Minireview

Prostaglandin E_2: the master of endometriosis?

Meng-Hsing Wu[1], Chun-Wun Lu[2], Pei-Chin Chuang[2] and Shaw-Jenq Tsai[2]

[1]Departments of Obstetrics and Gynecology; [2]Department of Physiology, College of Medicine, National Cheng Kung University, Tainan 701, Taiwan, Republic of China

Corresponding author: Shaw-Jenq Tsai, Department of Physiology, College of Medicine, National Cheng Kung University, 1 University Road, Tainan 701, Taiwan, Republic of China. Email: seantsai@mail.ncku.edu.tw

Abstract

Endometriosis is the primary cause of infertility in women, with a prevalence rate ranging from 5% to 10%. Women with endometriosis suffer from symptoms such as chronic pelvic pain, dysmenorrhea and dyspareunia, which significantly reduce the quality of life. Endometriosis is a polygenic disease with a complex, multifactorial etiology. The mechanism responsible for the initiation and development of this disease remains largely unknown. Prostaglandin E_2 (PGE_2), a versatile eicosanoid that exerts numerous physiological and pathological functions, has been implicated to play critical roles in the development of endometriosis. A growing body of evidence demonstrates that PGE_2 regulates many pathophysiological processes including cell proliferation, antiapoptosis, immune suppression and angiogenesis during the development of endometriosis. This review focuses on recent advances in cellular and molecular mechanisms triggered by PGE_2 that contribute to the pathological processes of endometriosis.

Keywords: angiogenesis, endometriosis, macrophage, phagocytosis, proliferation, prostaglandin, steroidogenesis, stromal cells

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Introduction

Endometriosis is one of the most common gynecological diseases in women of reproductive age. It is defined as the presence of endometria-like tissues outside of the uterine cavity commonly located in the gravitationally dependent area of the pelvis, including ovaries, peritoneum, bowel, cul-de-sac, uterosacral ligments and fallopian tubes.[1] Endometriosis is diagnosed in about 5–10% of women in the general population and in 20% of women who underwent laparoscopy for pelvic pain or infertility.[2] The prevalence rate of laparoscopically biopsy-proven endometriosis in patients with chronic pelvic pain can be as high as 28%.[3]

The most common symptoms for women who have endometriosis are pelvic pain and infertility, which adversely affect the quality of life. However, it is often difficult to evaluate the extent of endometriosis by physical examination and clinical history review. Dysmenorrhea is associated with cyclic recurrent micro-bleeding within various entities of ectopic endometriotic implants and the consequent inflammation.[4] In addition, endometriosis-related adhesions and compression or infiltration of nerves in the subperitoneal pelvic space by ectopic lesions also cause painful symptoms.[5] Infertility is another common outcome of women with endometriosis. The pregnancy rate in women with endometriosis is about half of that of women with tubal factor infertility.[6] Furthermore, studies on assisted reproduction treatment have shown that women with endometriosis have higher rates of pregnancy loss, complication of preterm delivery, pre-eclampsia and infants small for gestational age.[7] However, the mechanisms of these adverse outcomes remain unknown.

Current medical therapies for endometriosis, including the use of combined oral contraceptives, danazol, GnRH analogues, progestins and non-steroidal anti-inflammatory drugs (NSAIDs), primarily target suppressing the levels of prostaglandins (PGs) and thus reduce endometriosis-associated pain.[8] In the treatment for endometriosis-associated infertility, either operative laparoscopy or assisted reproductive techniques is recommended. If conservative treatment fails, laparoscopy is suggested. Laparoscopy is considered as a convenient and gold standard procedure in the diagnosis and treatment of endometriosis. However, the recurrence rate of surgical ablation is high. The short-term (2 y) recurrence rate is 5.7% for early stages of endometriosis and 14.4% for advanced stages[9] while the long-term (5 y) recurrence rate can be as high as 50%.[10,11] No effective therapeutic regimen has been

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developed thus far to cure or prevent the recurrence of endometriosis.

Etiology

The etiology of endometriosis is still poorly defined. Many hypotheses, including retrograde menstruation theory, embryonic rest theory, coelomic metaplasia theory and new composite theory, have been proposed to explain the development of endometriosis.[8,12–15] In addition, environmental[16,17] and genetic[18–20] factors have been proposed as contributing to the etiology of endometriosis. Nevertheless, no single theory can adequately explain the complex mechanisms of endometriosis.

Among the theories proposed to explain the pathogenesis of endometriosis, Sampson's transplantation and implantation hypothesis is by far the most widely accepted.[12] This hypothesis proposes that endometrial tissue fragments are spread by retrograde menstruation through the fallopian tubes into the peritoneal cavity. The pattern of endometriosis supports the theory of retrograde menstruation.[1] However, this theory seems to over-simplify the disease process. For example, the retrograded endometrial tissues must evolve a way to avoid being eliminated by the body's defense system and to survive and/or proliferate in the hostile microenvironment of the peritoneal cavity. How retrograded endometrial tissues develop such sophisticated systems remains an open question. Nevertheless, recent progress in investigating the molecular mechanisms of pathological processes of endometriosis implicated that prostaglandin E_2 (PGE_2) may play critical roles in the pro-survival and immune-privilege effects. It has been known for a long time that the concentration of PGE_2 in the peritoneal fluid of women with endometriosis is much greater than that in the peritoneal fluid of women without endometriosis.[21,22] Therefore, it would not be surprising if PGE_2 indeed plays such functional roles in the etiology of endometriosis. The following sections summarize recent advances in the cellular and molecular mechanisms triggered by PGE_2 that are implicated to contribute to the pathogenesis of endometriosis. For other aspects of endometriosis, readers should refer to some recently published reviews.[11,23–29]

Sources of PGE_2

PGs are unstable eicosanoids with a very short half-life.[30] It is generally believed that PGs in the peritoneal fluid are produced locally from peritoneal macrophages and ectopic endometriotic tissues.[22,31–33] The rate-limiting step in the biosynthesis of PGs, including PGE_2, is regulated by cyclooxygenase (COX), which catalyzes the conversion of arachidonic acid to PGH_2. PGH_2 is further converted to PGE_2 and $PGF_{2\alpha}$ by PGE synthase and PGF synthase, respectively. Two isoforms of COX exist: the constitutively expressed COX-1 and the inducible COX-2. COX-2 is normally undetectable in most tissues under physiological conditions but is rapidly induced by cytokines, endotoxins, proinflammatory agents, tumor promoters and certain hormones.[34,35] COX-2 is over-expressed in peritoneal macrophages derived from women with endometriosis while COX-1 is only over-expressed in severe stage endometriosis.[22] The expression of COX (especially COX-2) in peritoneal macrophage is highly associated with both PGE_2 concentrations in the peritoneal fluid and the severity of endometriosis.[22] In contrast, peripheral blood-derived monocytic cells (precursor of macrophages) express similarly low levels of COX-2 irrespective of their origins, suggesting that the over-expression of COX-2 in peritoneal macrophages is controlled by local factors present in the peritoneal fluid. Several proinflammatory cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-α) and PGE_2, have been found to induce COX-2 expression in peritoneal macrophages derived from endometriosis-free women, suggesting that they are the local factors that cause COX-2 over-expression in women with endometriosis.[22]

Besides peritoneal macrophages, an elevated expression of COX-2 in ectopic endometriotic lesions has been detected by several groups.[31–33] The over-expression of COX-2 in ectopic tissues is mirrored by an increased production of PGE_2 in primary cultured stromal cells derived from ectopic endometriotic lesions. This phenomenon was indisputably observed in unpaired normal and endometriotic samples as well as in paired eutopic and ectopic samples collected from the same individual.[33] Many factors including IL-1β, vascular endothelial growth factor (VEGF), 17β-estradiol and even PGE_2 have been shown to stimulate COX-2 expression in endometrial stromal cells.[33,36,37] More intriguingly, the *cox*-2 gene in ectopic endometriotic stromal cells is much more sensitive to stimuli compared with its eutopic counterpart.[33] Considering the homogeneity of the genetic background of paired eutopic and ectopic endometrial stromal cells, distinct responses of the COX-2 promoter to IL-1β imply that epigenetic regulation of gene expression and/or post-translational modification of chromatin leading to distinct promoter activity of a gene could be the underlying mechanism.

