Estrogen signaling in the proliferative endometrium: implications in endometriosis

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Summary

Even though the physiological role of estrogen in the female reproductive cycle and endometrial proliferative phase is well established, the signaling pathways by which estrogen exerts its action in the endometrial tissue are still little known. In this regard, advancements in cell culture techniques and maintenance of endometrial cells in cultures enabled the discovery of new signaling mechanisms activated by estrogen in the normal endometrium and in endometriosis. This review aims to present the recent findings in the genomic and non-genomic estrogen signaling pathways in the proliferative human endometrium specifically associated with the pathogenesis and development of endometriosis.

Keywords: endometrium, estrogens, signal transduction, endometriosis.

Introduction

Endometriosis is an estrogen-mediated benign inflammatory disease that affects about 10 to 15% of women during their reproductive years. The prevalence of this disease increases to about 30% in patients with infertility and to 45% in those with chronic pelvic pain.[1] Endometriosis is characterized by the presence of endometrial uterine tissue outside its normal location, especially in the pelvic peritoneum, although the location may also be in the ovaries, retrovaginal septum and, rarely, in the pericardium, pleura, and even in the brain.[2] Although the etiology of endometriosis has not been defined, the most widespread hypothesis for the development of endometriotic lesions is retrograde menstruation of endometrial tissue, as proposed by Sampson.[3] In addition to retrograde menstruation, factors such as heavy menstrual flow, abnormal eutopic endometrium, environmental dietary factors, among others, may also contribute to the adhesion and growth of the lesions.[4,5]

The physiological effects of estrogens in the female reproductive cycle are already well established.[6] With respect to its action in the endometrium in particular, the ovarian secretion of estrogen (mainly 17β-estradiol, E2) before ovulation triggers a marked proliferation of the endometrial stroma and increased development of the endometrial glands, preparing the tissue for the action of the hormone progesterone. Estrogen is considered the dominant hormone of the proliferative phase of the menstrual cycle.[7]

One of the main features of the eutopic endometrium in patients with endometriosis has been the incomplete transition of the proliferative (estrogen-dependent) to the secretory (progesterone-dependent) endometrium, leading to a persistent expression of genes involved in the synthesis of DNA and cellular mitosis in endometriotic lesions.[8,9]

In addition, latest research shows that the proliferative phase is not a uniform period of endometrial growth.[10] This represents without any doubt a great challenge in the search for the understanding of the mechanisms of cellular signaling by which estrogen regulates the human endometrium. Based on that, we will specifically cover in this study the events related to the proliferative phase.

Proliferative effects of estrogen in the human endometrium

Estrogen acts in the endometrium by linking to the estrogen receptor (ER), which may be either alpha or beta (ERα, ERβ) type, inducing mucosal proliferation during

the proliferative phase and synthesis of progesterone receptors, preparing the endometrium to the secretory phase. As transcription factors, these receptors have been found in the nucleus of glandular and endometrial stromal cells during the proliferative phase.[11-13]

Although morphological and ultrastructural observations and recent hysteroscopic analysis of the human endometrium have contributed significantly to the understanding of the effects of estrogen on the menstrual cycle,[14,15] much less is known about its mechanism of action in normal endometrial or endometriotic cells.

Genomic signaling in endometriosis

The classic model of the proliferative action of E2 in the endometrium involves the binding of E2 to the receptors ERα or ERb (residing in the nucleus or cytosol),[16] forming the complex E2-ER that interacts directly with specific sequences in the DNA (ERE) in promoter regions of genes related to the progression of the G1 phase of the cell cycle, regulating mainly the transcription of cyclins and cyclin-dependent kinases (CDK) (Figure 1a).[17] Descriptions of the ER residing close to the plasma membrane have also been reported.[18]

It has been recently verified in the normal human endometrium that expression levels of cyclin E and cyclin-dependent kinase inhibitor protein (p27) change during the progression of the menstrual cycle and show different patterns in fertile and infertile women.[19] In endometriosis, it has been suggested that high expression levels of cyclin B1 in ectopic endometrial tissue cells are associated with an abnormal regulation of the cell cycle (Figure 1a*).[20]

The transcription of cell cycle-related genes can also occur by interaction of the receptors ERα and ERβ with coactivator proteins. These proteins do not bind to the DNA, but are rather recruited to the promoter site through protein-protein interactions with the ER.[21,22] Examples of coactivators include the proteins of the p160/steroid receptor coactivator (SRC) family.

