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Review article

p27^{kip1} as a key regulator of endometriosis

G.A. Gonçalves

Faculdades Integradas Padre Albino (FIPA), Rua dos Estudantes, 225, Cep - 15.809 -144, Catanduva, São Paulo, Brazil

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ABSTRACT

p27^{kip1} as a key regulator of endometriosis Gonçalves GA p27^{kip1} is a cyclin-dependent kinase (CDK) inhibitor whose specific late G1 destruction allows progression of the cell across the G1/S boundary. There is a direct relationship between low level of p27 and rapid proliferation occurring in several benign states and in many malignances. In the glandular cells of the normal endometrium, the level of p27^{kip1} is exceedingly low during the proliferative phase, whereas it is markedly increased during the secretory phase. The expression of p27^{kip1} in endometriosis is very low but has been found to increase following treatment with progesterone. However, estrogen exposure is considered as a major risk factor in developing endometrial cancer. Endometriosis endometrial cells cultures have also lower levels of p27^{kip1} compared to heath endometrial cells cultures and restore the cell cycle balance when transduced with an adenoviral vector carring the p27^{kip1} coding gene (Adp27EGFP). More uniform and rigorous studies are required to confirm these and additional markers utility in a diagnostic and possible treatment panel. As a major clinical priority is to determine which lesions can be treated medically and which require surgical intervention, focusing future studies on markers that distinguish response to hormone therapy or are involved in hormone regulation, will be important future considerations. The goal of this highlight review is to provide a broad overview of the advancements in studies about endometriosis mainly correlating the cytokine p27^{kip1} expression with the diagnostic and disease treatment.

Contents

Introduction

Endometriosis establishment requires the implantation and survival of endometrial cells outside of uterus, which is supposed to be a polygenic inherited disease with multifactorial etiology related to genetic, immunologic, hormonal and environmental factors [1–3]. Molecular disorders in the topic endometrium from women with endometriosis, such as escape from apoptosis, degradation of extracellular matrix, invasion, recruitment of inflammatory cells, acquisition of steroidogenic capacity, evasion from immune system and enhanced angiogenesis capacity have already been established [4–7]. Several factors, such as increased of inflammatory activity in the peritoneal fluid, angiogenesis and up-regulating of pro-inflammatory cytokines may facilitate the pathogenesis of endometriosis, which is assumed to be a complex process [8].

An important cytokine in the development of endometriosis is p27^{kip1}. This protein plays a key role in cell cycle regulation by controlling the G1 to S phase transition, binding to numerous complex cyclins/CDKs (cyclin dependent kinases) throughout the cell cycle, and is an example of a CDK inhibitor, whose maladministration is found in several types of cancer [9–13]. The expression of p27^{kip1} not only has a significant relationship

E-mail address: goncalves.giovana2@gmail.com (G.A. Gonçalves).

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with cell differentiation, but when bound to cyclin E/CDK2, it also prevents the cell cycle from starting in the absence of external stimulus [14]. An important regulatory mechanism to control the inhibitory function of $p27^{kip1}$ is to regulate p27 protein levels through transcription, translational and post-translational mechanisms [15–17] (Fig. 1). The critical function of $p27^{kip1}$ is to inhibit CDK-cyclin E complex by controlling a checkpoint in the G1 in normal cells. When $p27^{kip1}$ is not present in the cells, cells are not follow a cell cycle control signal and proliferation [9]. Because endometrial cell cycle changes may be involved in cell cycle regulation of endometriosis in women with diseases significance of $p27^{kip1}$ protein level it may be seen that a change in the lining of a particular cell cycle is important [18].

The goal of this highlight review is to provide a broad overview of the advancements in studies about endometriosis mainly correlating the cytokine $p27^{kip1}$ expression with the diagnostic and disease treatment.

$p27^{kip1}$ and endometriosis

Schor et al. [19] demonstrated a significant decrease in the levels of p27 protein in the epithelium and stroma in the second phase of the cycle in women with endometriosis. These data suggest an alteration in the cell cycle of endometrial cells and suggest alteration of a signaling pathway. Decreased levels of this protein, in both the epithelium and the stroma, in endometriosis suggests that various cell types maybe involved in the genesis of the disease. These results corroborate an earlier report by Johnson et al. [20] which found that expression of c-Myc, transforming growth factor β1, and Bax genes resulted in a significant increase in the proliferation index of endometrial epithelia of women with endometriosis. This study was the first one to emphasize an alteration in the expression of a specific cell cycle regulatory protein, which suggested a signaling pathway that may be altered in endometriosis. This alteration was observed in the multiple stages of the disease, suggesting that it may be an initiation factor and that progression of the disease to advanced stages is probably due to other factors.