The half-lives of COX-2 mRNA and protein are very short and the transcription of the *cox*-2 gene is tightly controlled. However, continuous over-expression of COX-2 has been observed in both peritoneal macrophages and ectopic endometriotic stromal cells derived from patients with endometriosis.[31–33] This is due to the existence of positive feedback loops between COX-2–PGE_2–estrogen in ectopic endometriotic stromal cells[38] and COX-2–PGE_2–pro-inflammatory cytokines such as IL-1β and TNF-α in peritoneal macrophages.[22] As a result, the concentration of PGE_2 in the peritoneal fluid is constantly elevated in patients with endometriosis, which leads to more severe pathological processes. Figure 1 summarizes the sources and levels of PGE_2 in normal women and in those with endometriosis.

Stimulation of steroidogenic capacity

Endometriosis is a highly estrogen-dependent disease. How do endometriotic cells survive during the period of menstruation when ovarian estrogen is not available? This question was

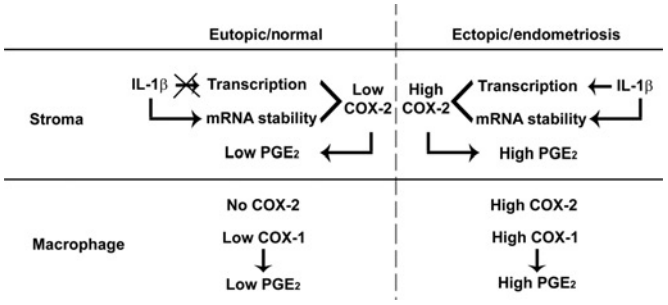

Figure 1 Sources and concentrations of PGE_2 in the peritoneal fluid of women with or without endometriosis. In normal women, the basal level of COX-2 is undetectable in endometrial stromal cells. Even with the stimulation of proinflammatory cytokines (such as IL-1β), the COX-2 level is low due to a lack of transcription. Similarly, COX-2 is not expressed in peritoneal macrophages while COX-1 is expressed at a low level. Therefore, the concentration of PGE_2 in peritoneal fluid is low. In contrast, COX-2 is over-expressed in both stromal cells and peritoneal macrophages of women with endometriosis. In addition, the COX-1 level is elevated in peritoneal macrophages. In the presence of inflammatory cytokines (e.g. IL-1β), COX-2 is dramatically induced due to increases in gene transcription and mRNA stability. As a result, the concentration of PGE_2 in the peritoneal fluid of women with endometriosis is very high. PGE_2, prostaglandin E_2; COX-2, cyclooxygenase-2; IL-1β, interleukin-1β

answered by the discovery that endometriotic stromal cells are capable of producing estrogen aberrantly.[39–42] It has been shown that concentrations of progesterone and 17β-estradiol are greater in the peritoneal fluid, but not in the peripheral blood of women with endometriosis.[42,43] The expression of all proteins/enzymes required for *de novo* synthesis of estrogen, i.e., steroidogenic acute regulatory protein (StAR), P450 side-chain cleavage enzyme (P450scc), 3β-hydroxysteroid dehydrogenase (3β-HSD) type 2, 17α-hydroxylase 17,20 lyase (P450c17), P450 aromatase and 17β-hydroxysteroid dehydrogenase (17β-HSD) type 1, has been detected in ectopic but not in eutopic endometrial stroma.[42,43] Although the existence of aromatase in endometriotic tissues has been recently questioned,[44] articles from several independent groups reported the detection of aromatase activity in endometriotic stromal cells.[45–47]

De novo biosynthesis of estrogen is controlled at two committed steps by StAR and aromatase, respectively. StAR transports cholesterol across the mitochondrial membrane to the inner mitochondrial leaflet, where the first enzymatic reaction occurs.[48] Aromatase catalyzes the conversion of androstenedione to estrone, which is further converted to estradiol by 17β-HSD type 1. Laboratory evidence shows that both StAR and aromatase are upregulated by PGE_2 in primary cultured endometriotic stromal cells.[41,42,49,50] PGE_2 binds to G protein-coupled plasma membrane receptors. Four distinct PGE_2 receptors (EP1–4), encoded by different genes, have been identified in human tissues.[51] In human endometrial and endometriotic stroma, expressions of EP2, EP3 and EP4 have been confirmed while the mRNA of EP1 is undetectable.[49] PGE_2-induced StAR expression is mediated via binding to the EP2 receptor, which then activates adenylyl cyclase and the protein kinase A (PKA) signaling pathway via Gs activation. Interestingly, the induction of StAR by PGE_2 is restricted to ectopic endometriotic stromal cells; it does not occur for eutopic endometrial stromal cells or epithelial cells.[42] In ectopic endometriotic stromal cells, treatment with PGE_2 or EP2 receptor agonist activates the PKA signaling pathway leading to the phosphorylation of the cAMP response element binding protein (CREB) in the nucleus (Figure 2). Because there is no consensus cAMP response element (CRE) in human StAR promoter,[52] CREB binds to the CCAAT/enhancer binding protein (C/EBP) response element instead of its cognate responsive element, CRE.[50] Upon phosphorylation by PKA, phosphorylated CREB recruits CREB binding protein (CBP), which then causes histone acetylation.[49] The acetylation of histone by CBP results in its dissociation from DNA, which provides space for C/EBP binding. The binding affinity of C/EBPβ to its cognate binding site is greater than that of CREB.[50] Thus, C/EBPβ replaces CREB in the StAR promoter and initiates the formation of transcription complex (Figure 2). The timing of increased C/EBPβ binding to the StAR promoter (at 30 and 60 min after PGE_2 treatment) nicely correlates with nascent StAR RNA synthesis and reflects the increased transcription activity of StAR promoter.[49]

The regulation of aromatase expression by PGE_2 in ectopic endometriotic stromal cells is also mediated via EP2/EP4 receptor-coupled signaling pathways.[53,54] Similar to what is seen in StAR regulation, the expression of aromatase in response to PGE_2 stimulation occurs only in ectopic endometriotic stromal cells. Aberrant expression of steroidogenic factor-1 (SF-1) in ectopic endometriotic stromal cells contributes to the transition of this cell from PGE_2-insensitive to PGE_2-sensitive status.[53] In ectopic endometriotic stromal cells, SF-1 competes against the inhibiting factor chicken ovalbumin upstream promoter transcription factor (COUP-TF) for the same DNA binding site of the aromatase promoter II upon PGE_2 treatment.[53] In contrast, in the eutopic endometrium, only COUP-TF binds to the aromatase promoter due to the lack of SF-1 expression. Besides COUP-TF, the other SF-1 corepressor, Wilms' tumor-1, has been reported to bind to aromatase promoter in eutopic endometrial but not in ectopic endometriotic stromal cells.[43] This redundant mechanism is likely a safe-guarding system that silences the aromatase promoter in normal endometrial stromal cells.

Besides StAR and aromatase, a recent study reported that mRNA encodings for other steroidogenic enzymes, including P450scc, 3β-HSD type 2 and P450c17, are also upregulated by PGE_2 in ectopic endometriotic stromal cells.[43] Taken together, these data indicate that PGE_2 alone, via activation of the EP2/EP4 receptor, is sufficient to induce *de novo* synthesis of estrogen from cholesterol, an unique feature only observed in ectopic endometriotic stromal cells. The synthesized estrogen can act via autocrine/paracrine pathways to affect stromal and epithelial cells of endometriotic lesions. By doing so, the ectopic endometriotic tissue can avoid apoptosis and/or maintain proliferation even during menstruation and the early follicular phase when the ovarian estrogen supply is not possible or limited.

