act as a "decoy" receptor (31). Several studies indicate the role played by IL-1 in the pathophysiology of endometriosis (32-35). Works from our laboratory described for the first time the existence of the decoy IL-1RII receptor in endometrial tissue and a drastic lack of its expression in eutopic endometrium from women with endometriosis (36). Defective IL-1RII gene expression observed during the menstrual cycle in women with endometriosis reduced the capability of endometrial tissue to down-regulate IL-1 activity and may, in view of IL-1 immuno-modulatory and growth promoting effects, account for the capability of endometrial cells of women with endometriosis to interact differently with the stimuli present in the peritoneal environment and facilitate their own abnormal growth (37)

5.3. Prostaglandins (PGs)

The PGs are involved in many aspects of physiological processes such as ovulation, pregnancy, but also in inflammatory pathological disease including arthritis, rheumatoid polyarthritis, cancer end endometriosis. There are three isoforms of cyclooxygenases enzymes (COX-1, COX-2 and COX-3) reported to catalyze the steps involved in prostanoid (prostaglandins and thromboxanes) biosynthesis (38, 39). COX-1 is long believed to be a constitutive enzyme involved in performing normal physiological functions, but has now been shown to be up-regulated particularly in

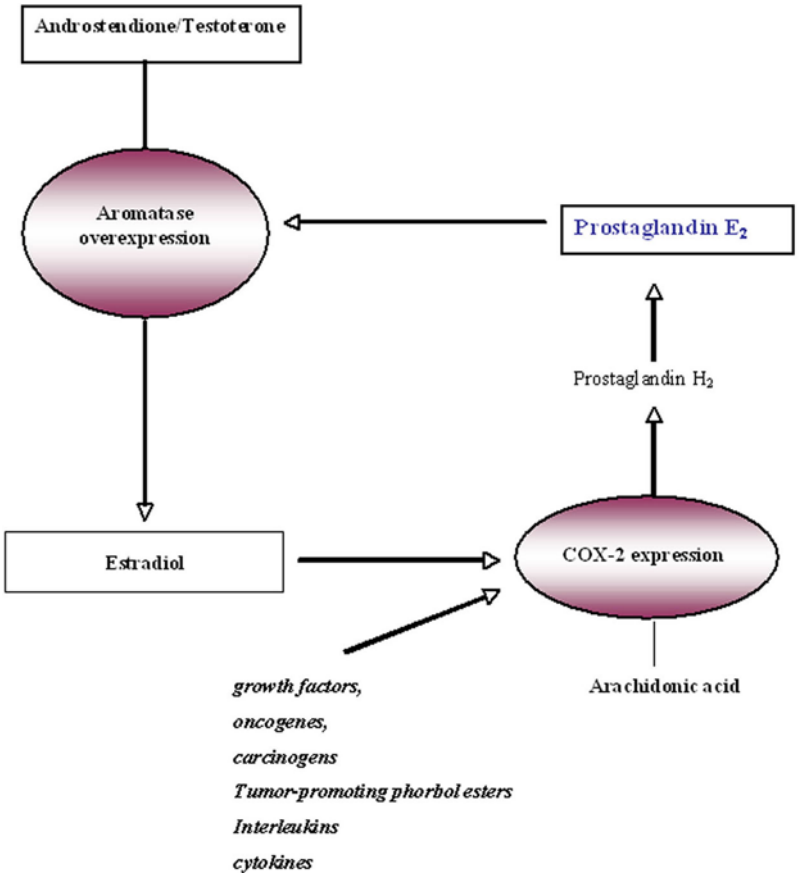

Figure 1. Simplified model of Aromatase – COX-2 interactions showing a positive feedback loop observed for continuous local production of estrogen and PGE_2 production.