Recent studies have demonstrated expression of SRC1 in the normal and ectopic endometrium.[23] Additionally, an important role not only in the development but also in the pathogenesis of endometriosis has been attributed to the interaction between the nuclear ER and the coactivator proteins.[24,25] A new SRC1 isoform is also presented as crucial to the pathogenic progression of endometriosis.[26] (Figure 1a1).

Both ERα and ERβ can also regulate the transcription of genes through interaction with other transcription factors, including specific protein-1 (SP1), nuclear factor kappa-B(NF-kappaB), activator protein-1(AP-1), CCAAT/enhancer binding protein b (C/EBPb), GATA-binding protein 1(GATA 1), and signal transducer and activator of transcription 5 (STAT5), enabling the activation or repression of target genes, which significantly amplifies the regulatory influence of estrogen.[27,28]

One of the best known examples of interaction between the ER and other transcription factors is that of the complex ER-estrogen with Finkel-Biskis-Jinkins (FBJ)-osteosarcoma homolog (FOS) protein and JUN protein at the DNA binding site of the transcription factor AP-1. It has been recently shown that the transcriptional activation of cyclin D1 occurs through binding of the complex ER-estrogen with the JUN protein in endometrial glandular cells.[29] A high expression of cyclin protein D1 has been previously observed in peritoneal endometriosis (Figure 1b*).[30]

Non-genomic signaling in endometriosis

Since growing evidence shows the importance of the non-genomic estrogen signaling pathway in endometriosis, we will discuss for comparison purposes the main known mechanisms of this type of regulation in the normal endometrium and in endometriosis.[31]

Nuclear ERs trigger relatively long (30 to 60 minutes) events. In the 1960s, researchers had already observed the rapid elevation in uterine levels of cyclic adenosine monophosphate (cAMP) in female rats after IV administration of physiological doses of E2.[32] This was one of the pioneering studies on the other type of mechanism of action of estrogen which does not require binding of the complex E2-ER to the DNA, known as non-genomic mechanism or non-genomic signaling. Although the presence of these receptors was demonstrated in the membrane of endometrial cells in 1997,[33] progress in this area was very slow until the past decade, but thank to accumulated evidence at a cellular level, this new mode of estrogen action could be elucidated.

One of the most clearly defined pathways in non-genomic estrogen regulation is the activation of the RAS protein via ERK (extracellular regulated kinase). Thus, cytosolic ERα binds to insulin-like growth factor 1 (IGF-1) receptors stimulating phosphorylation of these receptors and transmission of signals to activate extracellular-signal-regulated kinase (ERK) proteins (Figure 1c). ERβ is unable to interact with IGF-1 receptors or activate ERK proteins.[34]

Endometrial stromal cells produce IGF-1 and present the receptor for IGF-1 in their plasma membranes.[35] It has been demonstrated in endometrial carcinoma that regulation of IGF-1 by E2 and autocrine stimulation via IGF-1 receptor with participation of the ERK pathway is important for proliferation of these cells.[36] However, there is no evidence to date that this pathway participates directly in the pathogenesis of endometriosis (Figure 1c*).

