IntechOpen

Molecular Bases of Endometriosis

The Integration Between Research and Clinical Practice

Edited by Giovana Aparecida Gonçalves

Molecular Bases of Endometriosis - The Integration Between Research and Clinical Practice

Edited by Giovana Aparecida Gonçalves

Published in London, United Kingdom







IntechOpen






Supporting open minds since 2005

Molecular Bases of Endometriosis – The Integration Between Research and Clinical Practice
http://dx.doi.org/10.5772/intechopen.79037
Edited by Giovana Aparecida Gonçalves

Contributors
Alfredo Borges, Ningning Wang, Manuela Cristina Russu, Giovana Gonçalves

First published in London, United Kingdom, 2019 by IntechOpen
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, The Shard, 25th floor, 32 London Bridge Street
London, SE19SG – United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Molecular Bases of Endometriosis – The Integration Between Research and Clinical Practice
Edited by Giovana Aparecida Gonçalves
p. cm.
Print ISBN 978-1-78984-607-2
Online ISBN 978-1-78985-135-9
eBook (PDF) ISBN 978-1-78985-136-6

Meet the editor

Dr. Giovana Aparecida Gonçalves is a professor of Medicine and Nursing Courses at Padre Albino University Center, UNIFIPA, and a collaborating professor of Master's and Doctorate Degrees at the Department of Gynecology, UNIFESP (Federal University of São Paulo). Dr. Gonçalves has a PhD in Sciences from the Heart Institute, Medical School, São Paulo University, USP (2008); a postdoctoral degree from the Department of Gynecology, UNIFESP (2009–2014); and a postdoctoral degree from the Laboratory of Angiogenesis and Neurovascular Link, Vesalius Research Center, KU Leuven, Leuven, Belgium, under the supervision of Prof. Peter Carmeliet (2014–2015).
Dr. Gonçalves develops projects in the area of gene therapy and cell therapy with a focus on angiogenesis.

Contents

Chapter 1

Introductory Chapter: Changes in the Approach of the Patient with Endometriosis and the Development of Genetics and Molecular Biology in Gynecology

Giovana Aparecida Gonçalves

1. Introduction

Endometriosis is characterized by the presence of endometrial-like functional tissue located outside the uterine cavity, most commonly in the pelvic peritoneum, ovaries and rectovaginal septum, and more rarely in the pericardium, pleura and central nervous system. The studies indicate a prevalence of up to 20% of women of reproductive age [1] and 30–50% of infertile women with endometriosis [1].

2. Clinical condition

The clinical condition of the patient with endometriosis is quite variable. The patient may be asymptomatic, refer only to infertility, or have symptoms such as severe dysmenorrhea, profound dyspareunia, chronic pelvic pain, ovulatory pain, urinary symptoms or peri-menstrual bowel movements, and chronic fatigue. Gynecological examination may be normal, but the presence of pain in uterine mobilization, uterine retroversion, or increase in ovarian volume is suggestive of endometriosis, although it is not specific. Other conditions, such as irritable bowel syndrome, pelvic inflammatory disease, and interstitial cystitis may present similar symptoms and should be included in the differential diagnosis. Signs suggestive of deep infiltrative endometriosis are palpable nodulations in the posterior vaginal fornix or rectovaginal septum, thickening of the uterosacral ligaments, or violaceous lesions in the vagina [2].

3. Diagnostic evaluation of endometriosis

Although the definitive diagnosis of endometriosis requires surgical intervention, preferably through videolaparoscopy, several findings in physical, imaging, and laboratory tests can already predict, with a high degree of reliability, that the patient has endometriosis. To date, no biochemical marker can be considered as an endometriosis endpoint, but Ca-125, when collected on the first or second day of the menstrual cycle, may be useful for the diagnosis of advanced stage endometriosis when the values are higher than 100 IU/mL [3]. Although normal

concentrations do not exclude the disease, cases with elevated preoperative levels may aid in patient follow-up and clinical suspicion of recurrence of endometriosis. More recently, some cytokines have been studied as new nonsurgical endometriosis markers. Interleukin-6 (IL-6) appears to perform better than other cytokines in discriminating patients with endometriosis [4]. The first imaging test to be applied to the patient with a history and physical examination suggestive of endometriosis is transvaginal pelvic ultrasound, preferably with intestinal preparation. A study by Abrão et al. [5], evaluating the accuracy of this test, demonstrated a sensitivity of 94% and a specificity of 98% in the identification of foci of deep endometriosis. If the test is normal, the patient may not have endometriosis or have noninfiltrative initial disease. On the other hand, if the test is conclusive for ovarian endometriosis, rectovaginal septum or rectosigmoid, or urinary tract, treatment may be indicated without additional imaging tests. For evaluation of endometriomas larger than 2 cm, transvaginal ultrasonography is an efficient method, according to Moore et al. [6]. The presence of ovarian masses with a doubtful diagnostic hypothesis can be better evaluated with magnetic resonance imaging (MRI). Changes suggestive of rectovaginal septum disease, uterosacral, or rectosigmoid ligaments may be confirmed by rectal echoendoscopy or MRI. Rectal echoendoscopy allows the identification of the distance between the lesion and the rectal lumen as well as extrinsic compressions and submucosal lesions of the rectum [7]. MRI also allows the identification of deep disease with invasion of the intestinal tract, but it does not make it possible to specify the intestinal layer affected by the lesion [8]. Transvaginal ultrasonography for the diagnosis of bladder endometriosis has been reported as an effective method, with sensitivity of 71.4% and specificity of 100% [9]. Ultrasonography suggestive of bladder or ureteral endometriosis can be complemented with excretory urography, which may show ureteral narrowing. Uro-resonance can be used as an alternative method to excretory urography for evaluation of renal collecting system dilatations. Although the available imaging exams presented good accuracy in the diagnosis of endometriosis, laparoscopy with lesion biopsy for anatomopathological analysis is still the gold standard in the diagnosis of endometriosis.

4. Classification of endometriosis

After videolaparoscopy, endometriosis can be classified according to the histological type of the implants, with the anatomical location of the disease—peritoneum, ovary or rectum—or by the extension of the disease to the pelvic organs. The most commonly used classification is that of the American Society of Reproductive Medicine—revised in 1996 [10]. This classification rates minimal, mild, moderate, or severe endometriosis due to the extent of disease in the peritoneum and ovaries, as well as the presence of tube-ovarian and Douglas sack bottom block. This classification, although with some limitations, is quite useful in the orientation of postsurgical treatment, especially when the patient's complaint is infertility.

5. Critical analysis of treatments for endometriosis

The therapeutic approach to endometriosis varies, depending on the patient's complaint, pelvic pain or infertility, although such complaints are often associated. Gonadotropin-releasing hormone (GnRH) analogues, GnRHa, may be indicated for three months and then continue with oral contraceptives. If the patient has

recurrence of pain, or an image suggestive of endometrioma greater than 3 cm or suspected of adherence, surgery should be indicated.

6. Surgical treatment of endometriosis

Surgical treatment of endometriosis involves procedures of low complexity, such as the treatment of superficial foci and the release of tendon adhesions, to complex interventions in the ovaries, Douglas sacs, intestines, bladder, and ureters, requiring, in some cases, a multidisciplinary team. For several years, the surgical treatment of endometriosis was based on the oncological principles of radical removal of the lesions. This principle is still used when it comes to cases of intestinal or ureteral stenosis or ovarian masses of doubtful characteristics. However, we currently know that there is no correlation between the disease with the severity of the symptoms, as well as the reproductive prognosis and long-term recurrence of pain [11]. In addition, many patients present infertility associated with pain, requiring that the surgical procedure be conservative. Based on these considerations, some authors recommend surgical treatment only for patients who do not respond to drug treatment, as well as for those who wish to become pregnant spontaneously [12]. There are few published randomized clinical trials evaluating the outcome of surgical treatment of symptomatic endometriosis. A review by Vercellini et al. [12] describes symptomatic improvement after conservative treatment of around 60–80%, with recurrence of symptoms and reoperation rate ranging from 12 to 58% between studies.

For the patient with infertility, follicle ablation and adhesiolysis appear to improve fertility in the minimal and mild degrees of disease [1]. In cases of moderate or severe degrees, there are no randomized clinical trials or meta-analyses available to answer if resection of foci would increase gestation rates.

7. Molecular genetics and endometriosis

Endometriosis exhibits similarity with cancer since endometrial cell implants require neovascularization to establish, grow, and invade tissues. In addition, the etiopathogenic theories of endometriosis involve growth factors and cytokines associated with regulation of cell multiplication and neoangiogenesis that may act on carcinogenesis. It is estimated that 1% of cases of endometriosis is related to cancer, and for some types of endometriosis, its benign nature has been questionable [13, 14].

Although the definitive diagnosis of endometriosis necessitates a surgical intervention, called video-laparoscopy, several findings in the physical, imaging and laboratory examinations can already predict, with a high degree of reliability, that the patient has this disease. During this surgical procedure, it is possible to visualize lesions suggestive of the disease and to obtain a tissue specimen for anatomopathological analysis and confirmation of the diagnosis of endometriosis [15]. The classification used for endometriosis is that of the American Society of Reproductive Medicine (ASRM), revised in 1996, which rates this disease in minimal (stage I), mild (stage II), moderate (stage III), or severe (stage IV) [16]. Currently, the most common treatments are surgery, ovarian suppression therapy, or the association of both [13, 15].

The cause of endometriosis remains unknown. However, there is evidence of immunological [17, 18], environmental [19], and genetic [18, 20] factors involved in its pathogenesis.

Regarding the immune response, the role of cytokines in the development of endometriosis [21–23] is highlighted, and elevated levels of several of them have been found in patients with endometriosis [23, 25]. The same group of investigators [24, 26] evaluated the levels of cytokines involved in the Th1 immune response patterns (interleukin (IL)-2, tumor necrosis factor (TNF)-alpha and interferon (IFN) and Th2 (IL-4 and IL-10) in patients with endometriosis (n = 65) and in those without the disease (n = 33). Podgaec et al. [24] observed elevation in IFN-gamma and IL-10 levels in patients with endometriosis, evidencing the coexistence of both responses. However, when considering the ratio of cytokine levels to these responses, IL-4 and IL-10 predominated, thus reflecting a possible shift to the Th2 immune response component. In the subsequent study, 18 cytokine levels were associated with the clinical symptoms of endometriosis. Patients with endometriosis who had depth dyspareunia and infertility exhibited elevated levels of TNF-alpha and IL-2, respectively. These cytokines are related to the Th1 immune response, and almost 70% of the patients who presented these results exhibited deep endometriosis. The authors conclude that when specific clinical data are associated with elevated production of certain cytokines, there is a Th1 response pattern that may be associated with deep endometriosis. Induction of Th1 immune response was also reported by Fairbanks et al. [25], who showed elevated levels of IL-12 in patients with severe endometriosis.