One of the critical steps around endometriosis pathogenesis is an active angiogenic process [21], highlighted by increased levels of growth factors in peritoneal fluid [22] and angiogenic potential of eutopic endometrium [23]. Thus, an intricate network of host angiogenic and immune responses are activated in pelvis of endometriosis patients, which allow the implanting and growing of ectopic endometrial cell. However, the molecular process of angiogenic activation is still unclear.

VEGF (Vascular Endothelial Growth Factor) is a secreted heparin-binding homodymeric glycoprotein of z 46 kD, with several protein variants resulting from alternative splicing of VEGF mRNA [14,24]. It action is triggered by tyrosine kinase receptors, fms-like tyrosine kinase (flt) or kinase domain receptor (KDR), present predominately on endothelial cells [25]. The VEGF is not only a potent endothelial cell mitogen, morphogen, and vascular permeability–inducing agent, but it activation also leads to the expression of a number of proteolytic enzymes involved in the process of angiogenesis [26]. VEGF is expressed by eutopic endometrium [27], and is overexpressed in peritoneal fluid from endometriosis patients [28] may indeed contribute to the development of vasculature and the subsequent maintenance of endometriotic explants [29].

Members of the Cip/Kip family of cyclin-dependent kinase inhibitors are well characterized for their roles as negative regulators in the G1-phase cell cycle progression. However, emerging studies suggest that $p27^{kip1}$ play roles additional to cell cycle controls [30,18].

$p27^{kip1}$ as a key regulator

Studies showing the high proliferation rates and lower expression levels of $p27^{kip1}$ in eutopic endometrial cells suggest that $p27^{kip1}$ maybe take part in this cell cycle disorder [31,19,32,33]. Both the processes, diminished $p27^{kip1}$ and enhanced

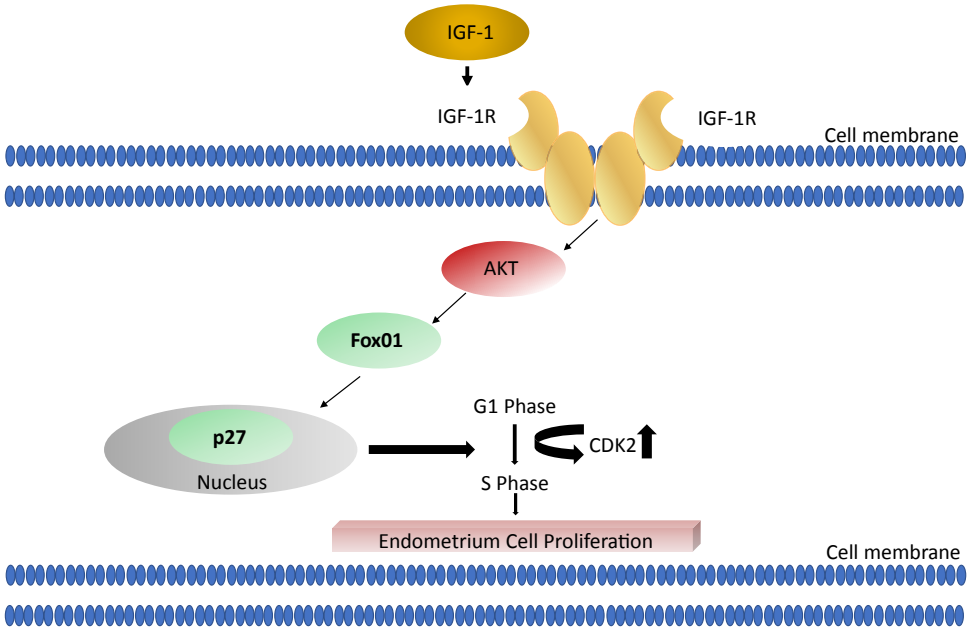

Fig. 1. Schematic diagram of proposed molecular mechanism of p27 downregulation in cell proliferation in endometriosis. We proposed that downregulation of p27 plays an essential role and may also estimulate growth factors and their receptor, such as IGF-1 and IGF-1 receptor (IGF-1R), which are known regulators of uterine leiomyoma growth. The downregulation of p27 leads to G1/S phase transition with accompanying increases in cyclin-dependent kinase 2 (CDK2) expression. The promotion of G1 to S phase cell cycle progression results in cell proliferation, which can contribute to tumor growth and expansion. AKT, also known as protein kinase B, is a serine/threonine-sprecific protein kinase. p27, also known as $p27^{kip1}$, is a CDK inhibitor.