Induction of cell proliferation

It is well known that the proliferation of endometriotic cells is estrogen-dependent; however, estrogen *per se* is not a

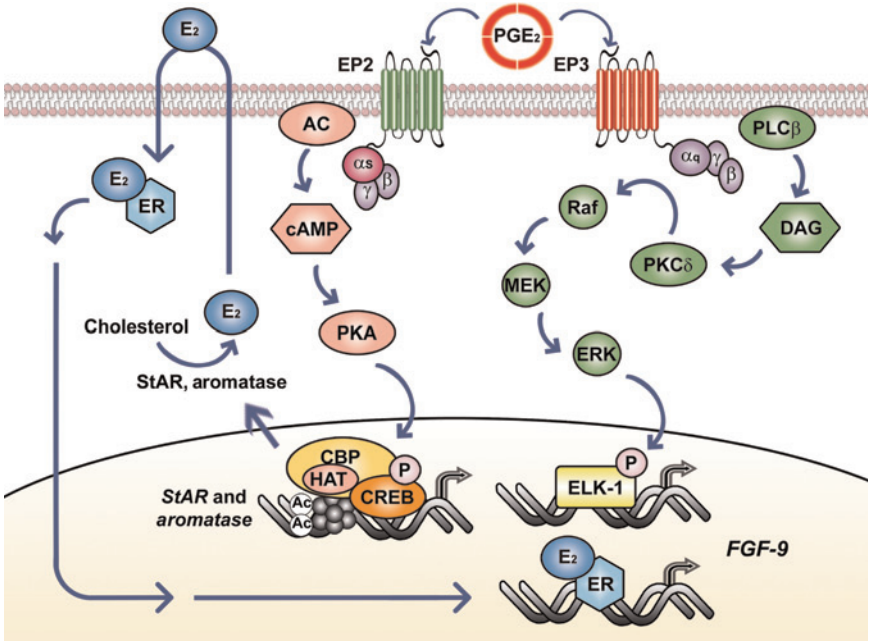

Figure 2 Biphasic activation of fibroblast growth factor (FGF)-9 by prostaglandin E_2 (PGE_2). Right panel: PGE_2 binds to the EP3 receptor and activates its downstream signaling cascade, including phospholipase C (PLC), protein kinase Cδ (PKCδ), Raf, MEK and ERK, to phosphorylate the transcription factor Elk-1. Phosphorylated Elk-1 binds to FGF-9 promoter and induces the transcription. This pathway is used to synthesize FGF-9 in an acute phase (within 12 h). A delayed phase of producing FGF-9 is shown in the left panel. PGE_2, via binding to the EP2 receptor, activates Gs protein, which causes the activation of adenylyl cyclase to increase cAMP production. Protein kinase A (PKA) is activated by cAMP binding and translocates to the nucleus to phosphorylate the DNA-bound cAMP response element binding protein (CREB). Phosphorylated CREB recruits CREB binding protein (CBP), which contains histone acetyltransferase (HAT) activity. The CREB–CBP complex induces histone acetylation (Ac) to create a space for C/EBP binding. The C/EBP bound promoter recruits the basic transcription factors and initiates the transcription of StAR mRNA. This pathway usually induces FGF-9 synthesis after 24 h of PGE_2 treatment. (A color version of this figure is available in the online journal)

mitogen. The mitogenic effect of estrogen is usually mediated by the upregulation of peptide growth factors.[55–58] Several such peptide growth factors, such as fibroblast growth factor (FGF), hepatocyte growth factor, insulin-like growth factor, epithelial growth factor, stromal-derived growth factor and vascular endothelial cell growth factor, have been reported to contribute to the proliferation of endometrial cells.[55–59] However, attempts to characterize the expression patterns of these growth factors in association with the severity of endometriosis have not found strong correlations.[60–64] Nevertheless, the lack of positive correlation does not exclude the importance of these peptide growth factors in the development of endometriosis.

A recent study has revealed that FGF-9 is an important peptide growth factor in the development and persistence of endometriosis.[65] FGF-9 is an estromedin that is indispensable for endometrial stromal cell proliferation.[66] The expression of FGF-9 can be inhibited by the administration of estrogen receptor antagonist, ICI182,870.[65] ICI182,870-blocked endometriotic stromal cell proliferation can be rescued by the addition of FGF-9 but not FGF-7 or FGF-10.[65] The mitogenic effect of FGF-9 is mediated through binding to its high-affinity receptor, especially FGFR2IIIc. The binding of FGF-9 to FGFR2IIIc activates two parallel but additive signaling pathways, the Ras-Raf-MEK-ERK and PLC-calcium-mTOR pathways, to induce cell proliferation.[67] Thus, the aberrant production of estrogen due to stimulation by PGE_2 results in increased levels of FGF-9 and its high-affinity receptor expression in ectopic endometriotic stromal cells. FGF-9 then acts in an autocrine manner to stimulate stromal cell proliferation.

The induction of FGF-9 expression by PGE_2 can also be mediated in an estrogen-independent manner.[68] Again, this phenomenon only occurs in ectopic endometriotic stromal cells but not in eutopic endometrial stromal cells. The induction of FGF-9 by PGE_2 is mediated via the EP3 receptor-activated PKCδ signaling pathway. The activation of PKCδ by PGE_2 leads to the phosphorylation of ERK, which then phosphorylates Elk-1, a member of the ETS-domain containing transcription factor.[69] The binding of Elk-1 to the binding sites in the human FGF-9 promoter region (between −886 and −1346) directly increases FGF-9 transcription.

The finding that the EP3 receptor mediates the action of PGE_2 in stimulating FGF-9 expression is interesting because PGE_2 induces estrogen biosynthesis via the EP2 receptor coupled PKA signaling pathway.[41,49] Since estrogen also induces the expression of FGF-9,[66] these data reveal that PGE_2 simultaneously activates two distinct pathways via binding to different receptor isoforms to exert the same function (Figure 2). The effect mediated by EP3 receptor signaling pathways represents the acute action of PGE_2 while the upregulation of FGF-9 via the EP2 receptor-dependent estrogen action represents a delayed response to PGE_2. Considering that FGF-9 is an important survival and mitogenic factor, the induction of FGF-9 by PGE_2 at different points in time may have different functions. However, the

underlying mechanism behind PGE_2 inducing FGF-9 expression via two different signaling pathways remains an open question and further investigation is needed to determine the significance of actions mediated by different EP receptors in the induction of FGF-9 gene expression.

Suppression of phagocytosis

As has been described above, although retrograde menstruation is the crucial constituent in the development of endometriosis, factors allowing the implantation and propagation of endometriotic lesions are largely unclear. Although aberrant production of steroids by ectopic endometriotic lesions is an important factor leading to the survival and proliferation of endometriotic tissues,[42,70] alteration/dysfunction of the immune system that results in decreased phagocytic ability of immune cells may be another critical factor in the development of endometriosis.[71,72] During endometriosis development, immune cells are recruited to the peritoneal cavity due to inflammation. Among these immune cells, macrophages are the dominant cell type in the peritoneal cavity; they are involved in phagocytosis, especially in cleaning retrograded endometrial debris.[73,74] In the homogenous animal model, it has been reported that the number of peritoneal macrophages increases within four hours after injections of endometrial epithelial and stromal cells into the peritoneal cavity of mice.[75,76] Ideally, peritoneal macrophages recruited to the peritoneal cavity would remove retrograded red blood cells and endometrial debris.[74,77] However, in the endometriosis cases, macrophages may fail to phagocytose the retrograded tissues and thus allow the implantation and proliferation of endometriotic lesions.