The contribution of environmental factors to the development of endometriosis was reviewed by Bellelis et al. [19] who related their influence and diet to the genetics of this disease. They concluded that the mechanism by which dioxin and its similes (2,3,7,8-tetrachlorodibenzo-p-dioxin/TCDD and polychlorinated biphenyls/PCBs) act to alter endometrial physiology is uncertain and speculative. They also state that there is insufficient evidence regarding the use of diets as preventive factors or even adjuvants in the treatment of endometriosis.

The genetic and hereditary basis of endometriosis was evidenced in the study by Bellelis et al. [19] in which approximately 5.3% of the patients reported a first-degree family history with a history of endometriosis. Familial aggregation, a high concordance rate in monozygotic twins, and a 4–7% risk for first-degree relatives support a contribution of genetic factors to the pathogenesis of this disease [14]. In this context, the identification of genetic variants or single nucleotide polymorphisms (SNPs), responsible for susceptibility to endometriosis, has been the subject of investigation in recent years [26–28]. Different classifications were proposed for endometriosis candidate genes.

8. Conclusions

What are the objectives of the genetic study of individuals? There is a great interest of the medical community and also much concern of the lay press about the potential benefits and harms of genetic screening, gene therapy, and even the possibility of cloning individuals. The current use of genetic tests for the detection and treatment of endometriosis is still at an early stage, but very important. The determination of susceptibility markers will be increasingly explored in clinical studies, and their uses will be much more defined.

Still, it seems increasingly likely that major changes will occur over the next decade in how we evaluate and treat our patients. In particular, surgeons and clinicians will have the opportunity to use a number of new tests to predict the future appearance of endometriosis in patients still free of the disease. They may have the power to explore the best therapeutic modality for a particular patient according to their genetic makeup. And they will be able to more specifically target prevention

measures for family members of people already affected by the disease. It should be understood that molecular diagnosis, especially in asymptomatic individuals, does not mean disease, but an increased risk of developing this disease. Ethical implications exist and should not be underestimated. Patients should be advised about the likely implications of such tests not only after, but especially before the achievement of these.

A major step has already been overcome and we currently have basic tools for a new leap in understanding human pathologies responsible for much of the world's mortality. Bridging the great barrier that still separates this basic knowledge from clinical practice is still a much greater challenge.

Author details

Giovana Aparecida Gonçalves

1 Federal University of São Paulo (UNIFESP), Brazil

2 Father Albino University Center (UNIFIPA), Brazil

*Address all correspondence to: goncalves.giovana2@gmail.com

References

[1] Jacobson TZ, Barlow DH, Koninckx PR, Olive D, Farquhar C. Laparoscopic surgery for subfertility associated with endometriosis. Cochrane Database of Systematic Reviews. 2002;**4**:CD001398

[2] Kennedy S, Bergqvist A, Chapron C, D'Hooghe T, Dunselman G, Greb R, et al. ESHRE guideline for the diagnosis and treatment of endometriosis. Human Reproduction. 2005;**20**(10):2698-2704

[3] Abrão MS, Podgaec S, Pinotti JA, de Oliveira RM. Tumor markers in endometriosis. International Journal of Gynaecology and Obstetrics. 1999;**66**(1):19-22

[4] Othman E-D, Hornung D, Salem HT, Khalifa EA, El-Metwally TH, Al-Hendy A. Serum cytokines as biomarkers for nonsurgical prediction of endometriosis. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2008;**137**(2):240-246

[5] Abrão MS, Gonçalves MO, Dias JA Jr, Podgaec S, Chamie LP, Blasbalg R. Comparison between clinical examination, transvaginal sonography and magnetic resonance imaging for the diagnosis of deep endometriosis. Human Reproduction. 2007;**22**(12):3092-3097

[6] Moore J, Copley S, Morris J, Lindsell D, Golding S, Kennedy S. A systematic review of the accuracy of ultrasound in the diagnosis of endometriosis. Ultrasound in Obstetrics & Gynecology. 2002;**20**(6):630-634

[7] Roseau G, Dumontier I, Palazzo L, Chapron C, Dousset B, Chaussade S, et al. Rectosigmoid endometriosis: Endoscopic ultrasound features and clinical implications. Endoscopy. 2000;**32**(7):525-530

[8] Bazot M, Daraï E. Sonography and MR imaging for the assessment of deep pelvic endometriosis. Journal of Minimally Invasive Gynecology. 2005;**12**(2):178-185

[9] Bazot M, Thomassin I, Hourani R, Cortez A, Darai E. Diagnostic accuracy of transvaginal sonography for deep pelvic endometriosis. Ultrasound in Obstetrics & Gynecology. 2004;**24**(2):180-185

[10] Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertility and Sterility. 1997;**67**(5):817-821

[11] Vercellini P, Fedele L, Aimi G, Pietropaolo G, Consonni D, Crosignani PG. Association between endometriosis stage, lesion type, patient characteristics and severity of pelvic pain symptoms: A multivariate analysis of over 1000 patients. Human Reproduction. 2007;**22**(1):266-271

[12] Vercellini P, Crosignani PG, Abbiati A, Somigliana E, Viganò P, Fedele L. The effect of surgery for symptomatic endometriosis: The other side of the story. Human Reproduction Update. 2009;**15**(2):177-188

[13] Baldi A, Campioni M, Signorile PG. Endometriosis: Pathogenesis, diagnosis, therapy and association with cancer (review). Oncology Reports. 2008;**19**(4):843-846

[14] Bassi MA, Podgaec S, Dias Júnior JA, Sobrado CW, D Amico Filho N, Abrão MS. Bowel endometriosis: A benign disease. Revista da Associação Médica Brasileira. 2009;**55**(5):611-616

[15] Nácul AP, Spritzer PM. Current aspects on diagnosis and treatment of endometriosis. Revista Brasileira de Ginecologia e Obstetrícia. 2010;**32**(6):298-307

[16] Johnson N, Hummelshoj L, Adamson GD, Keckstein J, Taylor HS,

Abrao MS, Bush D, Kiesel L, Tamimi R, Sharpe-Timms KL, Luk R, Giudice LG. World Endometriosis Society Sao Paulo Consortium. World Endometriosis Society consensus on the classification of endometriosis. Human Reproduction. 1 February 2017;**32**(2):315-324

[17] Mathur SP. Autoimmunity in endometriosis: Relevance to infertility. American Journal of Reproductive Immunology. 2000;**44**(2):89-95

[18] Berkkanoglu M, Arici A. Immunology and endometriosis. American Journal of Reproductive Immunology. 2003;**50**(1):48-59

[19] Bellelis P, Podgaec S, Abrão MS. Environmental factors and endometriosis. Revista da Associação Médica Brasileira. 2011;**57**(4):448-452

[20] Zondervan KT, Cardon LR, Kennedy SH. The genetic basis of endometriosis. Current Opinion in Obstetrics & Gynecology. 2001;**13**(3):309-314

[21] Bischoff F, Simpson JL. Genetic basis of endometriosis. Annals of the New York Academy of Sciences. 2004;**1034**:284-299

[22] Simpson JL, Bischoff FZ, Kamat A, Buster JE, Carson SA. Genetics of endometriosis. Obstetrics and Gynecology Clinics of North America. 2003;**30**(1):21-40

[23] Harada T, Iwabe T, Terakawa N. Role of cytokines in endometriosis. Fertility and Sterility. 2001;**76**(1):1-10

[24] Podgaec S, Abrao MS, Dias JA Jr, Rizzo LV, Oliveira RM, Baracat EC. Endometriosis: An inflammatory disease with a Th2 immune response component. Human Reproduction. 2007;**22**(5):1373-1379

[25] Fairbanks F, Abrão MS, Podgaec S, Dias JA Jr, de Oliveira RM, Rizzo LV. Interleukin-12 but not interleukin-18 is associated with severe endometriosis. Fertility and Sterility. 2009;**91**(2):320-324

[26] Falconer H, D'Hooghe T, Fried G. Endometriosis and genetic polymorphisms. Obstetrical & Gynecological Survey. 2007;**62**(9):616-628

[27] Montgomery GW, Nyholt DR, Zhao ZZ, Treloar SA, Painter JN, Missmer SA, et al. The search for genes contributing to endometriosis risk. Human Reproduction Update. 2008;**14**(5):447-457

[28] Tempfer CB, Simoni M, Destenaves B, Fauser BC. Functional genetic polymorphisms and female reproductive disorders: Part II-endometriosis. Human Reproduction Update. 2009;**15**(1):97-118

Chapter 2

The Role of the Molecular Genetic Approach in the Pathogenesis of Endometriosis

Alfredo Borges Garnica

Abstract

Advances in cytogenetic, molecular genetic, and molecular cytogenetic techniques have provided convincing evidence in favor of a genetic basis for endometriosis and corroborating the higher prevalence of the disease in first-degree relatives of affected women. The regulatory mechanisms involved in the morphological and biochemical differentiation of the uterine endometrium are obviously complex, but consistent somatic genetic alterations have been identified. A higher percentage of aberrant metaphases showing aneuploidy, dicentric chromosomes, endomitosis, and chromosomal spraying have been detected in several trials. These results were further amplified by multicolored fluorescent in situ hybridization (FISH) analysis demonstrating the presence of alterations, where at least chromosomes 1, 16, 17, and 22 show structural aberrations containing genes that could play a role in the development and/or progression of endometriosis. Overall, the non-random distribution along with the subchromosomal location of the genetic alterations strongly supports the idea that these anomalies are relevant and are associated with the endometriotic process.

Keywords: molecular genetic, endometriosis, chromosome, hybridization

1. Introduction

Endometriosis is a systemic, multisymptomatic, and disabling condition for women. Even when endometriosis originates from pelvic implantation, it can spread to other bodily surfaces outside of it.

