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A potential role of cyclin-dependent kinase inhibitor 1 (p21/WAF1) in the pathogenesis of endometriosis: Directions for future research

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ABSTRACT

Endometriosis is a common gynecological disorder that affects approximately 6–10% of the female population impairing the quality of life of patients. Several pathophysiologic pathways have been proposed as potential regulators of its severity; however, to date, the processes that trigger the onset and that influence the severity of the disease are not fully understood; hence, leading to disease recurrence in approximately 10–67% of cases. Cyclin-dependent kinase inhibitor 1 (p21/WAF1) is a protein that is a major target of p53 and is related to cell cycle arrest (it regulates transition from the G1 to the S phase) when DNA damage is detected. Its activity has been also linked to the angiogenic potential of tumors as it promotes the expression of various kinases that are responsible for endothelial development and function. Although several articles have underlined the importance of this protein in cancer cell development and tumor growth, there are no relevant data in the field of endometriosis. Indirect evidence suggests, however, that it may be involved in the pathogenesis of endometriosis as it inhibits the activity of various kinases which have been correlated with the course and severity of the disease. The present article investigates the background theory that implies the potential role of cyclin-dependent kinase inhibitor 1 (p21/WAF1) in the pathogenesis of endometriosis. Implications for future research are also provided given that indirect evidence seem to associate downregulation of p21 with decreased growth and invasiveness of human endometrial stromal cells.

Introduction

Endometriosis constitutes a common gynecological disorder that affects approximately 6–10% of reproductive-age women worldwide [1,2]. Various theories have been proposed concerning the pathophysiological background of the disease. Recently researchers proposed that endometriosis has a genetic/epigenetic background that may be transmitted during birth, thus, explaining the hereditary nature of the disease [3]. The perpetual bleeding of endometriotic lesions provokes local inflammatory reactions, such as scar tissue formation and adhesions which in turn result in various symptomatology including dysmenorrhea, dyspareunia, chronic pelvic pain, infertility, tenesmus etc [4]. These symptoms have a direct impact on patients' quality of life and it is estimated that the quality-adjusted life-year (QaLY) of endometriosis reaches 0.82 [5]. It is estimated that women with significant symptomatology may have decreased work productivity that reaches approximately 10 working hours per week with a cost burden that ranges between US$4 in Nigeria to US$456 in Italy [6]. Consequently, studies investigating the economic burden of the disease report that the total annual costs per woman may reach 10,000 euros [7].

Thorough understanding of the pathogenesis and the pathophysiology of endometriosis is fundamental and crucial, since it constitutes the cornerstone for the development of novel diagnostic techniques and effective treatments of this gynecological disorder [8,9]. Herein, there is an urgent need for novel research activities that emphasize in the identification of specific genes and biological pathways that are responsible for increasing disease risk.

The present article investigates the background theory that implies the potential role of cyclin-dependent kinase inhibitor 1 (p21/WAF1) in the pathogenesis of endometriosis.

The hypothesis

Endometriosis refers to the ectopic presence of functioning

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endometrium outside the uterine cavity [2]. Excessive proliferation of endometriotic cells in addition to apoptosis-evading properties seem to participate in the development of endometriosis [10]. Previous articles underlined the importance of this latter characteristic of endometriotic cells as these seem to exhibit increased expression of anti-apoptotic factors and decreased expression of pro-apoptotic factors compared to the eutopic endometrium of healthy women [11].

Endometriosis is also associated with augmented angiogenesis since the creation of novel vascular source in ectopic endometrium presupposes vascular proliferation and differentiation [12]. Current evidence suggests that uncontrollable angiogenesis in several tumors is correlated with a dysregulation of the gene of the protein p21(Cip1/Waf1) [13] and it may also be an important factor that accentuates the development and expansion of endometriotic lesions. Confirmation of the key-role of protein p21 in angiogenesis and endometriosis might lead to the subsequent development of therapies that could be used in combination with existing strategies in order to block the expansion of endometriotic lesions and achieve the definitive treatment of endometriosis.

Current evidence

The investigated protein p21 is a cyclin-dependent kinase inhibitor, related with multiple functions in the cell, and a transcriptional target of the p53 protein [14]. In the nucleus p21(Cip1/Waf1) inhibits the activity of cyclin dependent kinases (Cdk's) including Cdk1 and Cdk2 and subsequently blocks the transition from G1 phase into S phase or from G2 phase into mitosis when the DNA is damaged (Fig. 1) [15,16]. Its role is similar to that of p27 which has already been investigated in the field of endometriosis and which seems to be downregulated in endometriotic lesions [17].