Strong evidence shows that E2 interacts with a transmembrane receptor coupled to the G protein called G pro-

tein-coupled receptor 30 (GPR30). This receptor, discovered relatively recently,[37] has a curious intracellular location (in the membrane of the endoplasmic reticulum, although there are reports of the receptor residing in the plasma membrane).[38,39] GPR30 was recently found in normal endometrium and in the endometrium of women with endometriosis.[40] High levels of GRP30 transcripts have been observed in eutopic endometrium during the proliferative phase, although a higher expression has been shown in the ectopic endometrium in women with endometriosis.[41]

Following the E2-GPR30 interaction, the alpha subunit (Gα) stimulates the enzyme adenylate cyclase (AC), producing cAMP. The elevation in cAMP levels stimulates the transcription of genes related to the CRE (cyclic-AMP responsive element) sequence through activation of protein kinase A (PKA) (Figure 1d). Depending on the amount of cAMP produced, activation of cyclin D/E (leading to cell cycle progression) or p27Kip1 (decreasing cellular proliferation) can occur.

Despite the demonstration in 1994 that this pathway may be activated by E2 in uterine cells of female rats,[42] its importance in endometriosis was only demonstrated recently. Thus, in cells not affected by endometriosis, stimulation of the cAMP/PKA pathway leads to a decrease in the speed of the cell cycle progression due to a lower expression of cyclin D1 (Figure 1d*). However, in stromal cells of women with endometriosis, cyclin D1 levels remain unchanged, resulting in an increased proliferative potential when compared with stromal cells in women without endometriosis, which contributes to the establishment, survival, and proliferation of endometriotic lesions.[43]

Stimulation of GPR30 can also activate c-Src (Figure 1e). This protein in turn activates the matrix metalloproteinase (MMP), triggering the release of epidermal growth factor (EGF) from its form connected to the membrane. This transactivates the EGF receptor (EGFR) (Figura 1e2), leadingo to activation of the mitogen-activated protein kinase (MAPK) (Figure 1e3) and phosphatidylinositol-3-phosphate kinase (PI3K) (Figure 1e4).

The activation of the MAPK signaling cascade through the GPR30 receptor and activation of the Src protein has been observed mainly in endometrial cancer cells.[44] Several studies have reported the importance of the activation of components of this pathway in the development of endometrial cancer.

However, studies identifying the role of MAPKs in the etiology of endometriosis have been described especially in ectopic lesions, in which the increased activity of proteins from the MAPK pathway is responsible for growth control and maintenance of ectopic endometrial tissue.[45]

More recently, in an attempt to understand the molecular mechanisms that control not only the baseline proliferation of endometrial human cells but also their migration, it was demonstrated for the first time that the protein rho-associated kinase II (ROCKII) acts as a point of integration between cell proliferation and migration, and that the protein Rapidly accelerated fibrosarcoma-1 (Raf-1) regulates negatively the activity of ROCKII in endometrial cells.[46] Still, since levels of Raf-1 are lower and B-Raf activity is higher in eutopic endometrial cells than in normal cells, the B-Raf-MAPK and Rho/ROCKII pathways are abnormally activated, leading to a greater proliferation ability and increased migratory potential, which explains the incomplete transition in the damaged endometrium and the high proliferative migratory phenotype (Figure 1e*).

Cell signaling involving PI3K is related to the processes of normal decidualization of the endometrium in the secretory phase of the menstrual cycle in preparation for the implantation of the embryo. Although the activation of the PI3K pathway due to mutations is mainly related to the development of ovarian carcinomas,[47,48] it is possible that changes in the PI3K pathway cause an incomplete transition from the proliferative to the secretory endometrium, leading to the characteristic progesterone resistance in the development of endometriosis.[49]