These endometriosis cells do not shed and migrate like endometrial cells. They remain in situ, causing hemorrhage and an inflammatory response during each hormonal cycle, conditioning the different symptoms and complications in each affected organ.

Although the pathophysiological mechanism has been well studied, the cause of endometriosis remains uncertain. This fact has motivated the development of multiple and diverse theories that have tried to explain this pathology. The biggest problem is that they have been exclusionary theories, so they have not been able to define a cause that fits different scenarios.

It is under this concept and with the advent of new techniques of genetic and molecular study that new theories have been developed based on genetic changes and molecular alterations, which, being inclusive, provide a better vision of the origin of endometriosis and of the way it manages to develop much more effective therapeutic strategies.

In this chapter, we will discuss some aspects of the molecular genetic approach, with relevant findings on the definition and pathogenesis of endometriosis

2. Understanding endometriosis

2.1 Theories of endometriosis

Endometriosis is an estrogen-dependent chronic inflammatory disease that primarily affects women of reproductive age and is defined as the presence of endometrial glands and stroma outside the uterine cavity [1].

The first reference of endometriosis appeared in 1690, when Daniel Shroen described the presence of "ulcers" disseminated in the pelvic cavity and that appeared only in women of reproductive age. From that date, different theories have been developed in an attempt to explain its origin; however, even today, endometriosis remains an enigmatic disease.

The pathogenesis of endometriosis can be included in six theories, which in turn can be subdivided into two groups depending on whether the implants come from the uterine or extrauterine endometrium [2].

2.1.1 Origin of uterine endometriosis

2.1.1.1 Theory of retrograde menstruation

It is the most widely accepted theory about the etiopathogenesis of endometriosis. It was proposed in 1920 by Sampson. Menstruation ascends from the ostium, passes through the tubes and empties into the peritoneal cavity, with the subsequent implantation and growth of the endometrial fragments deposited in the peritoneal cavity. They represent an autotransplant, in which normal endometrial tissue is transplanted to an ectopic location in the body. This theory explains the higher frequency of endometriosis and the anatomical distribution of the lesions. However, it is not able to demonstrate the presence of endometriosis outside the peritoneal cavity, the appearance in early puberty, newborns, women affected by the Mayer-Rokitansky-Küster-Hauser syndrome or in the male [2, 3].

2.1.1.2 Theory of hematogenous or lymphatic dissemination of endometrial tissue (Halban theory)

It is based on the demonstration of ectopic endometrial tissue in locations distant from the uterus such as brain, lung, inguinal region, etc. However, on its own it is not capable of resolving its adhesion and progression capacity [2].

2.1.2 Origin of extrauterine endometrial endometriosis

2.1.2.1 The theory of coelomic metaplasia (Meyer's theory)

The peritoneum and endometrium appear to have a common embryological precursor that is the coelomic epithelium. Dioxins are proposed as a possible external chemical agent that acts as an endocrine disruptor. This theory could explain why although most women have some degree of retrograde menstruation only a small percentage have endometriosis, as well as the presence of the disease in the absence of menstruation [3–5].

2.1.2.2 Theory of endometrial stem cells

Stem cells in the circulation from bone marrow or from the basal layer of the endometrium could differentiate into endometrial tissue at different locations even from a distance. This theory would explain why women without endometriosis can have endometriosis, men with prostate cancer or after treatments can have high doses of estrogen [3, 4].

2.1.2.3 Theory of the Müllerian remains

Residual cells during embryonic development maintain the ability to develop endometriotic lesions influenced by estrogen stimulation [7].

2.1.3 Genetic predisposition

Endometriosis has a hereditary component. Susceptibility loci of the disease have been established in the 10q26 and 7p15 chromosomal regions. The endometrial cells need to adhere to each other and to the peritoneum, and integrins and cadherins participate in this process. Likewise, there is an upregulation of the antiapoptosis gene BCL-2 [5–7].

2.1.4 Hormone dependence

Endometriosis is an epigenetic disease in which the steroidogenesis factor 1, the estrogen, and progesterone receptors are hypomethylated in the ectopic endometrium causing a greater estrogenic effect locally. The endometriotic implants express aromatase and dehydrogenase of 17B-hydroxysteroid type 1, which are the enzymes responsible for converting androstenedione to estrone and estrone to estradiol. At the same time, there is a deficit of 17B-hydroxysteroid type 2, an enzyme responsible for deactivating estrogen (passes estradiol to estrone), and estrogen receptors α and β are expressed differently, with a marked increase in β receptors, favoring all this a greater estrogenic environment [8–10].

2.1.5 Resistance to progesterone

Normal endometrial tissue does not express aromatase and produces abundant dehydrogenase and 17B-hydroxysteroid type 2 in response to progesterone, ensuring the attenuation of estrogenic effects at the endometrial level during the luteal phase. In endometriosis, there is a relative resistance to progesterone that prevents the attenuation of the estrogenic stimulus. Prostaglandin E2 is the most potent inducer of aromatase activity in endometrial stromal cells and acts through the PGE2 receptor. The estradiol produced by the increased activity of aromatase increases the production of PGE2 for the stimulation of cyclooxygenase 2 in the endometrial cells of the uterus, causing a positive feedback that accentuates the estrogenic effects on the production of endometriosis [9].

2.1.6 Immunological factors

Different studies have shown the possibility that alterations in the immune system are responsible for the persistence of the ectopic endometrium, preventing immune mechanisms from eliminating endometrial cells within the peritoneal cavity [2].

These studies have shown a greater number of macrophages with altered function and alterations in the function of the natural killer cells, with a lower cytotoxic

activity, in turn an alteration in the humoral immunity to observe an increase in the concentration of IgG endometrial antibodies as well as IgG and IgA antibodies against endometrial and ovarian tissue.

2.2 Pathogenesis of endometriosis

Regardless of the mechanism that promotes endometriosis, it is a fact the presence of endometrial cells with the potential to be implanted in the receptors tissues, especially in the mesothelium of the pelvic cavity.

In the pathological process of endometriosis in the pelvic organs such as the ovary, the clinical consequence is the formation of chocolate cysts, which can be explained in different ways: (i) during each hormonal cycle, bleeding occurs in the endometriosis accumulation, with an inversion and invagination of the epithelium. At the same time, a cellular inflammatory process begins with adhesions to the surrounding peritoneum. (ii) Chocolate or endometriosis cysts affect the follicular cyst of the ovarian epithelium. (iii) A process of celomic/endometriosis metaplasia of the ovarian epithelium.

At the peritoneal level, especially in the rectovaginal septum, a natural evolution of the peritoneal endometriosis of the Douglas pouch may be caused by secondary infiltration of endometriosis emboli or metaplasia of embryonic/müllerian remains located in the rectovaginal septum [10].

A permissive peritoneal environment for the initiation and progression of endometriotic lesions may also be associated with the altered function of immune cells, together with local pelvic inflammatory processes that aid in the evasion of clearance by the immune system. In addition to the amount of menstrual endometrium reflux present in the peritoneal cavity, the altered secretion of immune factors, the formation of autoantibodies, impaired immune recognition, and the elimination of ectopic endometrial cells facilitate the initiation and progression of endometriosis. Laschke and Menger suggest that the "gut microbiome" or "microenvironment" could be crucial in the pathogenesis of endometriosis through the aberrant priming of immune responses [11].

2.3 Local microenvironment, exosomes

The endometrial lesions are composed of the same structural units as the lining of the uterus, the endometrium. This glandular epithelium is positive for cytokeratin and is apparently composed of two cell types, namely, positive E-cadherin and very few negative E-cadherin cells. The endometriotic stromal cells express mesenchymal markers such as vimentin and THY-1 and can be distinguished from the surrounding fibroblasts by, for example, expression of the CD10 metallo-endopeptidase membrane (common acute lymphocytic leukemia antigen).

The adhesive, proliferative, and invasive properties of the endometriosis conjunctive tissue, as well as the cellular functions of this epithelium can be related to the components of its extracellular matrix [13, 14].

Thus, in the pathogenesis and progression of endometriosis, the local microenvironment is vital to understand how endometriosis cells adapt to the control mechanisms of their host, escaping from immunological detections. The cell-stromal intercommunication through paracrine, hormonal, and angiogenic messengers is vital for the perpetuity of endometriosis tissue [15–17].

In a model by Hull et al., comparing the microarray data obtained from a xenotransplant model and eutopic versus ectopic endometrial paired samples, they identified alterations in four pathways: cell injury (ubiquitin/proteasome), inflammation (NFκB), tissue remodeling (TGF-β), and cell proliferation (KRAS). There is

thus an extensive metabolic reprogramming and the acquisition of changes similar to cancer that are reflected in an increase in the capacity of penetration and cellular penetration, a reduced apoptotic potential and an altered immune function [16].

The local microenvironment could also influence by altering gene expression through, for example, epigenetic changes (DNA methylation), histone modifications, and miRNA. The molecular networks associated with endometriosis are regulated by miRNA at the posttranscriptional level. In fact, 22 miRNAs aberrantly expressed in endometriotic lesions have been identified. In addition to differentially expressed miRNAs, the altered DNA methylation pattern occurs during the onset and/or progression of endometriosis. Since endometriosis is an estrogen-dependent but progesterone-resistant condition, it is not surprising that its respective promoter regions are affected accordingly [17].

In addition to the epigenetic modifications, endometriotic cells also present chromosomal abnormalities and instability that could alter gene expression by loss or mutations of DNA sequences expressed as alterations in the signaling pathways in endometriotic cells (regulatory proteins) [8, 12, 13].

Endometriosis cells release extracellular vesicles, such as exosomes and microvesicles, composed of various types of plasma membranes and origin of endosomal membrane, which are an alternative source for intercellular communication as they contain, for example, miRNAs with target genes in signaling pathways connected to the embryo-endometrial interface or enzymes and are capable of modulating cellular responses, for example, survival, differentiation, or modulation of immunogenic responses, important for endometriosis during implantation [18–23].

Exosomes containing ectonucleotidase could contribute to the progression of endometriosis and the local suppression of immune responses by regulating extracellular ATP and increasing extracellular adenosine levels [17].