Beta cell lymphoma 2 (Bcl-2) protein is another regulatory protein that is responsible for programming of apoptosis and which seems to be associated with p21 activity. Specifically, evidence in the field of breast cancer suggests that an interplay between Bcl-2 and p21 exists as reduced p21 protein expression was associated with over-expression of Bcl-2 [18]. Endometriotic lesions seem to have increased activity of Bcl-2 which results in enhanced cell survival due to reduced apoptotic index [19].

In the cytoplasm, p21(Cip1/Waf1) protein seems having an anti-apoptotic effect, as it inhibits caspase 3, as well as the apoptotic kinases ASK1 (Apoptosis signal-regulating kinase 1) and JNK (c-Jun N-terminal kinase) [20]. Recently, caspase 3 (Casp 3) has been implicated in the pathophysiology of endometriosis as researchers observed that patients with higher levels of this protein seem to have higher stages of the disease [21]. In a previous systematic review, McKinnon et al proposed that kinase signaling pathways should be viewed as viable targets for endometriosis treatment [22] and in this line we believe that targeting of cyclin-dependent kinase inhibitors including p27 and p21 (which are regulators of several kinases) may help succeed remission of the disease.

The actual role of p21-activated kinases (Paks) remains relatively unexplored, however, a previous experimental study suggested that their role is crucial during early endothelial development and function and is essential as well for adult blood vessel maintenance [23]. It is, therefore, anticipated that p21 has also a regulatory effect in the angiogenic process as well. This is confirmed through several pathophysiological pathways. For instance, p21(Cip1/Waf1) also induces activin A-mediated growth inhibition in vascular endothelial cells [13]. Activin A is a key regulatory protein in the pathophysiology of endometriosis as previous experimental studies have found that intraperitoneal administration of this protein promotes the development of the disease, together increasing the levels of cytokines including TNF-a (Tumor necrosis factor-a) and interleukin 6 [24]. Another potential protein that regulates the process of angiogenesis in endometriotic lesions is thioredoxin (Trx) which has been proved to be downregulated in p21 knockdown cancer cells [25,26].

Indirect evidence in the field of breast cancer also suggest p21 has the ability to trigger the activation of the estrogen-signaling pathway via a route that is independent of the action of estrogen receptors [27]. The whole process seems to be mediated by cyclins and particularly by overexpression of the G1/S cyclins D1, E or A [28]. Taking this information in mind, one could assume that loss of function of the p21 gene could partially suspend this process; thus, depleting the estrogenic environment that is required to sustain growth of endometriotic lesions.

Evidence in the field of endometriosis is extremely limited, however, current data seem to be promising. Specifically, researchers observed that ectopic endometrium retrieved by adenomyotic lesions has increased expression of the p21-activated kinase 4 (Pak4) compared to eutopic endometrial stromal cells [29]. The pathway seems to be regulated by the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) as in vivo supplementation of these cultures with pyrrolidinedithiocarbamate (an NF-kB inhibitor) seems to inhibit the increase of Pak4. Recently, Li et al also observed that p21 is involved in the process of granulosa cell proliferation in endometriotic lesions and its action is triggered following activation of the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway [30].

On the other hand, single nucleotide polymorphisms of the p21 gene do not seem to be important according to Ying et al as neither of the investigated haplotypes of codon 31 (a locum that has been implicated in ovarian cancer progression) seems to be associated with the development of endometriosis [31].

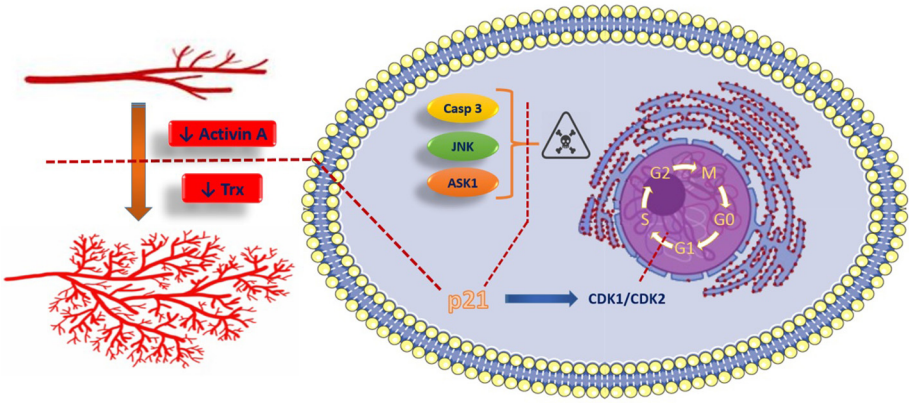

Fig. 1. Pathophysiological pathways in which p21 is presumed to be involved. Activation of the CDK1/2 system leads to cell cycle arrest in the G1/S transition. Deactivation of Casp 3, JNK and ASK1 reduces apoptosis and downregulation of Activin A and thioredoxin (Trx) reduces vascular growth.