various carcinomas and to play an important role in tumorigenesis (40-42). COX-2 is known as enzyme rapidly induced by growth factors, oncogenes, carcinogens and tumor-promoting phorbol esters, with a major involvement by this enzyme in many pathological processes such as rheumatic disease, inflammation and tumorigenesis (39). PGE_2 biosynthesis and secretion are mediated by an inducible cyclooxygenase type-2 (COX-2). The roles played by COX enzymes in reproductive biology have been established using COX-deficient mice. In fact, studies with COX-1-deficient mice have shown a prolonged gestation period and a reduced number of viable offspring. Nevertheless, no alteration in conception and fetal development was observed, suggesting that prostanoids produced by COX-1 are not crucial for ovulation, fertilization or implantation processes but may only be essential for bringing on normal labour at term (43, 44). On the contrary, suppression of COX-2 gene in mice results in many reproductive failures, including ovulation, fertilization, implantation and decidualization, bringing compelling evidence that PGs produced by COX-2 play a fundamental role in these processes (45, 46). Studies related to the COX-2 expression in the eutopic endometrium, endometriotic lesions and adenomyosis have been recently described and revealed an overexpression of this inducible enzyme in endometriotic lesions as well as in the endometrium of patients with endometriosis compared to healthy women (47, 48). PGE_2 was identified as the most potent inducer of aromatase activity in endometriomas and extraovarian endometriotic implants which express high levels of aromatase (49). COX-2 is upregulated in endometrium of patients with endometriosis compared to controls and in endometriotic lesions themselves. This results in higher PGE_2 levels required for aromatase and vascular endothelial growth factor (VEGF) stimulation (48). On the other hand, VEGF upregulates COX-2 expression at both transcriptional and post-transcriptional levels, thereby creating a positive feedback loop, which may contribute to ectopic endometrial tissue growth and endometriosis-associated inflammatory changes (Figure 1). However, the interplay between E2 and COX-2 in endometriosis remains to be further elucidated. Recent study reported that expression of COX-2 protein in endometrial stromal cells was induced by LH, but not by E2 or P4 (50).

6. ABNORMAL GENE EXPRESSION, PROTEIN PRODUCTION FROM EUTOPIC AND ECTOPIC ENDOMETRIUM OF WOMEN WITH ENDOMETRIOSIS

6.1. Matrix metalloproteinases (MMPs)

MMPs and their inhibitors, tissue inhibitors of the matrix metalloproteinase (TIMPs) are expressed during

menstruation and play a fundamental role in the breakdown of the extracellular matrix (ECM) and tissue repair (51-54). In physiological conditions, the MMPs expression is highly regulated by steroid hormones, growth factors (53) and cytokines such as IL-1, IL-6, TNF-α (55, 56). Using *in situ* hybridization, Rodgers *et al.* (53) identified a cell-specific and menstrual cycle-dependent expression for various members of the MMPs family in the cycling human endometrium. The MMPs and their inhibitors TIMPs are strongly required for the ECM degradation to facilitate the endometrial cells invasion and attachment into the peritoneal mesothelium (51). Several studies have shown an aberrant increase in the mRNA expression of several MMPs in endometriotic and endometrial tissues, notably MMP-1 (57), MMP-2, MMP-3 and MMP-7 and MMP-11 (58-60). Reduced levels of the MMP inhibitors, TIMP-1 and TIMP-2 (61) were observed in eutopic and ectopic endometrial tissues of women with endometriosis. Taken together, these data point to the invasive nature of endometriotic implants.

A considerable number of studies pointed out the ability of steroid hormones (E2, P4) to regulate endometrial MMPs and theirs inhibitors. This reflects the key role of these steroids in endometriosis development. Using nude mice as model for endometriosis, Bruner and al. (62) demonstrated that estrogen treatment of human endometrial tissue favored MMPs expression and promoted the establishment of ectopic lesions in mice. In contrast, *in vitro* blockade of MMPs expression and activity (MMP-3 and MMP-7) by either progesterone treatment of human tissue prior to injection in mice or natural inhibitor of MMP (TIMP-1) resulted in a marked inhibition of endometriosis development. The same authors also showed that *in vitro* treatments of these endometrial tissues with E2, P4, retinoic acid and transforming growth factor-β (TGF-β) prior to mice injection reduced MMPs expression and limited the development of endometriotic lesions *in vivo* (63). Further investigations of the molecular interactions between MMPs and endocrine factors indicated that suppression of both MMP-3 and MMP-7 expressions in endometrial cell culture treated with E2 and P4 was prevented by the addition of a pan-specific antibody raised against TGF-β or an endogenous inhibitor of TGF-β (EBAF, endometrial bleeding-associated factor) (59, 64). Multiple proinflammatory cytokines produced by endometrial epithelial cells have been thoroughly examined with respect to the contribution to MMPs in endometriosis-related eutopic endometrium dysfunctions, notably IL-1, IL-6, IL-8 and TNF-α. *In vitro* studies showed that TNF-α stimulates MMPs expression in endometrial tissue and suppresses endogenous TIMPs (61, 65). *In vivo* investigations also showed that the inhibition of TNF-α action, using a TNF-binding protein (r-hTBP-1), resulted in a considerable reduction of endometriosis (66). Taken together, these studies support a role for MMPs in the establishment of ectopic endometriotic lesions as well as in the development, the progression and the severity of the disease.