They can also exert enhanced angiogenic effects. It is likely that the endometrial exosomes can be directed retrogradely in the pelvic cavity or can be detached by the menstrual cells and influence the fate of the ectopic cells. Thus, in the control of the local microenvironment, exosomes could be an important factor in allowing a temporary endometriotic lesion to establish a sufficient blood supply to grow and survive in the ectopic site. The endometrial exosomes of women with endometriosis act in an autocrine, paracrine, and endocrine fashion but in turn may play a role in the manifestation of endometriosis as a disease.

Diaphonia within the local microenvironments through the exosomes can represent the union intersection where the different theories about the pathogenesis of this entity converge. They could, through their ability to send information between tissue strains, to induce changes such as metaplasias, tissue remodeling, and even represent a mechanism of regulation/alteration in signal transduction [18].

2.4 Phenotype and cytogenetics of endometriosis

Ectopic endometrial cells have been little investigated, mainly due to the rare availability of endometriotic tissue required for cell culture and the limited number of cells, particularly those of epithelial phenotype. The proposed in vitro cell models have their limitations since the endometriotic lesions are histologically complex and contain both glandular and stromal elements. Therefore, cell lines immortalized with a cell type, which normally exhibit characteristics of undifferentiated cells, do not accurately represent the situation in vivo. Thus, to evaluate the endometriosis phenotype, the cultures are prepared in general from biopsies of the various lesions [22, 23].

Among epithelial markers, cytokeratin expression remains one of the most specific characteristics of endometriosis cells. More than 40% of the cells were

immunoreactive with the anti-progesterone receptor (PR) antibody which shows a brown nuclear stain produced by the diaminobenzidine colorimetric reaction. Less than 15% are immunoreactive with the anti-androgen receptor antibody. Cytokeratins such as vimentin were expressed in endometriotic stromal cells. Therefore, most of the phenotypic characteristics of the normal endometrium are conserved in the endometriosis cells.

The existence of genomic aberrations in the tissues of endometriosis is probably related to genes involved in the development of the disease. Most genetic changes occur as germ line defects that can result in a hereditary predisposition to the development of endometriosis with a genetic basis for endometriosis. The prevalence of the disease is higher in first-degree relatives of affected women than in the general population [21–23].

2.4.1 Chromosomal abnormalities observed in endometriotic cells, loss of heterozygosity

For decades, it has been a challenge to obtain consistent results on genetic abnormalities in endometriosis cells. The information produced by conventional cytogenetic and karyotypic studies is limited. The culture of pure endometriotic cells is hampered by the mixture of epithelial and stromal cells in addition to the inflammatory infiltrate containing fibroblasts and histiocytic cells; in addition, there may be excessive growth of normal cells [24].

Despite the difficulties, cytogenetic analysis plays an important role in the understanding of endometriosis, being the only technique that has the capacity to identify new chromosomal translocations, monosomies, and trisomies in chromosomes 11, 16, and 17.

Somatic genetic changes have been detected, distributed along several chromosomes, including chromosome 9p, 11q, and 22q. There have been allelic imbalances in 82% of endometriotic lesions diagnosed simultaneously with ovarian carcinoma.

These genetic studies have the limitation of having an adequate amount of endometriosis tissue from the patient and a simile of normal tissue to be used as control tissue, and in turn they have been limited to evaluating specific areas of the genome (detect loss of part of a chromosomal arm). But even so, they have been sufficiently useful to define the importance, in the development of endometriosis, of the inactivation of one or more suppressor genes [24–28].

2.4.2 Genetic aberrations by FISH: fluorescent in situ and hybridization comparative genomic hybridization

FISH is a technology that uses DNA probes labeled with a fluorophore to detect or confirm gene or chromosomal abnormalities that are generally beyond the resolution capability of routine cytogenetics. First, the DNA sample (metaphase chromosomes or interphase nuclei) is denatured, a process that separates the complementary strands of the double-stranded structure in the DNA double helix. To the denatured sample is then added the probe of interest, which will be associated to the DNA of the sample at the target site, in the process called hybridization, where a double helix is re-formed. The probe is covalently linked (labeled) with a fluorophore, which emits an observable signal through a fluorescence microscope; thus the DNA sample can be classified according to the presence or absence of the signal, which reveals the presence or absence of the target sequence in the chromosomal DNA.

Comparing the genetic analysis with the FISH analysis, we can see that the FISH, by not requiring endometriosis cell culture and avoiding the inconveniences of cellular heterogeneity, has been more effective in revealing clonal aberrations such as

monosomy for chromosomes 16 and 17 and an increase in the number of cells with trisomy 11 [29–33].

The comparative genomic hybridization by arrays (CGH-a) allows to realize a molecular karyotype and detect alterations inferior to 10 Mb throughout the genome. The genomic DNA of the sample and a control sample are differentially labeled with fluorescent dyes and hybridized with the oligonucleotides. The results are analyzed using quantitative methods with analysis software to determine the number of copies. It will not detect low-level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype.

This microarray analysis uses approximately 180,000 oligonucleotides that cover the entire genome at an average resolution of 30 KB, 1714 genes with all the exons covered, 700 microRNAs, and the entire mitochondrial genome [38, 39].

Through CGH-a, primary endometriotic lesions have been examined for gains and/or chromosomal losses. The losses that predominated over the gains showed a grouping in certain chromosomal regions that suggests a recurrent non-random pattern of chromosomal alterations.

The average number of alterations of the copy in our series of endometriotic tissues was 3.1 per injury, which is low compared to malignant tissues [21].

The most common regions of loss of genomic material have been located in 1p involving at least 1p32–36 (50%), 5p (33%), 6q (27%), 7p14-p22ter (22%), 16qter (22%), and 22q12.3-qter (50%) segments. The other less common changes in the number of copies included the loss involving the arms of chromosomes 9q (22%), 16q (22%), and 17q [21, 22, 24–29, 33].

Chromosome 1 deletions were particularly common in all types and stages of endometriosis tissues, including peritoneal implants, endometriomas, and umbilical nodules. The gains were found less frequently and were located on chromosomes 6q and 17q. Several novel regions located on chromosomes 1p, 6q, and 22q that could harbor single or multiple tumor suppressor genes involved in the pathogenesis of endometriosis have been identified [38, 39].

2.4.3 Chromosomal instability in endometrial lesions

Chromosomal instability in endometrial lesions is the alteration of the chromosomal constitution that takes place in diverse pathological conditions: fundamental characteristic of the neoplasic cells (the majority of the malignant and benign tumors), precancerous lesions (dysplasia, leukoplakia, and cystically altered tissues), chronic inflammatory conditions, infectious diseases, and diseases induced by viruses (herpes, HPV, EBV, etc.).

Genomic instability is mainly caused by chromosomal alterations in non-neoplastic precursor lesions and mutation of the P53 gene, and in errors in DNA replication detected by the instability of microsatellites (deficiency in the repair mechanism of DNA mismatch) [34, 35].

Endometriosis tissues present this instability, through the presence of chromosomal copies, numerical changes, chromosomal deletions, translocations, the presence of endomitosis, premature centromeric dislocations, and the presence of micronuclei.

The loss of essential genes or even of whole chromosomes explains the high invasive potential of endometriotic cells. The genomic alterations (rearrangements) initiated can be a primary event that facilitates the initiation and dissemination of endometriosis. It is the alteration of the chromosomal constitution that takes place in diverse pathological conditions: fundamental characteristic of the neoplasic cells (the majority of the malignant and benign tumors), precancerous lesions (dysplasia, leukoplakia, and cystically altered tissues), chronic inflammatory conditions, infectious diseases, and diseases induced by viruses (herpes, HPV, EBV, etc.) [34–37].

3. Conclusions

Endometriosis, even when in essence it is not a mortal disease, is a major health problem in general due to the disability it causes in young and fertile women, in full reproductive stage.

Genetic factors play a predominant role in approximately one-third of chronic disorders in adulthood; so, it is logical to think that endometriosis in turn presents a genetic etiology. Genetic diseases in general can be chromosomal, monogenic, or multifactorial. Currently, epigenetics attempts to explain genetic and environmental interactions and studies changes in gene expression mediated by mechanisms other than the sequence changes of their nucleotides. These epigenetic changes include DNA methylation, histone modification, and interfering RNA. Epigenetic alterations are associated with inflammation and persistence of the lesions. The results of research on the role of these changes in endometriosis are very valuable in the design of future therapeutic strategies.

Currently, epigenetic studies based on FISH technology and comparative genomic hybridization have shown important chromosomal alterations, especially in chromosomes 1, 16, 17, and 22, and it is believed with greater certainty that they are the loci involved in the persistence and progression of chromosomes. Endometriosis cells.

It is in these locations where there has been an instability of DNA information through mechanisms of deletions, translocations, which have led on the one hand to the loss of important genomic information (loss of heterogizity) in endometrial diploid cells, fundamental alterations in the self-regulation, and apoptosis of these aberrant endometrial cells, by mutations as occurs with the P53 gene. These alterations not only occur in the endometriosis cell but are capable of being transmitted to other cells either in an autosomal manner when replicated, or through shared information through a microenvironment mediated by exosomes.

For the researchers, the process of analyzing cultured cells that reproduce the epigenetic changes of an endometriosis cell in vivo has been a feat, but the results of the investigation of the role of these changes in endometriosis have been very valuable and will be useful in the design of future therapeutic strategies (**Figures 1–3**).

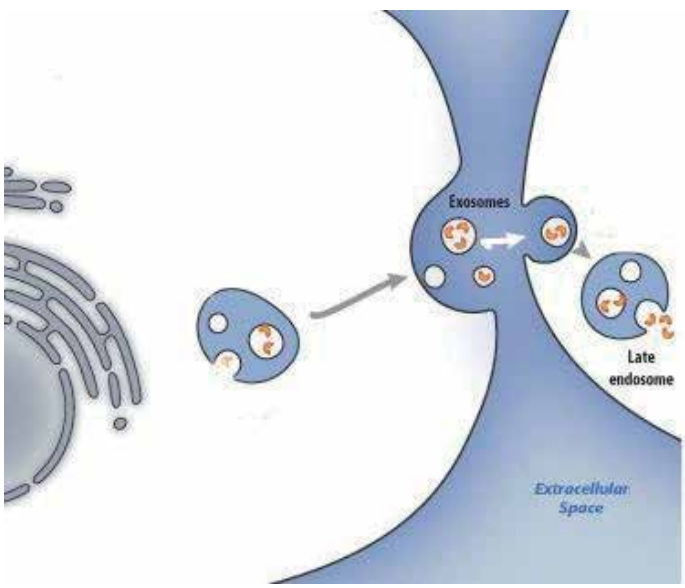

Figure 1.
Exosomes containing ectonucleotidase could contribute to progression of endometriosis.