Animal research models

Nowadays, experimental animal research, which is primarily based in rodents, has established several models for endometriosis, with the autologous or syngeneic rodent model being the most frequently used [32]. p21 deficient mice have been previously used in the field of cancer and seem to exhibit enhanced tumor suppressive activity [33]. Cell growth arrest that is exhibit in these cells is believed to be owed in impaired angiogenesis which is the result of the activation of p21 [34]. Deactivation of p21 in transgenic mice restores this process, thus, underlining the important role of this protein in the whole process.

Implications for future research

Current evidence which is primarily drawn from the field of cancer suggests that p21 is a key regulatory protein that is involved in tumor growth and the mechanisms involved seem to be similar to those encountered in the field of endometriosis. Its function seems to trigger several processes including regulation of the cell cycle, induction of programmed cellular death and angiogenesis control. To date, however, its potential role in the field of endometriosis remains relatively unexplored and deserves further investigation as it may become an adjunct target therapy which could be used together with established therapies in this field. Experimental models based in transgenic mice may be used to understand the actual role of p21 in tumor growth and angiogenic potential of endometriotic lesions.

Consequently, if the results of experiments in p21(Waf1/Cip1)-deficient are promising the development of novel therapies may be proposed in the field. Although, to date, specific drugs that target the function of p21 are not commercially available at 2012 Liu et al proposed that small-molecule inhibitors of p21 (like UC2288) may have tremendous potential in cancer cells [35]. Their findings were recently confirmed in two studies and the whole process seems to be triggered by G1 cycle arrest, although the effects of the drug in the angiogenic potential of tumors was not investigated [36,37]. Moreover, a recent experimental study in HeLa (human cervical carcinoma) cells suggested that fluvastatin and lovastatin may regulate p21 function and, thus, serve as medications that could be used indirectly in the field of endometriosis [38]. Sokalska et al also recently suggested that growth and invasiveness of human endometrial stromal cells may be downregulated by lipophilic statins and that the process was mediated by caspases 3/7; however, they did not report data on the activity of p21 (a key regulator protein for the expression of several caspases) [39].

Given these, further research is deemed necessary in the field to help elucidate the complex pathway that involves the function of p21 in endometriosis and experimental models may form the foundation which will provide adequate evidence to support the use of novel therapies in the clinical setting. Transgenic $p21^{-/-}$ mice have been already in the field of cancer [40,41] and these may be used in the field of endometriosis as well, by using established experimental methods [42]. Pathologic analysis of tissue specimens as well as proteomic and transcriptomic analysis of developed lesions may help elucidate the actual role of p21 in the pathophysiology of endometriosis as well as in the numerous clinical aspects of the disease. This should ideally target the angiogenic potential and the mitotic activity of lesions developed in transgenic mice and compare them to non-transgenic strains. Following that, interventional experiments may be planned using described p21 inhibitors to elucidate their potential role in the course of the disease as well as their mode of function.

Conclusion

Cyclin-dependent kinase inhibitor 1 (p21/Waf1) is a protein that regulates cell cycle, angiogenic potential and programmed cell death in various cell lines. Its effect in the field of endometriosis remains to date poorly explored, although at least two studies seem to suggest that it may be involved in the process of ectopic endometrial tissue formation. Indirect evidence is also abundant in this field and suggests that p21 regulates growth of endometriotic lesions via cyclin dependent kinases and by modulating the function of several cytokines (including activin A, TNF-a and IL-6) as well as of proteins that seem to be involved in the process of angiogenesis. Future research is needed to shed light in this interesting field as modification of the activity of p21 with novel drugs may help limit the extent of the disease and alleviate the accompanying symptoms which are debilitating in several cases and have a detrimental impact on the patients' quality of life.

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Consent

Written consent was not provided as this paper describes a medical hypothesis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2019.109414.

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