The phagocytic function of macrophages is mediated via at least two lines of mechanisms (Figure 3). The first line of mechanism is the secretion and activation of matrix metalloproteinases (MMPs) to break down the extracellular matrix of foreign entities.[78] The second line of phagocytic activity involves the expression of scavenger receptors on the macrophages to enhance the uptake and degradation of cell debris.[79,80] Recent studies reveal that both MMP-9 and the scavenger receptor, CD36, are downregulated in peritoneal macrophages isolated from patients with endometriosis.[81,82] These discoveries provide evidence that explains why peritoneal macrophages isolated from patients with endometriosis have phenotypic and functional alterations leading to poor phagocytic capacity.[71,72,83]

MMPs are a large family of zinc proteases, including 22 human homologs, that can be divided into four major subgroups: interstitial collagenases, gelatinases, stromelysins and membrane-type MMPs.[84] It was found that peritoneal macrophages derived from patients with endometriosis have reduced MMP-9 enzyme activity compared with that from normal women.[81] The decreased activity of MMP-9 secreted by macrophages from women with endometriosis is due to a decrease in MMP-9 mRNA and protein expression. The expression of MMP-9 is suppressed by the peritoneal fluid derived from women with endometriosis but not by the peritoneal fluid derived from endometriosis-free women.[81] Through systemic screening, the factor that effectively decreased MMP-9 expression in the peritoneal fluid derived from women with endometriosis was identified to be PGE_2.[81] PGE_2, via the EP2/EP4 receptor-dependent PKA signaling pathway, effectively inhibits MMP-9 promoter activity to suppress its expression. MMP-9 can degrade the type IV collagen-containing basement membrane that separates the epithelial and stromal compartments.[85] In addition, MMP-9 can activate several cytokines such as latent transforming growth factor-β and pro-TNF-α to their active forms.[84,86] Thus, the reduced expression of MMP-9 in peritoneal macrophages not only decreases macrophage scavenger activity but also attenuates the immune system's defensive mechanism.

Scavenger receptors are a family of structurally diverse receptors with broad ligand specificity that includes low-density lipoprotein, phosphatidylserine, polyanion and apoptotic cells.[87–89] The known scavenger receptors that participate in the phagocytosis of apoptotic cells by macrophages include class A scavenger receptors (SR-AI, SR-AII and SR-AIII)[88] and class B scavenger receptors (SR-BI, SR-BII and SR-BIII).[90,91] The reduced expression of one of these scavenger receptors may result in the loss of phagocytic ability. In peritoneal macrophages derived from endometriosis patients, the expression of SR-BIII (better known as CD36) is reduced, which results in the loss of proper phagocytic ability.[82] Normal macrophages (with high CD36 levels and great phagocytic ability) can be converted to endometriotic-like macrophages by simply inhibiting CD36 expression or blocking the function of CD36. In contrast, the ectopic expression of CD36 is sufficient to restore the phagocytic ability of endometriotic macrophages. An in-depth investigation demonstrated that the expression of CD36 in macrophage is inhibited by PGE_2.[92] Again, the inhibitory effect is mediated via the EP2/EP4 receptor-dependent signaling pathway. These data demonstrate that CD36 is necessary and sufficient for the phagocytic capacity of peritoneal macrophages and that the reduced expression of CD36 plays an important role in the pathogenesis of endometriosis.

The direct evidence that PGE_2 inhibits the phagocytosis of peritoneal macrophages and thus contributes to the development of endometriosis was demonstrated using an autologous transplanted mouse model. Mice that received intraperitoneal injections of small pieces of endometrial tissues from the donor mice developed endometriotic lesion-like cysts.[92] In the transplanted mice, the concentration of PGE_2 in the peritoneal fluid was greater than that in the sham control mice. The injection of PGE_2 into the peritonea of recipients increased the number and size of cysts, while treatment with COX inhibitors inhibited the development of cysts. Consistent with these results, peritoneal macrophages isolated from PGE_2-treated mice express less CD36 protein and have reduced phagocytic ability, while those from COX inhibitor-treated mice have increased CD36 expression and phagocytic ability.[92]

As described above, macrophages secrete MMP-9 to destroy the basement membrane between stromal and epithelial cells, which breaks down the endometriotic tissues into small pieces. The expression of CD36 on the cell membrane of macrophages facilitates the engulfing of these small pieces of

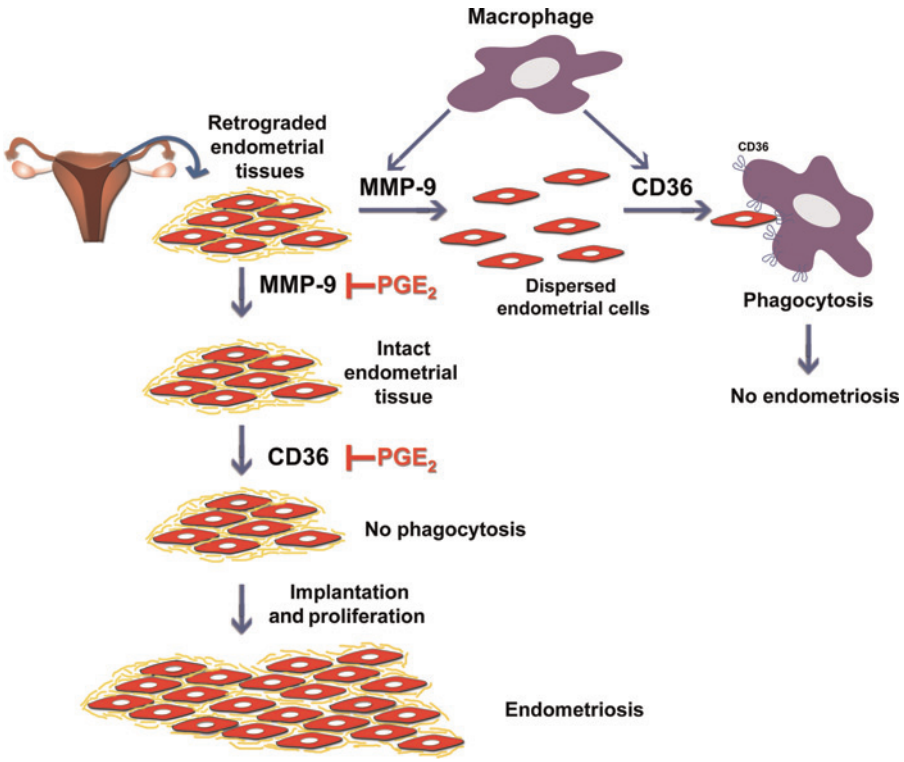

Figure 3 Prostaglandin E_2 suppresses the phagocytic ability of macrophages. Discharged endometrial tissues are retrogradely transported to the peritoneal cavity and cause sterile chronic inflammation. Immune cells, especially macrophages, are recruited to the peritoneum in response to inflammation. Under most circumstances, macrophages will secrete matrix metalloproteinase (MMP)-9 to destroy the extracellular matrix to disperse endometrial tissues into small pieces. In addition, the scavenger receptor CD36 is highly expressed in the plasma membrane of macrophages to facilitate phagocytosis of these small fragments of endometrial debris. However, in the presence of a high concentration of PGE_2, the expression of MMP-9 and CD36 is suppressed. This significantly inhibits the phagocytic ability of macrophage. As a result, the endometrial tissues become implanted and proliferate in the peritoneal cavity, causing endometriosis. (A color version of this figure is available in the online journal)

endometriotic debris. The downregulation of both MMP-9 and CD36 by PGE_2 impairs the first and second lines of phagocytic ability, thus allowing the retrograded endometrial tissues to survive in the peritoneal cavity (Figure 3).

Besides decreased MMP-9 and CD36 expression, peritoneal macrophages in women with endometriosis express greater amounts of estrogen receptor alpha and beta,[93] suggesting that estrogen may modulate the phagocytic ability of macrophage. Furthermore, it has been shown that peritoneal macrophages derived from women with endometriosis may undergo alternative activation, which is necessary for ectopic lesions to vascularize and grow.[94] Concurring with this notion, depleting peritoneal macrophages in a rat model attenuates the adhesion of endometriotic lesions.[95] All these findings suggest that peritoneal macrophages in women with endometriosis may have impaired phagocytic ability but have enhanced growth-promoting capability owing to the change in the microenvironment of peritoneal fluid. Further studies should focus on delineating local factors that cause the transition of peritoneal macrophages from 'suppressive' to 'permissive' phenotype during the development of endometriosis.