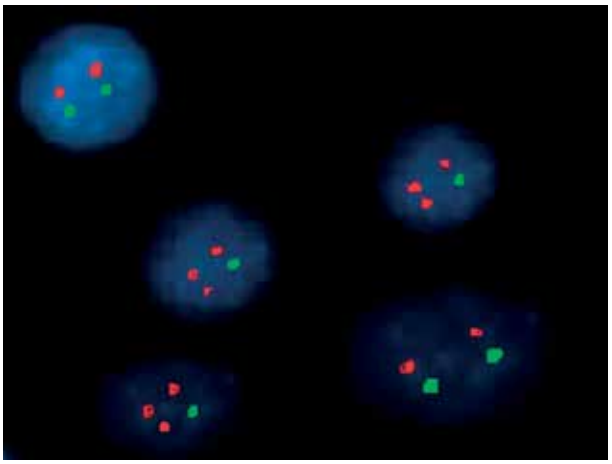

Figure 2.
Clonal aberrations detected by FISH.

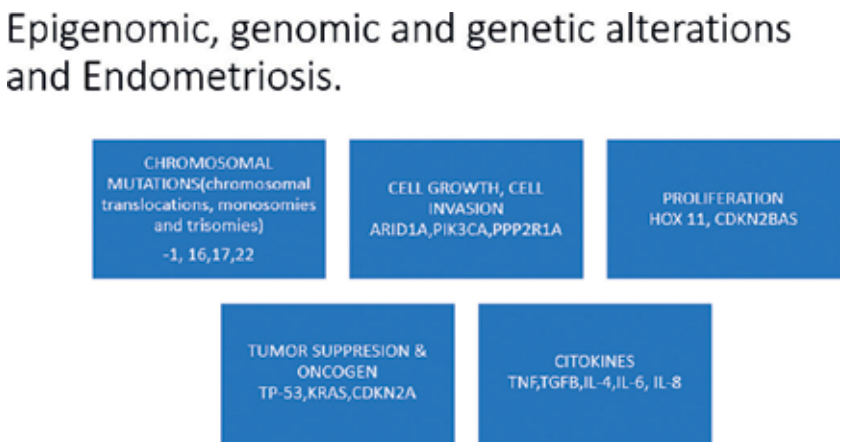

Figure 3.
Summary of alterations in endometriosis.

Conflict of interest

I certify that I do not have an actual or potential conflict of interest in relation to this article.

Appendices and nomenclature

Exosomes: are cell-derived vesicles that are present in many and perhaps all eukaryotic fluids, including blood, urine, and cultured medium of cell cultures.

Cadherin: is a group of cellular adhesive (membrane glycoprotein) that keeps cells tightly bound in time, favoring the organization of tissues and organs, facilitating the mobility of heterogeneous groups of cells.

Phenotype: A phenotype is the composite of an organism's observable characteristics or traits, such as its morphology, development, biochemical or physiological properties, behavior, and products of behavior.

Cytokeratin: are keratin proteins found in the intracytoplasmic cytoskeleton of epithelial tissue. They are an important component of intermediate filaments, which help cells resist mechanical stress.

Heterozigosity: A heterozygote is an organism that has different alleles in a gene. This organism carries different forms of a gene, where those forms produce different phenotypic results. In each case, the same gene has slight variations.

Hybridization: is the process of interbreeding individuals from genetically distinct populations to produce a hybrid. A genetic hybrid would therefore carry two different alleles of the same gene.

Monosomy: the condition of having a diploid chromosome complement in which one (usually the X) chromosome lacks its homologous partner.

Trisomy: is a type of polysomy in which there are three instances of a particular chromosome, instead of the normal two. A trisomy is a type of aneuploidy (an abnormal number of chromosomes).

Deletion: is a mutation (a genetic aberration) in which a part of a chromosome or a sequence of DNA is lost during DNA replication. Any number of nucleotides can be deleted, from a single base to an entire piece of chromosome.

Translocation: is a chromosome abnormality caused by rearrangement of parts between nonhomologous chromosomes. A gene fusion may be created when the translocation joins two otherwise-separated genes.

Author details

Alfredo Borges Garnica[1,2]

1 Biomedical Research Center of the University of Carabobo, Valencia, Carabobo, Venezuela

2 Clinical Researcher Womens Cancer and Surgical Care, Albuquerque, New Mexico, USA

*Address all correspondence to: aborges@wcscnm.com

References

[1] LaGrenade A, Silverberg SG. Ovarian tumors associated with atypical endometriosis. Human Pathology. 1988;**19**(9):1080-1084

[2] Watkins RE. Uterine retrodisplacements, retrograde menstruation and endometriosis. Western Journal of Surgery, Obstetrics, and Gynecology. 1938;**46**:490-494

[3] Halme J, Hammond MG, Hulka JF, Raj SG, Talbert LM. Rettograde menstruation in healthy women and in patients with endometriosis. Obstetrics and Gynecology. 1984;**64**(2):151-154

[4] Olive DL, Henderson DY. Endometriosis and mullerian anomalies. Obstetrics and Gynecology. 1987;**69** (3 Pt 1):412-415

[5] Frey GH. The familial occurrence of endometriosis; report of five instances and review of the literature. American Journal of Obstetrics and Gynecology. 1957;**73**(2):418-421. PubMedGoogle Scholar

[6] Moen MH. Endometriosis in monozygotic twins. Acta Obstetricia et Gynecologica Scandinavica. 1994;**73**(1):59-62. PubMedCrossRefGoogle Scholar

[7] Klemmt PAB, Starzinski-Powitz A. Molecular and cellular pathogenesis of endometriosis. Current Women's Health Reviews. 2018;**14**(2):106-116. DOI: 10.2174/1573404813666170306163448

[8] Kennedy S. Genetics of endometriosis: A review of the positional cloning approaches. Seminars in Reproductive Medicine. 2003;**21**(2):111-118. PubMedCrossRefGoogle Scholar

[9] Hadfield RM et al. Endometriosis in monozygotic twins. Fertility and Sterility. 1997;**68**(5):941-942. PubMedCrossRefGoogle Scholar

[10] Treloar SA et al. Genetic influences on endometriosis in an Australian twin sample. Fertility and Sterility. 1999;**71**(4):701-710. PubMedCrossRefGoogle Scholar

[11] Cramer DW et al. Endometriosis associated with the N314D mutation of galactose-1-phosphate uridyl transferase (GALT). Molecular Human Reproduction. 1996;**2**(3):149-152. PubMedCrossRefGoogle Scholar

[12] Baranova H et al. Possible involvement of arylamine N-acetyltransferase 2, glutathione S-transferases M1 and T1 genes in the development of endometriosis. Molecular Human Reproduction. 1999;**5**(7):636-641. PubMedCrossRefGoogle Scholar

[13] Bulun SE, Monsivais D, Kakinuma T, Furukawa Y, Bernardi L, Pavone ME, et al. Molecular biology of endometriosis: From aromatase to genomic abnormalities. Seminars in Reproductive Medicine. 2015;**33**:220-224

[14] Bukulmez O, Hardy DB, Carr BR, Word RA, Mendelson CR. Inflammatory status influences aromatase and steroid receptor expression in endometriosis. Endocrinology. 2008;**149**(3):1190-1204

[15] Smuc T, Hevir N, Ribic-Pucelj M, Husen B, Thole H, Rizner TL. Disturbed estrogen and progesterone action in ovarian endometriosis. Molecular and Cellular Endocrinology. 2009;**301**(1-2):59-64

[16] Otsuka J, Okuda T, Sekizawa A, et al. K-ras mutation may promote carcinogenesis of endometriosis leading to ovarian clear cell carcinoma. Medical Electron Microscopy. 2004;**37**(3):188-192

[17] Maruyama T, Yoshimura Y. Stem cell theory for the pathogenesis of endometriosis. Frontiers in Bioscience (Elite Edition). 2012;**4**:2854-2863

[18] Chan RW, Schwab KE, Gargett CE. Clonogenicity of human endometrial epithelial and stromal cells. Biology of Reproduction. 2004;**70**(6):1738-1750

[19] Schwab KE, Chan RW, Gargett CE. Putative stem cell activity of human endometrial epithelial and stromal cells during menstrual cycle. Fertility and Sterility. 2005;**84**(Suppl 2):1124-1130

[20] Du H, Taylor HS. Contribution of bone marrow- derived stem cells to endometrium and endometriosis. Stem Cells. 2007;**25**(8):2082-2086

[21] Leyendecker G, Herbertz M, Kunz G, Mall G. Endometriosis results from the dislocation of basal endometrium. Human Reproduction. 2002;**17**(10):2725-2736. 26. 27. 28. 29. 30

[22] Sasson IE, Taylor HS. Stem cells and the pathogenesis of endometriosis. Annals of the New York Academy of Sciences. 2008;**1127**:106-115

[23] Taylor HS. Endometrial cells derived from donor stem cells in bone marrow transplant recipients. JAMA. 2004;**292**(1):81-85

[24] Vercellini P, Vigano P, Somigliana E, Fedele L. Endometriosis: Pathogenesis and treatment. Nature Reviews Endocrinology. 2014;**10**(5):261-275

[25] Bouquet De Jolinière J, Ayoubi JM, Gianaroli L, Dubuisson JB, Gogusev J, Feki A. Endometriosis: A new cellular and molecular genetic approach for understanding the pathogenesis and evolutivity. Frontiers in Surgery. 2014;**1**:16. DOI: 10.3389/fsurg.2014.00016. eCollection 2014

[26] Li J, Fadare O, Xiang L, Kong B, Zheng W. Ovarian serous carcinoma: Recent concept on its origin and carcinogenesis. Journal of Hematology & Oncology. 2012;**5**:8