More recently, studies from our laboratory showed an imbalance between MMP-9 expression and that of its inhibitor TIMP-1 in the eutopic endometrium of women with endometriosis. These results may reflect the inherent capacity of endometrial tissue to breakdown the ECM and therefore enable ectopic implantation and growth (67).

6.2. Angiogenesis

Angiogenesis is defined as the growth of new capillaries from preexisting vessels. This term usually refers to important physiological events involved in embryogenesis and wound healing. Angiogenesis is also associated to pathological processes such as atherosclerosis, chronic inflammation, tumors malignancies and endometriosis (68, 69). Chesney *et al.* (70) have shown that angiogenesis is triggered by a variety of mediators and chemokines such as IL-8, macrophage migration inhibitory factor (MIF) and VEGF in tumor neovascularization. In human endometrium, angiogenesis occurs periodically, notably as part of the cyclical growth and shedding during the menstrual cycle (71).

VEGF is well known for being the most potent angiogenic factor. VEGF or VEGF-A belongs to a family of dimeric glycoproteins such as VEGF-B, -C, -D, -E and placenta growth factor (PlGF). Among the most important regulators of VEGF gene expression are cytokines, sex steroids, growth factors and hypoxia (72-74). The biological activity of VEGF depends mainly on its binding to different receptors VEGFR-1 (Flt-1), VEGFR-2 (Flk-1, KDR) and neuropillin-1 and -2. Alternative splicing of a single gene generates six isoforms of VEGF-A: $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{183}$, $VEGF_{189}$ and $VEGF_{206}$. Most isoforms are cell-associated or bound to extracellular matrix components, except for $VEGF_{121}$ and $VEGF_{165}$ which are freely secreted in the extracellular environment (75, 76). Dvorak (72) identified $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$ as the prominent isoforms in humans.

In human endometrium, VEGF protein expression was found to be higher but not significant during the early proliferative phase compared with the other phases (71). Shifren *et al.* (73) showed increased *VEGF* mRNA levels during the mid-proliferative, late proliferative and secretory endometrium (73). Recent *in vivo* studies, assuming that angiogenesis is of pivotal importance in the pathogenesis of endometriosis, have demonstrated that angiogenic inhibitors agents such as anti-hVEGF, TNP-470, endostatin and anginex were effective inhibitors of established endometriotic lesions in nude mice (77). In human, Donnez *et al.* (78) reported that eutopic glandular epithelium of women with endometriosis *versus* endometrium from healthy women, exhibited significantly increased VEGF levels, particularly during the late secretory phase of the menstrual cycle. In addition, high secretion of VEGF by endometriotic lesions, activated macrophages and neutrophils and increased levels of VEGF have been found in the peritoneal fluid of endometriosis patients compared to controls (73, 78-80).

Extensive studies regarding the regulation of VEGF and its receptors expression indicated that eutopic endometrium from women with endometriosis had

significantly higher expression of VEGF-A in glandular epithelium and VEGFR-2 in endometrial blood vessels than that from women without endometriosis (81). The authors also observed that endometriotic lesions with high proliferative activity exhibited higher vascular expression of VEGFR-2, accompanied by higher levels of VEGF-A in peritoneal fluid and serum. Using chorioallantoic chicken membrane as *in vitro* experimental model of endometriosis, Kressin *et al.* (82) measured the expression of different VEGF mRNA isoforms from human endometrial fragments and showed that although $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$ and $VEGF_{189}$ mRNA were detected at all time point, only $VEGF_{121}$ and $VEGF_{165}$ PCR products were always the most abundant.