Induction of angiogenesis

The establishment of an effective blood supply is a perquisite for the survival of retrograded endometrial tissue and the development of endometriosis.[96–98] Newly formed vessels play an indispensable role in the development and persistence of endometriosis by providing nutrients, growth factors and oxygen to endometriotic lesions. However, surprisingly little information is available on the mechanisms underlying the revascularization of endometriotic lesions given that many angiogenic factors associated with endometriosis have been identified. It appears that angiogenic processes in endometriosis share common markers with tumor angiogenesis as many factors over-expressed in endothelial cells from eutopic and ectopic endometrium of endometriosis patients, such as VEGF, VEGF receptor-2, endoglin, integrin, urokinase-type plasminogen activator, IL-8, MMP-2 and -9, and fibronectin, are also found in activated endothelial cells in tumors.[98] Estrogen and COX-2 have been shown to play critical roles in angiogenesis in various tumor models. Both can stimulate VEGF expression and induce endothelial cell proliferation.[99–101] The deletion of the *cox*-2 gene in mice results in reduced vascular network formation and tumor growth due to a lack of VEGF expression.[101] Two recent studies tested the effects of the selective COX-2 inhibitor on the growth of endometriotic lesions and the development of microvascular networks using xenografted animal models. Ozawa *et al.*[102] demonstrated that a selective COX-2 inhibitor, NS398, decreased the size of implants in an experimental endometriosis model that implants human ovarian

endometrioma into the peritonea of SCID mice. Laschke *et al.*[103] reported that the expression of proliferating cell nuclear antigen and VEGF is significantly reduced in endometrial grafts of NS398-treated golden hamsters. The microvessel density of newly developed microvascular networks within the endometrial grafts is also decreased in NS398-treated animals. These data suggest that COX-2-derived PGE_2 likely plays a key role in establishing an effective blood supply system either directly or indirectly via estrogen during the development of endometriosis. More studies on the cellular and molecular mechanisms of neoangiogenesis in endometriotic implants are needed for developing effective therapeutic strategies of endometriosis.

PGE_2 as a molecular target for endometriosis therapy

Accumulated evidence suggests that PGE_2 might be the master of endometriosis (Figure 4). According to Sampson's hypothesis, retrograded endometrial tissues accompanied by proinflammatory cytokines cause chronic inflammation in the pelvic cavity, which recruits macrophages and other immune cells. Infiltrated macrophages respond to the stimuli present in the peritoneal fluid and become hyperactive with the purpose to clean up the retrograded tissues. Considering that retrograde menstruation occurs in more than 90% of women and the prevalence rate of endometriosis is about 10%, it can be concluded that most macrophages have done their job. However, due to some as-yet unknown mechanism, macrophages fail to clean up all the retrograded tissues, which then implant themselves somewhere in the abdomen and initiate the process of endometriosis.

There are two positive feedback loops that keep the concentrations of PGE_2 persistently elevated in peritoneal fluid of women with endometriosis – the PGE_2–COX-2–PGE_2 pathway in peritoneal macrophages and the PGE_2–estrogen–COX-2–PGE_2 pathway in ectopic endometriotic lesions. An elevated concentration of PGE_2 in the peritoneal fluid governs numerous pathophysiological processes that contribute to the development and persistence of endometriosis. First, PGE_2 induces an aberrant expression of steroidogenic proteins such as StAR and aromatase, leading to an abnormal biosynthesis of estradiol, a critical survival factor for endometrium. The autonomous production of estradiol by ectopic tissues induces several known peptide growth factors such as VEGF and FGF that serve as autocrine (for endometriotic cells) and paracrine (for endothelial cells) factors to stimulate cell proliferation and angiogenesis. Second, PGE_2 exerts direct action on endometriotic and endothelial cell proliferation via the induction of FGF and VEGF. Third, PGE_2 inhibits the expression of MMPs (such as MMP-9) and CD36 by macrophages. The decreased MMP-9 activity and CD36 expression results in an attenuated scavenger function of macrophages, which in the long run benefits the survival and growth of endometriotic lesions.

Considering that PGE_2 can perform various functions to regulate the development of endometriosis, further investigations should focus on how to minimize or eradicate the effects triggered by PGE_2. Inhibiting the production of PGE_2 by ectopic endometriotic stromal cells and by peritoneal macrophages might be an option. However, given the short half-life of COX inhibitors and the unfavorable side-effects caused by the long-term use of NSAIDs, suppressing PGE_2 production to cure endometriosis may not be an ideal choice. An alternative approach is to block the downstream signaling pathways to terminate PGE_2 action. For example, it has been recently shown that blocking EP2/EP4 may prevent PGE_2 from transactivating the EGF receptor and inducing apoptosis in SV40-immortalized endometriotic stromal and epithelial cells.[104] A similar approach may be applicable to blocking the EP3 receptor on endometriotic stromal cells to inhibit the production of FGF-9 or to target EP2 on macrophages to prevent the suppression of phagocytic ability by PGE_2. The blockage of downstream signaling may also disrupt the positive feedback loops of COX-2 over-expression, thus reducing the concentration of PGE_2 in the peritoneal fluid. Therefore, developing effective small molecules such as those used against protein

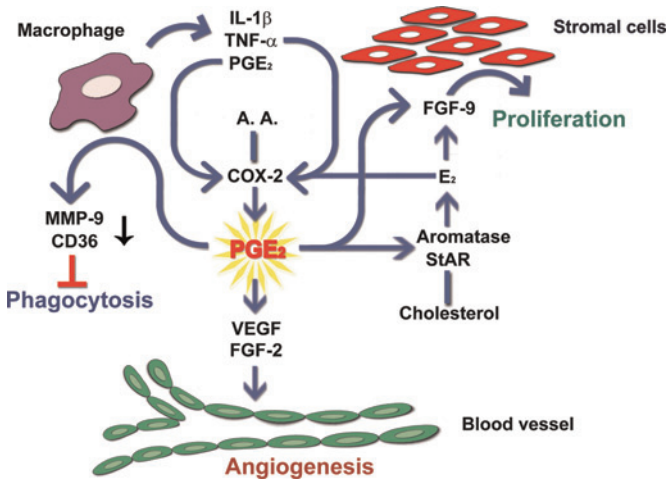

Figure 4 A composite model of PGE_2 actions in the development of endometriosis. Proinflammatory cytokines, such as IL-1β, TNF-α and PGE_2, induce the over-expression of COX-2 in macrophages and endometriotic stromal cells, leading to an elevated concentration of PGE_2 in the peritoneal fluid. In one end, the high level of PGE_2 acts on macrophage to suppress its phagocytic ability by the downregulation of MMP-9 and CD36. In the other end, the high level of PGE_2 stimulates the steroidogenic capacity of endometriotic stromal cells by the upregulation of StAR and aromatase, which enables aberrant biosynthesis of estrogen. Estrogen further stimulates the production of critical mitogens such as FGF-9 to induce endometriotic cell proliferation. PGE_2 can also induce FGF-9 expression by EP3-dependent transcriptional upregulation. These actions ensure the survival and proliferation of endometriotic cells. The third important function of PGE_2 is to induce the expression of angiogenic factors such as VEGF and FGF-2 to induce endothelial cell proliferation and migration, a process known as angiogenesis. The newly formed blood vessels provide nutrients and oxygen to support the continuous growth of endometriotic cells. Furthermore, the two positive feedback loops present in this microsystem keep the PGE_2 concentration consistently high in the peritoneal fluid. The first loop is formed by PGE_2–estrogen–COX-2–PGE_2 in endometriotic stromal cells while the second one consists of PGE_2–proinflammatory cytokines–COX-2–PGE_2 in the macrophages. As a result, a self-supporting survival system is established to support the growth and persistence of endometriosis. PGE_2, prostaglandin E_2; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-alpha; COX-2, cyclooxygenase-2; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor (A color version of this figure is available in the online journal)

kinases[105] and/or histone acetyltransferase[106] to terminate PGE_2-mediated signaling is the most promising therapeutic strategy for treating endometriosis.