In an interesting study, Zhang et al.[50] have shown that the PI3K pathway is effectively important in endometriosis and contributes not only to the maintenance, but also (and especially) to the proliferation of ectopic lesions. The authors demonstrated that the protein phosphatase and tensin homolog (PTEN, an endogenous inhibitor of the PI3K pathway that is normally expressed in endometrial glandular cells) is expressed at low levels and is even absent in the eutopic and ectopic endometrium. It has also been observed that E2 is able to decrease the mRNA expression of PTEN, leading to decreased protein expression. Therefore, during the proliferative phase of the menstrual cycle, PTEN expression is decreased. Furthermore, E2 is able to regulate the subcellular distribution of PTEN, and in eutopic cells this protein was observed to be virtually undetectable in the nucleus. It was also possible to demonstrate for the first time the presence in endometriosis of a positive feedback circuit created by a high concentration of E2 leading to stimulation of the PI3K pathway favoring the binding activity of the of NFkB to the DNA, with the consequent decrease in transcription and expression of PTEN protein and new activation of the PI3K pathway. This circuit is responsible for the continuous proliferation of endometrial cells, contributing both to the pathogenesis as well as to the development of endometriosis (Figure 1f*).[50]

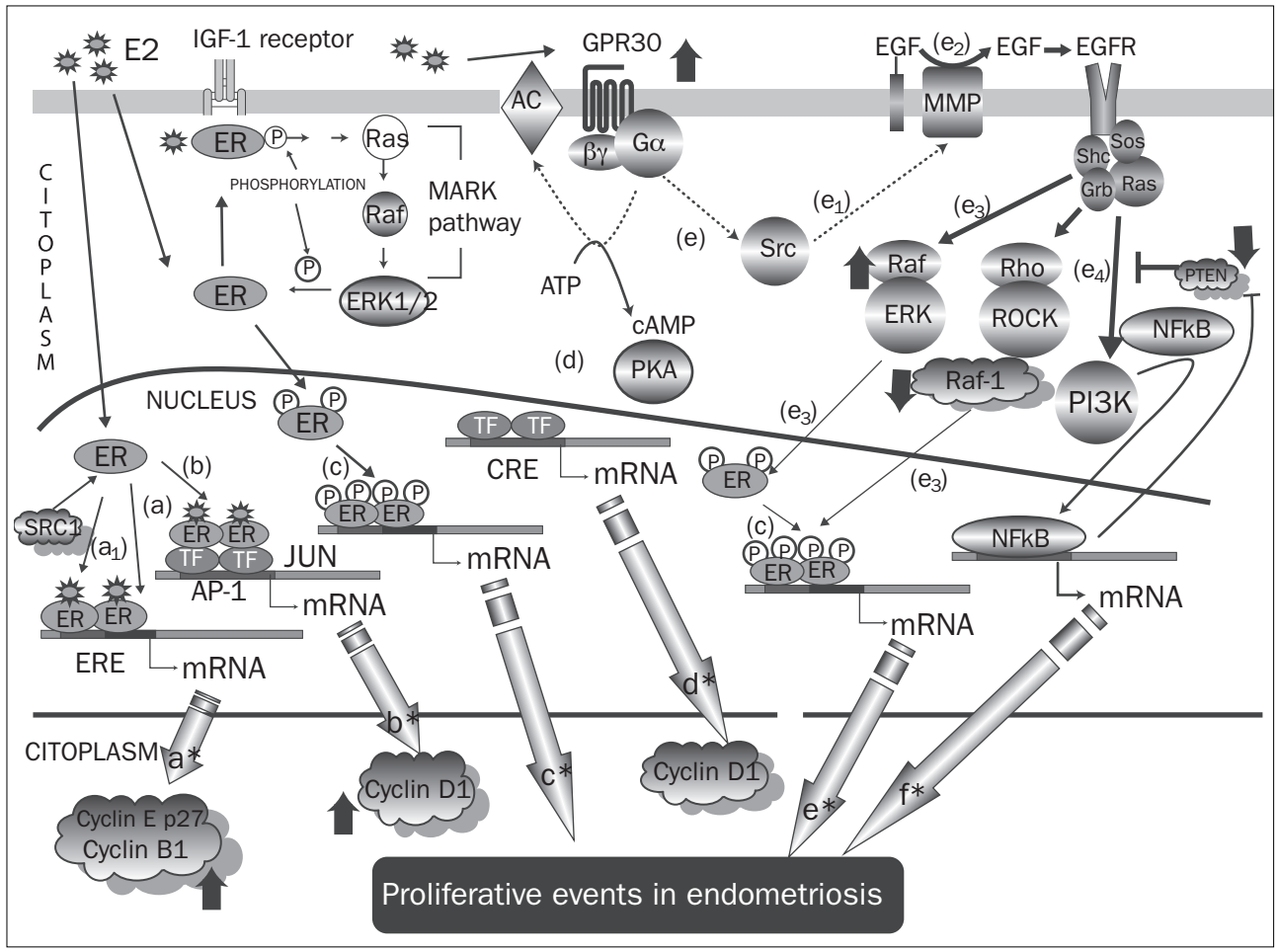

FIGURE 1 Major signaling pathways in the proliferative endometrium and in endometriosis. In the genomic model, e2 interacts directly with its receptor ER and binds to ERE sequences in the DNA (a). ER can also interact with coativactor proteins (a1) or with other transcription factors (b). In endometriosis: 1) high expression levels of cyclin B1 and SRC1 was observed (a*), 2) ER interacts with JUN at the AP-1 binding site and high expression of cyclin D1 was observed (b*). In the non-genomic model, ER can be phosphorylated by IGF-1 receptor that stimulates MAPK pathway (Ras - Raf- MAPKK- ERK1/2) leading to stimulation of proliferative events in endometrial carcinoma, but not confirmed in endometriosis (c*). Also w2 can interact with GPR30 that activates PKA pathway (d). In endometriosis, GPR30 is highly expressed and abnormal changes in cyclin D1 levels are observed (d*). GPR30 can also activate the protein c-Src (e, e1,e2). This results in activation of two important pathways: MAPK (e3) and PIK3 (e4). In endometriosis, low levels of Raf-1 and PTEN culminate in abnormal proliferative events e* and f*, respectively. Protein high levels in endometriosis: Pathways in endometriosis: a*– f*.