[27] Nothnick WB, Falcone T, Joshi N, Fazleabas AT, Graham A. Serum miR-451a levels are significantly elevated in women with endometriosis and recapitulated in baboons (Papio anubis) with experimentally-induced disease. Reproductive Sciences. 2017;**24**:1195-1202

[28] Shin JC, Ross HL, Elias S, Nguyen DD, Mitchell-Leef D, Simpson JL, et al. Detection of chromosomal aneuploidy in endometriosis by multi-color fluorescence in situ hybridization (FISH). Human Genetics. 1997;**100**(3-4):401-406

[29] Hsieh YY et al. Androgen receptor trinucleotide polymorphism in endometriosis. Fertility and Sterility. 2001;**76**(2):412-413. PubMedCrossRefGoogle Scholar

[30] Vlahos NF, Kalampokas T, Fotiou S. Endometriosis and ovarian cancer: A review. Gynecological Endocrinology. 2010;**26**(3):213-219

[31] Sampson J. Endometrial carcinoma of the ovary arising in endometrial tissue of that organ. Archives of Surgery. 1925;**10**:1-72

[32] von Rokytansky C. Uber uterusdrusen-neubildung in uterus inder ovarilsarcomen. Zeitschrift Ges Artze Wien. 1860;**37**:577-593

[33] Dzatic-Smiljkovic O, Vasiljevic M, Djukic M, Vugdelic AR, Vugdelic J. Frequency of ovarian endometriosis in epithelial ovarian cancer patients. Clinical and Experimental Obstetrics & Gynecology. 2011;**38**(4):394-398

[34] Ugur M, Turan C, Mungan T, et al. Endometriosis in association with mullerian anomalies. Gynecologic

and Obstetric Investigation. 1995;**40**(4):261-264

[35] Song GG, Lee YH. A meta-analysis of the association between p53 codon 72 polymorphism and susceptibility to endometriosis. Immunological Investigations. 2014;**43**(6):595-605. DOI: 10.3109/08820139.2013.833623

[36] Kitawaki J, Noguchi T, Amatsu T, et al. Expression of aromatase cytochrome P450 protein and messenger ribomnucleid acid in human endometriotic and adenomyotic tissues but not in normal endometrium. Biology of Reproduction. 1997;**57**(3):514-519

[37] Kim HS, Kim TH, Chung HH, Song YS. Risk and prognosis of ovarian cancer in women with endometriosis: A meta-analysis. British Journal of Cancer. 2014;**110**(7):1878-1890

[38] Georgiou I et al. Association of estrogen receptor gene polymorphisms with endometriosis. Fertility and Sterility. 1999;**72**(1):164-166 PubMedCrossRefGoogle Scholar

[39] Sun PR, Jia SZ, Lin H, Leng JH, Lang JH. Genome-wide profiling of long noncoding ribonucleic acid expression patterns in ovarian endometriosis by microarray. Fertility and Sterility. 2014;**101**(4):1038-46.e7. DOI: 10.1016/j.fertnstert.2013.12.035 Epub 2014 Feb 4

Chapter 3

Proteomics Research and Its Possibility of Application in Endometriosis

Ningning Wang

Abstract

The onset search for differential protein expression in endometriosis commenced more than 30 years ago, when the gel electrophoresis could not be available to distinguish serum from women with and without disease. Gradually as the proteomics allows the comprehensive analysis of peritoneal fluid, serum and tissue samples with good sensitivity and resolution, it has promised in delivering markers possibility associated with endometriosis. Cytokines and growth factors that are present in serum, peritoneal fluid, endometrium, endometriotic lesions tissues and involved in tissue implanting process including hormone regulation, angiogenesis, invading and malignancy may be the focus to develop the noninvasive diagnostic test and possible treatment target for endometriosis. Individual peptides or proteins that are present or absent (or up- or down-regulated) in various conditions can be assessed as possible biomarkers. Alternatively, proteomic profiling, using mass spectrometry in combination with bioinformatics software to identify the actual protein and peptide pattern can be used as a distinctive marker to diagnostic and treatment target contribution to the disease.

Keywords: diagnosis, treatment, endometriosis

1. Introduction

Endometriosis (EM) is defined as a benign condition of gynecological diseases when endometrial debris including gland and stroma components outside the uterus and spread to pelvis and extrapelvic sites. It affects at least 10% reproductive women with approximately 70% of cases developing pelvic inflammatory disease and 25–30% of cases associated with infertility [1]. The classical symptoms are dysmenorrhea, chronic pelvic pain, and ovarian chocolate cysts.

Till now, the pathogenesis of endometriosis is unclear. The retrograde menstruation is the basic theory which the menstrual fragments mostly attach to the peritoneum or ovaries area [2]. Whereafter endometriosis mainly responds to fluctuations in estrogen and progesterone by growth and inflammation. And endometriosis mostly involves in the reproductive tract components, such as ovaries, fallopian tubes, uterosacral ligaments, cervix, recto-vaginal septum and vagina. There are commonly exhibited with fibrous walls, adhering to the neighbor structures and usually containing chocolate-colored content. Therefore the affected organs are forming together with serial symptoms-dysmenorrhoea, ovarian chocolate cysts and infertility are often found in the patients. Recently, stem cell theory can be

considered as perspective view of the retrograde menstruation theory [3]. Other theories like immune system dysfunction, genetic susceptibility and exposure to the environment such as dioxins can devote to the progress of the disease [4, 5].

Generally, severity of endometriosis is classified by the revised American Fertility Society (rAFS) system [6], dividing patients into one of four stages (I–IV, minimal-severe) based on lesion size and pelvic adhesions associated with infertility. However, it remains uncertain whether the disease progresses through these stages. Actually some surgeon would prefer to stage on the basis of the sites and extent of the lesions under the laparoscopy or laparotomy and describe without AFS or r-AFS stage. Sometimes they may give some complementary suggestion [7]. Meanwhile, endometriosis has a variable symptom profile which does not relate with severity of this disease [8]. Furthermore, the patients often suffer from the symptoms of infertility or chronic pain for several years before the diagnosis is lately confirmed. So it makes the clinical diagnosis even more difficult. And actually there has been a lack of precise diagnosis method in previous researches. On the aspects of clinical application and financial consideration, more effective and noninvasive test will be needed in endometriosis disease.

Currently, laparoscopy offers the most widely accepted technique and method for evaluating and treating endometriosis. And most of endometriosis patients are treated by surgical removal of lesions and/or hormonal suppression focused on reducing estrogen, such as progestins, androgens, gonadotropin-releasing hormone (GnRH) agonists, and recent aromatase inhibitors. However, both approaches are associated with various side effects and a highly recurrent incidence [9, 10]. Therefore, identification of protein molecular mechanisms involved in the pathogenesis of endometriosis and strategic therapies for treatment are critical.

2. Protein molecular mechanism involving in endometriosis

There should be some factors present in the ectopic lesions of endometriosis, peritoneal fluid and blood that stimulate the lesion to proliferate, implant and invade [11, 12]. And comparing with eutopic endometrium, endometriosis lesions has different biochemical and functional expression including steroid receptivity and invasive potential [13]. Over all, the specific markers of endometriosis may be classified into three main categories: (i) serum and/or peritoneal: growth factors, cytokines, hormones, glycoproteins, proteolytic enzymes and their specific inhibitors, soluble adhesion molecules, autoantibodies and environmental contaminants. (ii) endometrial and biochemical-endometrial: stromal, glandular, neuronal, hormonal receptors, mesenchymal stem cells, proteolytic enzymes and their specific inhibitors, adhesion molecules, osteopontin.(iii) genetic types: oxidative stress genes, tumor suppressor genes, oncogenes, regulatory genes, DNA repair genes, chromosomal aberrations or amplifications, loss of heterozygosity, genetic polymorphism of variable genes, and genome-wide alterations. And we show the focus continuously.

2.1 Hormones regulation proteins in the pathogenesis of endometriosis

Steroid hormones play an important role in endometriosis physiology and pathology. The production of cytokines in endometriosis is the altered responsiveness to progesterone, showing a characteristic very low expression of progesterone receptor A (PR-A) and the absence of PR-B in rodent models, and with decreased PR-B/A ratio and reduced PR-B immunoreactivity, demonstrated in human endometriosis lesions [14, 15]. Hormonal dependence in endometriosis is demonstrated by an increased ER-β expression (approximately 100 times), due to altered

methylation in the ESR2 gene promoter [16]. In experimental model of endometriosis, ER-β may be involved in inhibiting apoptosis and increasing cytokine production in endometriosis, such as MCP-5, IL-1b and IL-16, which seem to enhance cells adhesion and proliferation [17]. The secretion of PGE2 is regulated by MIF through stimulating COX-2 activity. PGE2 is considered to be one of the main pro-inflammatory regulating factors which appears to be confirmed by its elevated level in endometriosis tissues of human being [18].

2.2 Proteins of angiogenesis process of endometrial tissue

On behalf of endometriosis lesions, neovascularization and angiogenesis factors should be introduced. Angiogenic factors such as VEGF [19], ENDO-I [20], angiogenin [21], pleiotrophin, midkine [22], PGF [23], angiopoietin [24], and glycodelin [25, 26] have been identified association with the lesions angiogenesis in endometriosis. Angiogenic activity is supplemented by the co-existence of pathologic angiogenesis, immune suppression, and immune activation. Study from human being indicated the correlation between high MVD and symptom of pelvic pain in patients with endometriosis by transvaginal color doppler ultrasound evaluation. MVD has been described using immunohistochemical evaluation with CD34-labeled endothelial cells of vessels [27, 28]. There was no significantly increased VEGF expression but it has been found in the involvement of other angiogenic factors with the active implants showing high mitotic index and increased MVD [27]. Further findings were reported in other articles that VEGF-A role was demonstrated by its increased concentrations in the endometrium of patients with endometriosis. Moreover, it was confirmed that the expression of VEGF-A gene was higher in peritoneal endometriosis compared with normal peritoneum [29].