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REFERENCES

1 Giudice LC, Kao LC. Endometriosis. *Lancet* 2004;**364**:1789–99
2 Eskenazi B, Warner ML. Epidemiology of endometriosis. *Obstet Gynecol Clin North Am* 1997;**24**:235–58
3 Stanford EJ, Koziol J, Feng A. The prevalence of interstitial cystitis, endometriosis, adhesions, and vulvar pain in women with chronic pelvic pain. *J Minim Invasive Gynecol* 2005;**12**:43–9
4 Brosens IA. Endometriosis – a disease because it is characterized by bleeding. *Am J Obstet Gynecol* 1997;**176**:263–7
5 Fauconnier A, Chapron C. Endometriosis and pelvic pain: epidemiological evidence of the relationship and implications. *Hum Reprod Update* 2005;**11**:595–606
6 Halis G, Arici A. Endometriosis and inflammation in infertility. *Ann N Y Acad Sci* 2004;**1034**:300–15
7 Pandian Z, Bhattacharya S, Templeton A. Review of unexplained infertility and obstetric outcome: a 10 year review. *Hum Reprod* 2001;**16**:2593–7
8 Crosignani P, Olive D, Bergqvist A, Luciano A. Advances in the management of endometriosis: an update for clinicians. *Hum Reprod Update* 2006;**12**:179–89
9 Parazzini F, Bertulessi C, Pasini A, Rosati M, Di F, Shonauer S, Vicino M, Aguzzoli L, Trossarelli GF, Massobrio M, Bracco G, Perino A, Moroni S, Beretta P. Determinants of short term recurrence rate of endometriosis. *Eur J Obstet Gynecol Reprod Biol* 2005;**121**:216–9
10 Cheong Y, Tay P, Luk F, Gan HC, Li TC, Cooke I. Laparoscopic surgery for endometriosis: how often do we need to re-operate?. *J Obstet Gynaecol* 2008;**28**:82–5
11 Guo SW. Recurrence of endometriosis and its control. *Hum Reprod Update* 2009;**15**:441–61
12 Sampson JA. Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am J Obstet Gynecol* 1927;**14**:422–5
13 Von Rocklinghausen F. Adenomyomas and cystadenomas of the wall of the uterus and tube: their origin as remanants of the wolffian body. *Wien Klin Wochenschr* 1896;**8**:530
14 Ferguson BR, Bennington JL, Haber SL. Histochemistry of mucosubstances and histology of mixed mullerian pelvic lymph node glandular inclusions. Evidence for histogenesis by mullerian metaplasia of coelomic epithelium. *Obstet Gynecol* 1969;**33**:617–25
15 Olive DL, Schwartz LB. Endometriosis. *N Engl J Med* 1993;**328**:1759–69
16 Koninckx PR, Braet P, Kennedy SH, Barlow DH. Dioxin pollution and endometriosis in Belgium. *Hum Reprod* 1994;**9**:1001–2
17 Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JL. Endometriosis in rhesus monkeys (*Macaca mulatta*) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Fundam Appl Toxicol* 1993;**21**:433–41
18 Hadfield RM, Mardon HJ, Barlow DH, Kennedy SH. Endometriosis in monozygotic twins. *Fertil Steril* 1997;**68**:941–2
19 Stefansson H, Geirsson RT, Steinthorsdottir V, Jonsson H, Manolescu A, Kong A, Ingadottir G, Gulcher J, Stefansson K. Genetic factors contribute to the risk of developing endometriosis. *Hum Reprod* 2002;**17**:555–9
20 Treloar SA, Wicks J, Nyholt DR, Montgomery GW, Bahlo M, Smith V, Dawson G, Mackay IJ, Weeks DE, Bennett ST, Carey A, Ewen-White KR, Duffy DL, O'Connor DT, Barlow DH, Martin NG, Kennedy SH. Genomewide linkage study in 1,176 affected sister pair families identifies a significant susceptibility locus for endometriosis on chromosome 10q26. *Am J Hum Genet* 2005;**77**:365–76
21 Dawood MY, Khan-Dawood FS, Wilson L Jr. Peritoneal fluid prostaglandins and prostanoids in women with endometriosis, chronic pelvic inflammatory disease, and pelvic pain. *Am J Obstet Gynecol* 1984;**148**:391–5
22 Wu MH, Sun HS, Lin CC, Hsiao KY, Chuang PC, Pan HA, Tsai SJ. Distinct mechanisms regulate cyclooxygenase-1 and -2 in peritoneal macrophages of women with and without endometriosis. *Mol Hum Reprod* 2002;**8**:1103–10
23 Bulun SE. Endometriosis. *N Engl J Med* 2009;**360**:268–79
24 Bulun SE, Utsunomiya H, Lin Z, Yin P, Cheng YH, Pavone ME, Tokunaga H, Trukhacheva E, Attar E, Gurates B, Milad MP, Confino E, Su E, Reierstad S, Xue Q. Steroidogenic factor-1 and endometriosis. *Mol Cell Endocrinol* 2009;**300**:104–8
25 Vercellini P, Somigliana E, Vigano P, Abbiati A, Barbara G, Crosignani PG. Endometriosis: current therapies and new pharmacological developments. *Drugs* 2009;**69**:649–75
26 Pitsos M, Kanakas N. The role of matrix metalloproteinases in the pathogenesis of endometriosis. *Reprod Sci* 2009;**16**:717–26
27 Taylor RN, Yu J, Torres PB, Schickedanz AC, Park JK, Mueller MD, Sidell N. Mechanistic and therapeutic implications of angiogenesis in endometriosis. *Reprod Sci* 2009;**16**:140–6
28 Tempfer CB, Simoni M, Destenaves B, Fauser BC. Functional genetic polymorphisms and female reproductive disorders: part II – endometriosis. *Hum Reprod Update* 2009;**15**:97–118
29 Gargett CE, Chan RW, Schwab KE. Hormone and growth factor signaling in endometrial renewal: role of stem/progenitor cells. *Mol Cell Endocrinol* 2008;**288**:22–9
30 Ferreira SA, Vane JR. Prostaglandins: their disappearance from and release into the circulation. *Nature* 1967;**216**:868–73
31 Ota H, Igarashi S, Sasaki M, Tanaka T. Distribution of cyclooxygenase-2 in eutopic and ectopic endometrium in endometriosis and adenomyosis. *Hum Reprod* 2001;**16**:561–6
32 Chishima F, Hayakawa S, Sugita K, Kinukawa N, Aleemuzzaman S, Nemoto N, Yamamoto T, Honda M. Increased expression of cyclooxygenase-2 in local lesions of endometriosis patients. *Am J Reprod Immunol* 2002;**48**:50–6
33 Wu MH, Wang CA, Lin CC, Chen LC, Chang WC, Tsai SJ. Distinct regulation of cyclooxygenase-2 by interleukin-1beta in normal and endometriotic stromal cells. *J Clin Endocrinol Metab* 2005;**90**:286–95
34 Smith WL, Garavito RM, DeWitt DL. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. *J Biol Chem* 1996;**271**:33157–60
35 Langenbach R, Morham SG, Tiano HF, Loftin CD, Ghanayem BI, Chulada PC, Mahler JF, Lee CA, Goulding EH, Kluckman KD, Kim HS, Smithies O. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell* 1995;**83**:483–92
36 Tamura M, Sebastian S, Gurates B, Yang S, Fang Z, Bulun SE. Vascular endothelial growth factor up-regulates cyclooxygenase-2 expression in human endothelial cells. *J Clin Endocrinol Metab* 2002;**87**:3504–7
37 Tamura M, Sebastian S, Yang S, Gurates B, Ferrer K, Sasano H, Okamura K, Bulun SE. Up-regulation of cyclooxygenase-2 expression and prostaglandin synthesis in endometrial stromal cells by malignant endometrial epithelial cells. A paracrine effect mediated by prostaglandin E2 and nuclear factor-kappa B. *J Biol Chem* 2002;**277**:26208–16
38 Bulun SE, Fang Z, Imir G, Gurates B, Tamura M, Yilmaz B, Langoi D, Amin S, Yang S, Deb S. Aromatase and endometriosis. *Semin Reprod Med* 2004;**22**:45–50
39 Noble LS, Simpson ER, Johns A, Bulun SE. Aromatase expression in endometriosis. *J Clin Endocrinol Metab* 1996;**81**:174–9
40 Bulun SE, Noble LS, Takayama K, Michael MD, Agarwal V, Fisher C, Zhao Y, Hinshelwood MM, Ito Y, Simpson ER. Endocrine disorders associated with inappropriately high aromatase expression. *J Steroid Biochem Mol Biol* 1997;**61**:133–9
41 Noble LS, Takayama K, Zeitoun KM, Putman JM, Johns DA, Hinshelwood MM, Agarwal VR, Zhao Y, Carr BR, Bulun SE. Prostaglandin E2 stimulates aromatase expression in