VEGF expression and previous studies in animal prompted us to investigate the relationship between $p27^{kip1}$ expression and VEGF synthesis by primary endometrial cells cultures from endometriosis patients. We firstly observed that primary culture cells from eutopic endometrium in endometriosis patients express higher basal levels of VEGF. Then, we demonstrated that overexpression of $p27^{kip1}$ in endometrial cells from women with endometriosis is able to regulate the VEGF expression to levels closely to those observed in women without endometriosis [18].

The expressions of a wide array of cytokines are abnormal in the processes of tumor invasion and metastasis as well as in endometriosis [34]. Among them, VEGF and MMP-9 play important roles in the processes. Progression of malignant tumor is characterized by the formation of new vessels and the newly formed vessels act as a bridge between tumor and circulation system, which facilitate the metastasis of cancer cells to distant parts of body. The formation of new vessels entails participation of a good many cytokines and among them, VEGF is believed to be the most potent vessel-forming factor. Chen [35] revealed in their study that Ad-p27 could inhibit the expression of VEGF and thereby suppressed the generation of tumor-feeding vessels and stopped the growth of tumors.

In vitro study demonstrated the MIF (Macrophage Migration Inhibitory Factor), VEGF and $p27^{Kip1}$ are molecules involved in a network of inflammatory and angiogenic systems [36], but mechanisms of regulation remain unclear. Also, down-regulation of $p27^{Kip1}$ appears to be early event in endometriosis and may be important for the establishment of the endometriotic implants [32].

The expression of $p27^{kip1}$ is subject to multiple mechanisms of control involving several transcription factors, kinase pathways and at least three different ubiquitin ligases, which regulate $p27^{kip1}$ transcription, translation, protein stability and subcellular localization [37]. We observed the control group also showed a small decrease of VEGF after $p27^{Kip1}$ transduction, while that difference on endometriosis cells presented a higher range. On the other hand, the longitudinal analysis showed the modification of VEGF levels according to culture time was not significant in control group. Those results suggest $p27^{kip1}$ regulation is not affected on control cells [18].

In women with endometriosis, the peritoneal fluid has high concentrations of cytokines, growth factors, and angiogenic factors [38], derived from the lesions themselves; secretory products of macrophages and other immune cells; and follicular fluid after follicle rupture in ovulating women. Once endometriotic lesions are formed, they secrete several pro-inflammatory molecules [39]. IL-1β has been suggested to support the development of endometriosis through the production of various inflammatory molecules including IL-6, IL-8, MCP-1 and COX-2 [40]. The present finding raises a novel notion that IL-1β may promote the disease through the microenvironment with stem cell. The presence of stem progenitor cells in endometrium and in menstrual blood led to the hypothesis that these cells could be at least in part responsible for the development of endometriosis. Lots of studies in the last decade have contributed to the consolidation of this hypothesis, through different approaches [41]. In one study, it was suggested that the basal layer of endometrium was significantly shed in menstrual flow in women with endometriosis, in comparison with control women [42]. Interestingly, endometrial stem cells are particularly frequent in endometrial tissue during menstruation. It has been speculated that endometrial stem cells may play an important role in the development of endometrial implants [43]. Moon & Mantzoros [44] demonstrated that adiponectin induces cell cycle arrest in certain cancer cell lines such as hepatoma HepG2, prostate carcinoma PC-3, and breast cancer MCF-7 by upregulating tumor suppressor genes such as p53, p21, and p27 [45–47]. Also, it has been demonstrated that these effects on cancer cell proliferation are associated with decreased expression of cyclins and increased expression of p27 [48,49]. Recently, we demonstrated that an increase in $p27^{kip1}$ expression leads to a decrease in IL-1ß expression [50].

Considerations and perspectives

A strong reduction in the tumor suppressor gene $p27^{kip1}$ results in a high resistance and poor prognosis in cancer. Also, $p27^{kip1}$ has an important role cell cycle regulatory factors. Recently, $p27^{kip1}$ has been reported that many women involved in diseases such as breast cancer, endometriosis and ovarian cancer.

Further studies strongly demonstrate the role of $p27^{kip1}$ as a key factor in regulating progression of endometriosis because those findings, in the future, might open up interesting perspectives for the use of $p27^{kip1}$ targeted gene therapy as an alternative or additional strategy in the treatment of endometriosis.

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