2.3 Proteins in invading process of endometrial tissue

In endometriosis, after the attachment to the ectopic sites, the epithelial endometrial cells invade to the extracellular matrix with MMPs and TIMPs secretion playing an important role in degradation in extracellular matrix and basement membrane components [30]. It has been proved that this process is related to the involvement of MMPs, which is stimulated by TNF-a and IL-1 at high concentrations in peritoneal fluid. Meanwhile, TNF-a inhibits TIMP-1 and TIMP-2, leading to an imbalance of MMPs/TIMPs ratio. In patients of endometriosis, MMP-2 and membranous type 1 of eutopic endometrium have been found higher, TIMP-2 was lower than normal women [31]. Increased MT5- MMP expression and alterations of the balances between MMP-9/TIMP-1, MMP-9/TIMP-3, MMP-3/uPA, VEGF/MMP-3/uPA, VEGF/ /MMP-2/CD44/Ki67, PAI/TIMP-1, and IL-1/ /MMP-1 indicated that implantation and invasion might participate the mechanism of endometriosis [32–38].

2.4 Proteins in carcinogenesis possibility of endometriosis

Clinicopathological, molecular, and genetic evidences support the hypothesis of endometriosis as a neoplastic process, with a potential to malignant transformation. Polypoid endometriosis, premalignant changes, borderline tumors and malignant tumors were described. Except for an increased MMPs expression associated to deregulation of the intercellular adherence signaling, tumor suppressor genes, oncogenes, CAMs, furthermore LOH and inflammatory immunomodulation were detected [39, 40]. The progressive accumulations of genetic alterations of tumor-suppressing genes and oncogenes are probably responsible for endometriosis development and its possible association with the development of malignancies [41–45].

2.5 Proteins involving in peritoneal fluid and plasma of endometriosis

Most of researches have demonstrated that macrophages, lymphocytes, endometrial cells and mesothelial cells are able to produce cytokines and inflammatory mediators such as ILs [3, 46, 47], TNF-a [48], PGF2, PGE2 and thromboxane B2 [49], MCP-1 [50], RANTES [51], eotaxin [52], GROa [47], SDF1 [53], and MIF [50, 54]. The main process is leading to the stimulation of endometriotic cells proliferation and adhesion to ectopic sites, angiogenesis, and stimulation of the release of other cytokines and chemokines, later amplifying their effects.

Macrophages can amplify the activity of COX-2 and PGE2, which results in VEGF stimulation in endothelial cells from endometriotic lesions, together with factor StAR, association with an increased estrogen level in the endometrial tissue [55–57]. PGE2 suppresses the activity of phagocytes, that allowing endometriotic implants formatting [58]. Estrogens and PGE2 can induce FGF-9 expression which can further activate endometrial cells proliferation, paralleling to the stimulation of angiogenesis and apoptosis inhibition at the same time. Lymphocytes are involved in various cytokines production with potential role in endometriosis lesions implanting. Whether in vitro study or in human endometriosis, Th2 cells of peritoneal fluid were shown to stimulate the secretion of IL-4 and IL-10. NK cell-mediated cytotoxicity, commonly manifested by lymphocytes adherence to endometrial cells through LFA-1 — ICAM-1 pathway and their presentation as targets to NK cells, may fail in endometriosis [59, 60]. This indicated the possible mechanism that sICAM-1 may bind to LFA-1 expressing in lymphocytes can prevent endometrial cells recognition involved in endometriosis pathogenesis.

TNF-a is a typical pro-inflammatory cytokine produced by macrophages, which can exhibit high levels in serum and peritoneal fluid of patients with endometriosis [61, 62]. Recent studies have shown that TNF-a-induced activation of IKKb complex leads to the initiation and progression of endometriosis by enhancing the survival rate of ectopic epithelial cells rather than stromal cells and not eutopic epithelial cells [63]. MIF, another cytokine, has been shown a high level in the peritoneal fluid, in serum samples, and in peritoneal macrophages, its secretion being regulated by estrogens in endometriosis [85]. MIF can stimulate endothelial cell proliferation, endometriotic lesions survival, expressing VEGF, IL-8, PGE2, COX-2, MCP-1, aromatase, and resulting back in stimulating TNF-a in endometrial cells [18, 64]. That is why in experimental models, MIF antagonist significantly reduces lesions size of endometriosis by inhibiting cell adhesion, tissue remodeling, angiogenesis, and inflammation, in addition to routine alteration of the balance between pro- and anti-apoptotic factors [65].

3. Progress in proteomics

It is recognized that gene expression changes does not reflect the changes in protein expression within the cell. Better tools are needed to accurately probe the protein activities and levels. Protein arrays are arguably underachieving their potentials as they are perceived not as precise as DNA arrays. Functional proteomics still holds great promises that it could result in greater understandings of the mechanism of disease. The goals proteomics of diseases are to improve molecular classification of diseases and to discover molecular biomarkers for their diagnosis, treatment target and following up [66–68]. Better tools are needed to accurately calculate the protein activity levels. Protein arrays (used synonymously with protein microarrays or protein biochips) are one of the solutions to the high throughput study of protein interaction networks or immune reactivity [69–71]. Mass spectroscopy (MS) [72], relies mainly

on the mass-to-charge ratios to distinguish different proteins. It is widely used as a discovery tool of diagnostic, prognostic, and therapeutic protein biomarkers. Moreover, it does not need to identify molecules. And protein arrays depend on the availability of specific recognition molecules. MS-based methods can simultaneously screen numerous proteins, but its sensitivity is approximately 1 ng/mL. Emerging protein arrays based on magneto-nanosensors enable to study many proteins to proteins interactions simultaneous easily with sensitivity low than about 1 pg/mL. Proteomic researches mainly includes the 2-DE reference map and a database by proteomic comparison between healthy persons and patients. Comparing to western blot, 2-DE can be used to find out autoantigens that may induced autoantibodies in some diseases.

3.1 Protein arrays

Protein arrays with antibodies are developing a tool for rapid measurement of abundance of proteins expression. It makes the possibility to screen the aimed proteins changes in different diseases and biological processes. In most cases, this approach depends on exposing serum samples from patients to an ordered array of antigens, capturing those antibodies that bind the antigens on the arrays. Immobilization of proteins on the surface of arrays and neutralizing reactive areas after the immobilization are important practical issues in protein array. Many different types of proteins arrays such as antibody arrays and peptide arrays have been reported [73]. It starts research in breast cancer and leukemia [74, 75]. Recently, one of the protein array technologies is the magneto-nanosensor array where giant magnetoresistive (GMR) [76] sensors are used to quantitatively measure analyte of interest proteins which are labeled with magnetic nanoparticles (MNP). Another emerging protein array technology is Nucleic Acid Programmable Protein Arrays (NAPPA) [77], which have thousands of protein features directly expressed by nucleic acids on array surface.

More attentions have been paid to the role of protein arrays in medicine. They can be used for early detection of diseases, diagnosis of stages, stratification of patients, and prediction of therapeutic effects, and are increasing realization of the vision of personalized medicine. For cytokine measurements, protein arrays should be improved in both functional sensitivity and probe density. Till now, the arrays have two major drawbacks: first, they are biased, because antigen selection is based on their potential to play a role in disease. Secondly, the analytical comprehensiveness of this technique is limited because only the molecules represented on the array can be identified.

3.2 Mass spectrometry (MS)

In the past few decades, the paradigm of biomarker research has shifted from a hypothesis-driven approach to a discovery-driven approach. Mass spectrometry and separation techniques and proteomics methods have been fully developed and have been common. Particularly when two-dimensional liquid chromatography/tandem mass spectrometry, or two-dimensional gel electrophoresis (2-DE) and matrix laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) combination, followed by database search (sequence tags or peptide mass fingerprints) are widely used. These methods greatly increase the comprehensiveness of protein identification.

Identify disease-associated antigens can elicit through immune responses by combining protein separation (2-DE, gel-free separation), immunological detection (Western blotting) and MS, or combining immunocapture and MS [78]. Proteins derived from cells or tissues (e.g., cancer cells) are separated by 2-DE, and antigen proteins are detected by applying patient serum, which may include disease-specific antibodies followed by enzyme-labeled secondary antibodies. In order to identify

immunogenic proteins, the corresponding spots are separated from the gel and gels were digested. MS or tandem mass spectrometry is used for analysis and then analyzed by peptide fingerprinting or sequence tags method.

3.2.1 Isotope-coded affinity tagging multidimensional LC-MS

LC–MS makes identification and quantification of target protein possible in a large and complex sample and most of the time it may not be achieved in the clinical work. However, it will be useful when proteins are limited in a sample and at present this method will be considered as a supplement to 2-DE gel [79, 80].

3.2.2 MALDI-MS

Antigen analysis of immunocapture MS is derived from the immobilization of antibodies in the serum of patients. Almost all antibodies are captured on protein A or G, which is a bacterial derived protein with specific affinity for the Fc domain of the antibody. Protein mixtures (cell or tissue lysates) are applied to a column or bead immobilized with antibodies to capture specific antigens of antibodies present in patient samples. Finally, the proteins were identified by MALDI-TOF-MS or surface enhanced laser desorption/ionization mass spectrometry (SELDI-MS). In SELDI-MS, the protein is enzymatically cleaved into a peptide mixture and undergoes MS. It can be a fully automated system allowing high-throughput and rapid identification. MS afford a method to identify a protein even from a complex mixture of proteins [81, 82]. It is preferred and an applicable for a pure protein or a single spot for 2-DE gel.

3.2.3 Surface-enhanced laser desorption/ionization (SELDI-MS)

Chemical defined or antibodies-coated protein biochip arrays for rapid protein detection. This system is used when small amount of samples is available. Very high surface expression of the immunoglobulin binding protein (proACTR) as the antigen capture and transfer reagent [83]. ProACTR can immobilize the antibody through the Fc region of antibody, and allows for higher capture capacity than antibody-coated beads. Captured antigens can be directly transferred to a platform for MS analysis (SELDI-TOF-MS). It starts its diagnosis research with post-translationally modified proteins and high-throughput technique in breast cancer, lung cancer and prostate cancer. Unfortunately, it does not allow reliable protein sometimes [84, 85]. To the best of our knowledge, immunoproteomics with proACTR has not yet been applied to profile antigens associated with a certain disease, but mainly to the quantification of a single target [86, 87].

4. Applications and experiences in the past

The 2-DE for tissue sample is more complex than that for serum. Tissue from ovarian chocolate cysts or from eutopic endometrium contains connective tissues, red blood cells, epithelial cells and stromal cells. Stringent protocols are adopted to ensure uniformity throughout the process to facilitate the protein maps. However, the development of robust assay platforms and standardized protocols are required before MS-based antigen profiling can be performed in the clinical setting.