endometriosis-derived stromal cells. *J Clin Endocrinol Metab* 1997;**82**:600–6
42 Tsai SJ, Wu MH, Lin CC, Sun HS, Chen SM. Regulation of steroidogenic acute regulatory protein expression and progesterone production in endometriotic stromal cells. *J Clin Endocrinol Metab* 2001;**86**:5765–73
43 Attar E, Tokunaga H, Imir G, Yilmaz MB, Redwine D, Putman M, Gurates B, Attar R, Yaegashi N, Hales DB, Bulun SE. Prostaglandin E2 via steroidogenic factor-1 coordinately regulates transcription of steroidogenic genes necessary for estrogen synthesis in endometriosis. *J Clin Endocrinol Metab* 2009;**94**:623–31
44 Colette S, Lousse JC, Defrere S, Curaba M, Heilier JF, Van Langendonckt A, Mestdagt M, Foidart JM, Loumaye E, Donnez J. Absence of aromatase protein and mRNA expression in endometriosis. *Hum Reprod* 2009;**24**:2133–41
45 Bukulmez O, Hardy DB, Carr BR, Word RA, Mendelson CR. Inflammatory status influences aromatase and steroid receptor expression in endometriosis. *Endocrinology* 2008;**149**:1190–204
46 Kyama CM, Overbergh L, Mihalyi A, Meuleman C, Mwenda JM, Mathieu C, D'Hooghe TM. Endometrial and peritoneal expression of aromatase, cytokines, and adhesion factors in women with endometriosis. *Fertil Steril* 2008;**89**:301–10
47 Matsuzaki S, Canis M, Pouly JL, Dechelotte PJ, Mage G. Analysis of aromatase and 17beta-hydroxysteroid dehydrogenase type 2 messenger ribonucleic acid expression in deep endometriosis and eutopic endometrium using laser capture microdissection. *Fertil Steril* 2006;**85**:308–13
48 Stocco DM, Clark BJ. Regulation of the acute production of steroids in steroidogenic cells. *Endocr Rev* 1996;**17**:221–44
49 Sun HS, Hsiao KY, Hsu CC, Wu MH, Tsai SJ. Transactivation of steroidogenic acute regulatory protein in human endometriotic stromal cells is mediated by the prostaglandin EP2 receptor. *Endocrinology* 2003;**144**:3934–42
50 Hsu CC, Lu CW, Huang BM, Wu MH, Tsai SJ. Cyclic adenosine 3′,5′-monophosphate response element-binding protein and CCAAT/enhancer-binding protein mediate prostaglandin E2-induced steroidogenic acute regulatory protein expression in endometriotic stromal cells. *Am J Pathol* 2008;**173**:433–41
51 Breyer RM, Bagdassarian CK, Myers SA, Breyer MD. Prostanoid receptors: subtypes and signaling. *Annu Rev Pharmacol Toxicol* 2001;**41**:661–90
52 Christenson LK, Johnson PF, McAllister JM, Strauss JF 3rd. CCAAT/enhancer-binding proteins regulate expression of the human steroidogenic acute regulatory protein (StAR) gene. *J Biol Chem* 1999;**274**:26591–8
53 Zeitoun KM, Bulun SE. Aromatase: a key molecule in the pathophysiology of endometriosis and a therapeutic target. *Fertil Steril* 1999;**72**:961–9
54 Yang S, Fang Z, Suzuki T, Sasano H, Zhou J, Gurates B, Tamura M, Ferrer K, Bulun S. Regulation of aromatase P450 expression in endometriotic and endometrial stromal cells by CCAAT/enhancer binding proteins (C/EBPs): decreased C/EBPbeta in endometriosis is associated with overexpression of aromatase. *J Clin Endocrinol Metab* 2002;**87**:2336–45
55 Haining RE, Cameron IT, van Papendorp C, Davenport AP, Prentice A, Thomas EJ, Smith SK. Epidermal growth factor in human endometrium: proliferative effects in culture and immunocytochemical localization in normal and endometriotic tissues. *Hum Reprod* 1991;**6**:1200–5
56 Croze F, Kennedy TG, Schroedter IC, Friesen HG, Murphy LJ. Expression of insulin-like growth factor-I and insulin-like growth factor-binding protein-1 in the rat uterus during decidualization. *Endocrinology* 1990;**127**:1995–2000
57 Pierro E, Minici F, Alesiani O, Miceli F, Proto C, Screpanti I, Mancuso S, Lanzone A. Stromal–epithelial interactions modulate estrogen responsiveness in normal human endometrium. *Biol Reprod* 2001;**64**:831–8
58 Cooke PS, Buchanan DL, Lubahn DB, Cunha GR. Mechanism of estrogen action: lessions from the estradiol receptor-α knockout mouse. *Biol Reprod* 1998;**59**:470–5
59 Khan KN, Kitajima M, Hiraki K, Fujishita A, Sekine I, Ishimaru T, Masuzaki H. Immunopathogenesis of pelvic endometriosis: role of hepatocyte growth factor, macrophages and ovarian steroids. *Am J Reprod Immunol* 2008;**60**:383–404
60 Huang JC, Yeh J. Quantitative analysis of epidermal growth factor receptor gene expression in endometriosis. *J Clin Endocrinol Metab* 1994;**79**:1097–101
61 Huang JC, Papasakelariou C, Dawood MY. Epidermal growth factor and basic fibroblast growth factor in peritoneal fluid of women with endometriosis. *Fertil Steril* 1996;**65**:931–4
62 Matalliotakis IM, Goumenou AG, Koumantakis GE, Neonaki MA, Koumantakis EE, Dionyssopoulou E, Athanassakis I, Vassiliadis S. Serum concentrations of growth factors in women with and without endometriosis: the action of anti-endometriosis medicines. *Int Immunopharmacol* 2003;**3**:81–9
63 Seli E, Zeyneloglu HB, Senturk LM, Bahtiyar OM, Olive DL, Arici A. Basic fibroblast growth factor: peritoneal and follicular fluid levels and its effect on early embryonic development. *Fertil Steril* 1998;**69**:1145–8
64 Di AM, Centinaio G, Carniti C, Somigliana E, Vigano P, Vignali M. Basic fibroblast growth factor messenger ribonucleic acid levels in eutopic and ectopic human endometrial stromal cells as assessed by competitive polymerase chain reaction amplification. *Mol Cell Endocrinol* 1995;**115**:169–75
65 Wing LYC, Chuang PC, Wu MH, Chen HM, Tsai SJ. Expression and mitogenic effect of fibroblast growth factor-9 in human endometriotic implant is regulated by aberrant production of estrogen. *J Clin Endocrinol Metab* 2003;**88**:5547–54
66 Tsai SJ, Wu MH, Chen HM, Chuang PC, Wing LY. Fibroblast growth factor-9 is an endometrial stromal growth factor. *Endocrinology* 2002;**143**:2715–21
67 Wing LY, Chen HM, Chuang PC, Wu MH, Tsai SJ. The mammalian target of rapamycin-p70 ribosomal S6 kinase but not phosphatidylinositol 3-kinase-Akt signaling is responsible for fibroblast growth factor-9-induced cell proliferation. *J Biol Chem* 2005;**280**:19937–47
68 Chuang PC, Sun HS, Chen TM, Tsai SJ. Prostaglandin E2 induces fibroblast growth factor 9 via EP3-dependent protein kinase Cdelta and Elk-1 signaling. *Mol Cell Biol* 2006;**26**:8281–92
69 Hipskind RA, Rao VN, Mueller CG, Reddy ES, Nordheim A. Ets-related protein Elk-1 is homologous to the c-fos regulatory factor p62TCF. *Nature* 1991;**354**:531–4
70 Bulun SE, Yang S, Fang Z, Gurates B, Tamura M, Sebastian S. Estrogen production and metabolism in endometriosis. *Ann N Y Acad Sci* 2002;**955**:75–85
71 Dmowski WP, Braun D, Gebel H. Endometriosis: genetic and immunologic aspects. *Prog Clin Biol Res* 1990;**323**:99–122
72 Dmowski WP, Gebel HM, Braun DP. The role of cell-mediated immunity in pathogenesis of endometriosis. *Acta Obstet Gynecol Scand Suppl* 1994;**159**:7–14
73 Haney AF, Muscato JJ, Weinberg JB. Peritoneal fluid cell populations in infertility patients. *Fertil Steril* 1981;**35**:696–8
74 Dunselman GA, Hendrix MG, Bouckaert PX, Evers JL. Functional aspects of peritoneal macrophages in endometriosis of women. *J Reprod Fertil* 1988;**82**:707–10
75 Cao X, Yang D, Song M, Murphy A, Parthasarathy S. The presence of endometrial cells in the peritoneal cavity enhances monocyte recruitment and induces inflammatory cytokines in mice: implications for endometriosis. *Fertil Steril* 2004;**82** (Suppl. 3):999–1007
76 Lin YJ, Lai MD, Lei HY, Wing LY. Neutrophils and macrophages promote angiogenesis in the early stage of endometriosis in a mouse model. *Endocrinology* 2006;**147**:1278–86
77 Khorram O, Taylor RN, Ryan IP, Schall TJ, Landers DV. Peritoneal fluid concentrations of the cytokine RANTES correlate with the severity of endometriosis. *Am J Obstet Gynecol* 1993;**169**:1545–9
78 Osteen KG, Yeaman GR, Bruner-Tran KL. Matrix metalloproteinases and endometriosis. *Semin Reprod Med* 2003;**21**:155–64
79 Febbraio M, Hajjar DP, Silverstein RL. CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism. *J Clin Invest* 2001;**108**:785–91
80 Linton MF, Fazio S. Class A scavenger receptors, macrophages, and atherosclerosis. *Curr Opin Lipidol* 2001;**12**:489–95
81 Wu MH, Shoji Y, Wu MC, Chuang PC, Lin CC, Huang MF, Tsai SJ. Suppression of matrix metalloproteinase-9 by prostaglandin E(2) in