Several studies have also provided evidence of potent angiogenic characteristics for macrophage migration inhibitory factor (MIF), mainly in tumorigenesis (83). In fact, MIF is required for tumor-initiated endothelial cell proliferation and tumor neo-vascularisation and anti-MIF antibody has been shown to significantly inhibit tumor growth and tumor-associated angiogenesis (70).

The important role of the pleiotropic cytokine MIF has been proved in a wide range of reproductive processes, such as ovulation and pregnancy and in inflammatory diseases (84, 85). MIF was originally described as a cytokine secreted by T lymphocytes that inhibits the random migration of macrophages *in vitro*. MIF also plays a pivotal role in stimulating the secretion of other cytokines such as TNF-α and IL-1β (86). MIF is present in a variety of tissues, including endometrium (87) and shows a cycle-phase-dependant expression during the menstrual cycle (87, 88). Our recent data showed an abnormal MIF expression in the peritoneal fluid, the peripheral blood and the endometrial tissue of women with endometriosis, particularly in those who were infertile (89-91). This suggests the key role of this cytokine in triggering off endometriosis pathogenesis.

For example, IL-1β produced by activated macrophages in patients with endometriosis has been shown to induce an increased expression of VEGF and IL-6 *in vitro* (92). More interestingly, Rossi *et al.* (93) have demonstrated that stimulation of endometrial stromal cells with IL-1β resulted in an upregulation of IL-8 gene.

IL-6 is known as a multifunctional cytokine produced by immune cells and endometrial epithelial and stromal cells (94, 95). Using a mouse model of endometriosis, Lin *et al.* (96) pointed out the angiogenic properties displayed by IL-6, showing that IL-6 and TNF-α secretion by peritoneal macrophages and neutrophils resulted in an upregulation of VEGF. IL-6 was highly expressed in endometriotic cysts ad eutopic endometrium of patients with endometriosis compared to healthy control (95).

IL-8 was initially described as a chemokine that induces a strong chemotaxis of lymphocytes and neutrophils, but lately has demonstrated its ability to stimulate angiogenesis (97). It has been shown that IL-8 significantly stimulates cell proliferation in endometrial and endometriotic stromal cells (98, 99). In addition, Gavzani *et al.* (100) observed that elevated IL-8 concentrations found in the peritoneal fluid of women with endometriosis were correlated with the severity of the disease. In endometriotic tissue, it has been demonstrated that invasion of neutrophils was due to high secretion of IL-8 and incubation of peritoneal fluid from patients with endometriosis (EPF) with neutrophils increases VEGF secretion (80). The same authors also reported that incubation of EPF-treated neutrophils with IL-8 and TNF-α stimulates VEGF secretion, but neutralizing antibodies against IL-8 and TNF-α only partially abrogate VEGF secretion. More recently, Ulukus *et al.* (101) investigated IL-8 receptors (IL-8R)-A and -B expression in eutopic and ectopic endometrium of women with endometriosis. The authors found higher levels of IL-8R-A in ectopic endometrium compared to their eutopic autologous and normal endometrium, whereas similar levels of IL-8R-B in both eutopic and ectopic tissue from women with endometriosis were found to be more elevated than in normal endometrium.

Epidermal growth factor (EGF), a 6-kd protein with homology to TGF-α, betacellulin proteins involved in embryonic development, has also an angiogenic activity. It has been shown that EGF and its receptor HER-1 are expressed in ectopic endometrium (102). EGF showed highly variable expression, with no difference found in the peritoneal fluid of women with or without endometriosis (103).