E2: 17β-estradiol; ERE: estrogen response element; TF: transcription factor; SRC1: steroid receptor coactivator protein; insulin-like growth factor 1 (IGF-1); MAPK mitogen-activated protein kinase; GPR30: G protein-coupled receptor 30; PKA: protein kinase A; c-Src: cellular sarcoma protein kinase; PI3K: phosphatidylinositol-3-phospahte kinase; PTEN: protein phosphatase and tensin homolog. Adapted partially from "Oestrogen-dependent regulation of miRNA biogenesis: many ways to skin the cat" by A Gupta, E Caffrey and S Gupta, 2012, Biochem Soc Trans, 40:752 and from: "Molecular mechanisms mediating the G protein-coupled receptor regulation of cell cycle progression", by DC New and YH Wong, 2007, J Mol Signal.

Final considerations

Since endometriosis is a disease mediated by estrogen, the medications used so far to treat the disease aim basically at decreasing the serum levels of this hormone through action at hypothalamic-pituitary level. Advancement in knowledge of the mechanisms of estrogen-mediated signaling in the endometrial cell will allow identification of new intracellular targets for therapies inhibiting the altered signaling pathways that contribute to the proliferation and migration of endometriotic cells.

Resumo

Sinalização pelo estrogênio no endométrio proliferativo: implicações na endometriose

Embora esteja bem estabelecido o papel fisiológico do estrogênio no ciclo reprodutivo feminino e na fase proliferativa do endométrio, as vias de sinalização por meio das quais a ação do estrogênio é exercida no tecido endometrial são ainda pouco conhecidas. Nesse sentido, o avanço nas técnicas de cultura celular e a manutenção de células endometriais em cultivo possibilitaram a descoberta de novos mecanismos sinalizadores ativados pelo estrogênio no endométrio normal e na endometriose. Esta revisão tem o objetivo de apresentar as descobertas recentes envolvendo as vias de sinalização genômica e não genômica do estrogênio no endométrio proliferativo humano, especificamente associadas à patogênese e ao desenvolvimento da endometriose.

Palavras-chave: endométrio, estrogênios, transdução de sinal, endometriose.

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