peritoneal macrophage is associated with severity of endometriosis. *Am J Pathol* 2005;**167**:1061–9
82 Chuang PC, Wu MH, Shoji Y, Tsai SJ. Downregulation of CD36 results in reduced phagocytic ability of peritoneal macrophages of women with endometriosis. *J Pathol* 2009;**219**:232–41
83 Raiter-Tenenbaum A, Baranao RI, Etchepareborda JJ, Meresman GF, Rumi LS. Functional and phenotypic alterations in peritoneal macrophages from patients with early and advanced endometriosis. *Arch Gynecol Obstet* 1998;**261**:147–57
84 Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 2001;**17**:463–516
85 McMillan JI, Weeks R, West JW, Bursten S, Rice GC, Lovett DH. Pharmacological inhibition of gelatinase B induction and tumor cell invasion. *Int J Cancer* 1996;**67**:523–31
86 Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. *Genes Dev* 2000;**14**:163–76
87 Krieger M, Herz J. Structures and functions of multiligand lipoprotein receptors: macrophage scavenger receptors and LDL receptor-related protein (LRP). *Annu Rev Biochem* 1994;**63**:601–37
88 Platt N, da Silva RP, Gordon S. Recognizing death: the phagocytosis of apoptotic cells. *Trends Cell Biol* 1998;**8**:365–72
89 Rigotti A, Acton SL, Krieger M. The class B scavenger receptors SR-BI and CD36 are receptors for anionic phospholipids. *J Biol Chem* 1995;**270**:16221–4
90 Savill J, Dransfield I, Hogg N, Haslett C. Vitronectin receptor-mediated phagocytosis of cells undergoing apoptosis. *Nature* 1990;**343**:170–3
91 Savill J, Hogg N, Ren Y, Haslett C. Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. *J Clin Invest* 1992;**90**:1513–22
92 Chuang PC, Lin YJ, Wu MH, Wing LYC, Shoji Y, Tsai SJ. Inhibition of CD36-dependent phagocytosis by prostaglandin E_2 contributes to the development of endometriosis. *Am J Pathol* 2010;**176**:850–60
93 Capellino S, Montagna P, Villaggio B, Sulli A, Soldano S, Ferrero S, Remorgida V, Cutolo M. Role of estrogens in inflammatory response: expression of estrogen receptors in peritoneal fluid macrophages from endometriosis. *Ann N Y Acad Sci* 2006;**1069**:263–7
94 Bacci M, Capobianco A, Monno A, Cottone L, Di Puppo F, Camisa B, Mariani M, Brignole C, Ponzoni M, Ferrari S, Panina-Bordignon P, Manfredi AA, Rovere-Querini P. Macrophages are alternatively activated in patients with endometriosis and required for growth and vascularization of lesions in a mouse model of disease. *Am J Pathol* 2009;**175**:547–56
95 Haber E, Danenberg HD, Koroukhov N, Ron-El R, Golomb G, Schachter M. Peritoneal macrophage depletion by liposomal bisphosphonate attenuates endometriosis in the rat model. *Hum Reprod* 2009;**24**:398–407
96 Donnez J, Smoes P, Gillerot S, Casanas-Roux F, Nisolle M. Vascular endothelial growth factor (VEGF) in endometriosis. *Hum Reprod* 1998;**13**:1686–90
97 Taylor RN, Lebovic DI, Mueller MD. Angiogenic factors in endometriosis. *Ann N Y Acad Sci* 2002;**955**:89–100; discussion 118, 396–406
98 Groothuis PG, Nap AW, Winterhager E, Grummer R. Vascular development in endometriosis. *Angiogenesis* 2005;**8**:147–56
99 Jones MK, Wang H, Peskar BM, Levin E, Itani RM, Sarfeh IJ, Tarnawski AS. Inhibition of angiogenesis by nonsteroidal anti-inflammatory drugs: insight into mechanisms and implications for cancer growth and ulcer healing. *Nat Med* 1999;**5**:1418–23
100 Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. *Cell* 1998;**93**:705–16
101 Williams CS, Tsujii M, Reese J, Dey SK, DuBois RN. Host cyclooxygenase-2 modulates carcinoma growth. *J Clin Invest* 2000;**105**:1589–94
102 Ozawa Y, Murakami T, Tamura M, Terada Y, Yaegashi N, Okamura K. A selective cyclooxygenase-2 inhibitor suppresses the growth of endometriosis xenografts via antiangiogenic activity in severe combined immunodeficiency mice. *Fertil Steril* 2006;**86**:1146–51
103 Laschke MW, Elitzsch A, Scheuer C, Vollmar B, Menger MD. Selective cyclo-oxygenase-2 inhibition induces regression of autologous endometrial grafts by down-regulation of vascular endothelial growth factor-mediated angiogenesis and stimulation of caspase-3-dependent apoptosis. *Fertil Steril* 2007;**87**:163–71
104 Banu SK, Lee J, Speights VO Jr., Starzinski-Powitz A, Arosh JA. Selective inhibition of prostaglandin E2 receptors EP2 and EP4 induces apoptosis of human endometriotic cells through suppression of ERK1/2, AKT, NFkappaB, and beta-catenin pathways and activation of intrinsic apoptotic mechanisms. *Mol Endocrinol* 2009;**23**:1291–305
105 Fedorov O, Marsden B, Pogacic V, Rellos P, Muller S, Bullock AN, Schwaller J, Sundstrom M, Knapp S. A systematic interaction map of validated kinase inhibitors with Ser/Thr kinases. *Proc Natl Acad Sci USA* 2007;**104**:20523–8
106 Biel M, Kretsovali A, Karatzali E, Papamatheakis J, Giannis A. Design, synthesis, and biological evaluation of a small-molecule inhibitor of the histone acetyltransferase Gcn5. *Angew Chem Int Ed Engl* 2004;**43**:3974–6