Hypoxia is a common pathophysiological feature that regulates gene expression and remains one of the most potent stimulus for new blood vessels formation and VEGF expression (104). Hypoxia is generally considered as the result of a physiological stress response that leads to stimulation of angiogenesis in endometrial tissue during the premenstrual period. The hypoxic effects are in large part mediated by a protein hypoxia-inducible factor-1 (HIF-1) protein, a heterodimeric transcriptional complex composed of HIF-1α and HIF-1β subunits (105). The HIF-1β is constitutively expressed whereas HIF-1α is specifically hypoxia-regulated. In fact, under hypoxic conditions, stabilization and accumulation of HIF-1α in cells allows its nuclear translocation where it binds HIF-1β/ARNT (aryl hydrocarbon nuclear receptor translocator) (105, 106). The HIF-1α/HIF-1β/ARNT complex targets gene transcription by binding to hypoxia responsive elements on various genes including VEGF. Studying the regulation of VEGF in human endometrium, Sharkey *et al.* (74) demonstrated that hypoxia results in a significantly increased VEGF secretion and mRNA levels in both stromal and epithelial cells isolated from human endometrium. In addition, the authors also found that increase in VEGF secretion in response to hypoxia was not disrupted by treatment with E2 or P4. A recent study by Wu *et al.* (107) examined the expression of HIF-1α in endometriotic tissue and provided evidence that aberrant expression of leptin in endometriotic cells was induced by elevated HIF-1α levels.

6.3. Apoptosis

Endometrial cells of women with endometriosis appeared to resist apoptosis and to survive in ectopic locations. The B-cell lymphoma/leukemia-2 well known as Bcl-2, is a proto-oncogene whose main function is to prevent apoptosis or "programmed cell death". The action of Bcl-2 depends on the expression and concentration of an antagonist pro-apoptotic protein Bax and the Bcl-2/Bax ratio expression determine the cell fate to survival or death. Study of their expression in endometrial cells showed an increased Bcl-2 expression concomitantly to an absence of Bax expression in the late proliferative phase of eutopic endometrium from women having endometriosis (108).

Taken together, these findings make therefore plausible the capability of endometrial cells to resist apoptosis, invade the host tissue, stimulate angiogenesis and favor their ectopic growth.

6.4. CA-125

CA-125 is a high molecular weight cell surface glycoprotein expressed in tissues derived from embryonic coelomic epithelium: endometrium, endocervix, fallopian tubes, peritoneum, pleura and pericardium. This factor is also expressed in many epithelial cancers and abnormal elevated CA-125 serum levels have been primarily associated with ovarian carcinoma and further with gynecologic and nongynecologic malignancies. In women with advanced endometriosis, it has been shown that the eutopic endometrium expressed two to four times more CA-125 in both early and late secretory phases of the menstrual cycle (109). In addition, a positive correlation has been found between serum and peritoneal fluid values of CA-125, particularly in women with advanced endometriosis (110).

7. ROLE OF OXIDATIVE STRESS IN THE EUTOPIC ENDOMETRIUM FROM WOMEN WITH ENDOMETRIOSIS

Inflammation in endometriosis is associated with the generation of free radicals. Elevated expression of inducible nitrogen oxide synthase (iNOS) and nitrogen oxide (NO) production were found in eutopic endometrial tissue and peritoneal fluid from women with endometriosis (111, 112). Findings from Omland *et al.* showed a significant increase in endothelial nitrogen oxide synthase (eNOS) expression in the glandular and luminal endometrium of patients with endometriosis (113). Antioxidant agents whose main role is to counteract oxidative damage showed altered expression patterns. In fact, Ota *et al.* (114) demonstrated an aberrant expression of defensive enzymes such as manganese, and copper/zinc superoxide dismutase (SOD) in the endometrium of women with endometriosis and adenomyosis throughout the menstrual cycle. Aberrations were also found for glutathion peroxidase (GPx) and xanthine oxidase (XO) expression in both eutopic and ectopic endometrium, supporting the evidence that altered expression of these antioxidant enzymes may widely contribute to the persistency of oxidative stress in endometriosis (115, 116).

There are many studies which have reported inconsistent results regarding the association between oxidative stress and endometriosis. While patients without the disease showed cyclic variations in both enzymes, significantly lower levels of SOD, GPx and higher levels of lipid peroxides and the subsequent release of aldehydes such as malondialdehyde were found in peritoneal fluid of women with endometriosis compared to fertile control (117, 118). However, Ho *et al.* (119) and Wang *et al.* (120) found no association between oxidative stress markers (ROS and total antioxidant agents) measured in peritoneal fluid from women with endometriosis when compared to tubal ligation controls.

8. GENE DYSFUNCTIONS IN THE EUTOPIC ENDOMETRIUM OF WOMEN WITH ENDOMETRIOSIS-RELATED INFERTILITY

There is growing evidence supporting abnormal eutopic endometrium and implantation failure in some women with endometriosis as an underlying cause of infertility in this population. In addition, evidence is accumulating of aberrant gene and gene-product expression in eutopic and ectopic endometrium that may be related to infertility or to the establishment of the disease. In many infertility-associated gynecological disorders, the failure of embryonic implantation into the endometrium has been considered as a limiting factor to the establishment of successful pregnancy (121). Embryonic implantation normally occurs during a defined period known as "window of implantation", which is spatially and temporally regulated by various factors such as integrins (122).

8.1. Integrins and NOS/NO

Integrins are known to play a crucial role during endometrial receptivity. Lessey *et al.* (123) reported that some integrins such as $\alpha v\beta 3$ and $\alpha 4\beta 1$ are the key molecules to frame the putative window of implantation. However, these authors have also found a lack of $\beta 3$ integrin subunit expression in the endometrium of women with endometriosis, during the period of implantation (124).

The relation between increased oxidative stress in endometriosis and reduced fertility has been extensively documented (125). In normal conditions, NO regulates endometrial stromal edema generation and uterine contractions. Excessive production of NO in endometriosis results in an altered regulation of NOS within endometrium that might favor a reduction in implantation rates success and therefore affect fecundity and pregnancy (112). It has also been proved that the endothelial NOS (eNOS) has a similar pattern of expression as $\alpha v\beta 3$ integrin throughout the menstrual cycle, both displaying a predominant location in endometrial glandular epithelium (126). These authors have provided evidence that in women with endometriosis, a prominent increase in eNOS during the mid-luteal phase was concomitant with a drastic decrease in the adhesion molecule $\alpha v\beta 3$. Such an imbalance may strongly contribute to implantation defects often occurred in endometriosis.

8.2. HOX genes

The transcriptional factors *HOX* genes play an essential role during the embryonic development. The two defined *HOXA10* and *HOXA11* are expressed in the adult uterus in human and mouse and have been reported to serve as key regulatory factors during embryonic implantation (127). However, a down-regulation of these genes expression was observed in the eutopic endometrium of women with endometriosis in comparison with endometrium of healthy women (128).

8.3. EMX2

The transcriptional factor EMX2 is necessary for the reproductive tract development and is negatively regulated by *HOXA10* gene. Recent data indicate that *EMX2* mRNA is aberrantly expressed in the eutopic endometrium from women with endometriosis during the peri-implantation phase, whereas a significant decrease of mRNA levels was observed in the endometrium of women without endometriosis (129).

8.4. Leukemia inhibitory factor (LIF)

LIF cytokine has been shown to be important in embryonic attachment to the epithelium and also for endometrial decidualization (130). In humans, LIF is expressed in endometrium and decidua (131, 132). Study of *LIF* mRNA level within normal endometrium showed a higher expression in glandular epithelium and during the secretory phase. In addition, *in vivo* studies demonstrated the importance of LIF cytokine and another cytokine IL-11 in the earliest stages of implantation since no functional *LIF* gene or mutation of IL-11Rα gene resulted in blastocyst implantation defect and infertility observed in female mice (133, 134). Recent studies suggest that reduced endometrial LIF may contribute to infertility in some endometriosis women (135).

9. CONCLUSION

Endometriosis remains an enigmatic gynecological disorder. There are fundamental differences between eutopic and ectopic endometrium of women with endometriosis and this is supported by the presence of hormonal dysfunctions, genes aberrantly expressed, immuno-inflammatory changes, abnormal growth, remodeling and angiogenesis. There are also major changes in the eutopic endometrium of women with endometriosis compared to normal women. It is now believed that all these dysfunctions play a central role in the multi-factorial molecular events underlying the pathogenesis of endometriosis and the appearance of clinical symptoms. Nevertheless, further investigations remain essential to improve endometriosis diagnosis and establish well-efficient clinical therapeutic approaches.

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