4.1 Expression and significance of peritoneal fluid protein in patients with endometriosis

In 2003, Tabibzadeh et al. [88] used two-dimensional gel electrophoresis to analyze the protein profile of peritoneal fluid in 12 patients with EMs (6 cases were mild and 6 cases were severe degree). However, 12 cases of non-EMs ascites were analyzed as control (6 cases of infertility, 6 cases of normal fertile). There was no significant difference between the infertile controls and the normal fertile control group. However, the patients with mild EMs had protein reductions associated with several peritoneal protein spots of approximate molecular weights of 35–40 kD and pI close to 5.7–6.0. and the reduction in severe EMs cases was more markedly apparent. Consistent with these data, enzyme-linked immunosorbent assay showed that severe endometriosis was associated with markedly elevated levels of IL-10 in the peritoneal fluid. Endometriosis maybe associated with disturbed secretion of proteins into the peritoneal cavity and with an elevated level of IL-10 in the peritoneal fluid. Most of these proteins have not been further described in the existing literatures, so it is still unclear whether the aforementioned results can be used as diagnostic markers for EMs.

Ferrero et al. [89] used two-dimensional gel electrophoresis, silver stained, semiquantitative computerized analysis the changes of protein expression profile in the peritoneal fluid and plasma of 72 patients with EMs and 35 infertile control patients. Compared with the controls, one beta chain isoform (HpbetaE; molecular weight 38.40 ± 0.94 kD; and isoelectric point, 5.63 ± 0.17) had significantly higher expression in women with endometriosis. HpbetaE level was found no significant difference between mild endometriosis (rAFS, stage I-II) and severe endometriosis (rAFS, stage III-IV). But the expression of HpbetaE in the control group was obtained to be related to the stage of menstrual cycle. The above studies indicate that changes in the protein expression profile of patients with endometriosis. In 2007, Liu et al. [90] used SELD I-TOF-MS technology and its associated protein chip to detect the plasma protein profiles from 36 patients of endometriosis and 35 healthy individuals. 21 differentially expressed protein peaks were found and three protein peaks were established. The endometriosis diagnostic model had a sensitivity of 91.7% and a specificity of 82.9%, and was performed on 16 healthy subjects and 15 patients. The sensitivity was 87.5% and the specificity was 80%. It provides an approach for screening the plasma markers of endometriosis. In 2007, Ferrero et al. [91] used two-dimensional gel electrophoresis; protein spots of interest were identified by liquid chromatography tandem mass spectrometry to study the differential expression of peritoneal fluid proteins in patients with and without endometriosis. Several molecules had aberrant expression in peritoneal fluid of women with endometriosis may be useful for a better understanding of the pathogenesis of this disease.

In 2006, Zhang et al. [92] applied two-dimensional gel electrophoresis (2-DE), Western blotting, and mass spectrometry (MS) technology to study proteins in endometriosis and normal controls, and analyzed differences using Western blots. The normal human serum and patient serum were compared with the total protein of endometriosis. In patients with endometriosis, 13 protein spots were associated with 11 known proteins, while 11 protein spots were found differently expressed in the endometrium of patients with and without endometriosis. Some proteins may be cytoskeleton, some may regulate in cell cycle, signal transduction or immune function participation. The hybridization of vimentin, beta-actin and ATP synthase beta subunit in serum of patients with endometriosis was significantly different from that of normal serum. Three different points were used to determine the protein expression profile, vimentin, β-actin, and ATP synthase β subunits respectively.

ATP synthase may play an important role in ectopic endometrium as it needs invasive, and cell adhesion and cytoskeletal remodeling. Vimentin and β-actin is a cytoskeletal protein. Studies have shown that the expression of these proteins is up-regulated in the endometrium in patients with endometriosis. These proteins have a certain effect on the formation of endometriotic lesions. Given that the occurrence of endometriosis may be due to an abnormality of eutopic endometrium itself. In 2007 and 2008, Liang [93] and others used surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) technology to study serum protein expression in patients with endometriosis and healthy controls. A diagnostic model consisting of 5 protein peaks was established with a sensitivity of 91.7% and a specificity of 90.0% provides new prospect for screening endometriosis markers. In 2007, Liu et al. [90] also used SELDI-TOF-MS technology to study plasma protein expression in patients with endometriosis and healthy controls. It was found that 20 protein peaks were elevated or decreased in both of them. The sensitivity was 87.5% and the specificity was 80.0%.

4.2 Expression and significance of tissue protein in patients with Endometriosis

In 2006, Kyama et al. [5] used SELD I-TOF-MS technology to find that the expression of proteins and peptides in the eutopic endometrium of patients with endometriosis weight range of 2.8–12.3 kDa was 3–24 times lower than the molecular with non endometriosis. The expression of proteins and peptides in the lesions of patients with endometriosis had a tendency to increase within the molecular weight range of 3–96 kDa, and especially an up-regulated cluster of proteins between 22 and 23 kDa, identified to be transgelin, a smooth muscle actin-binding protein. In 2007 Fowler et al. [94] used 2-DE and peptide mass (MS) technology to confirm that the protein associated with endometriosis. Several deregulated proteins are identified: (1) chaperones and calcium binding proteins include heat shock protein 90 (HSP90) and annexin A2. (2) Cell oxygenation status related to protein, such as peroxiredoxin and thioredoxin-1, -2 (thioredoxin reductase-1, -). (3) Proteins associated with protein/DNA synthesis/decomposition, such as nucleoside diphosphate reductase, prohibitin, and proline-4-hydroxylase. (4) Secreted proteins, such as apolipoprotein A1. (5) Structural proteins such as vimentin and actin, whose function suggests that they play a role in the pathogenesis of endometriosis. At the same time, it was believed that the differentially expressed protein spots produced are identical whether the lysate of the endometrium was aggregated or isolated. Immunohistochemistry, Western blotting, and biological effects were also used to validate differential proteins and achieve desirable results. This study demonstrated that 2-DE gel analysis and mass spectroscopic protein identification are suitable for the identification of proteins with candidate associations with endometriosis.

4.3 Endometriosis infertility protein detection

Previous result of our group indicated [95] that 76 eutopic endometrial polyps cases of endometriosis group histologically resembled endometrial polyps but the majority of endometrial polyps with endometriosis occurred in primary infertility cases and in fewer pregnancy rate women who had stable and smaller EPs without association with the AFS stage. The recurrence rate of endometrial polyps with endometriosis group was higher than that in non endometriosis group. In 2009, Ferrero et al. [96] reported the peritoneal fluid proteome collected under laparoscopy from 26 fertile women and 26 infertile ones With endometriosis. One isoform of immunoglobulin light chain spot and 9 protein spots had been found significantly higher expression in PF of infertile patients than infertile controls by applied with

2-dimensional gel electrophoresis (2-DE) with computerized analysis and protein spots were identified by liquid chromatography tandem mass spectrometry (MS). No protein spots had significantly lower expression and 3 protein spots remain unidentified.

4.4 Proteomics detection of animal models of endometriosis

Many animals such as rhesus monkeys, rabbits, and nude mice have been used as models for studying endometriosis, but most of these animals have no menstrual cycle, and only primates can spontaneously produce endometriosis with regular menstrual cycles and menstrual blood flow. Their pathogenesis and pathological features are similar to those of humans. Monkey experiments have confirmed that current menstrual bleeding can cause pelvic lesions. In 1991, Sharpe et al. [97] first reported using two-dimensional electrophoresis (2-DE) to observe that surgically induced ectopic endometrium in rats lacked progesterone-induced secretory uterin protein 21 (PUP21, MW 70 kDa, p I 5. 7), whereas normal endometrium expression of the protein suggests that PUP21 deficiency is associated with reduced fertility in patients with endometriosis. In 1993, Sharpe et al. [98] also used two-dimensional electrophoresis to study the rat endometriosis implanted by surgery. It was found that ectopic endometrium specifically expressed two groups of proteins: ENDO I (MW 40–50 kDa, p I 4. 0–5. 2) and ENDO II (MW 28–32 kDa, p I 7.5–9.0). Further studies using amino acid sequence analysis confirmed that ENDO-2 is TIMP-1 and ENDO-1 is haptoglobin-like. Studies have shown that endometriotic lesions secrete haptoglobin in combination with macrophages to reduce their adherent phagocytic capacity, so that intimal ectopic cells cannot be eliminated, but haptoglobin can stimulate macrophages to secrete inflammatory cytokines such as IL-1, − 6 and TNF, IL-6 can up-regulate ectopic endometrium cells to express haptoglobin, forming a positive feedback in the lesion, thereby promoting the progression of endometriosis. In the peritoneal fluid and serum of patients with endometriosis, the concentration of TIMP-1 was significantly reduced. Therefore, the abnormal expression of TIMP-1 may be one of the causes of the onset of endometriosis, and may become a potential marker for diagnosing this disease.

5. Possible directions in the future

Once we have discovered one or more proteins that are specifically expressed in patients with endometriosis, the next step is to develop them as a diagnostic test for it. The diagnostic method must have good sensitivity, specificity, positive predictive value, and negative predictive value. What we need to overcome is not only the individual differences in the population, but also the differences in specific tissue protein components and the effects of natural menstrual cycles or hormone effects. Protein chip, protein array mass spectrometry technique have been used to perform a comprehensive search of protein expression profiles of endometriosis patients in order to find a group of proteins with high sensitivity and specificity. Applying the changes in the expression of this group of proteins to diagnose and predict disease will undoubtedly bring about a new endometriosis diagnosis field.

Acknowledgements

This chapter was co-funded by the National Natural Science Foundation of China (No. 30762222, No. 81170550).

Conflict of interest

None.

Notes/thanks/other declarations

We thank Professor Guanglun Zhuang from the Reproductive Center of First Affiliated Hospital of Sun Yat-sen University for his support. Thanks for Huijuan Ding-one of my postgraduates' collecting part materials. And some sentences polished were under the suggestion by Jianing Donna Li from UC Davis.

Author details

Ningning Wang
The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China

*Address all correspondence to: wangnn@mail.sysu.edu.cn

References

[1] De Ceglie A, Bilardi C, Blanchi S, et al. Acute small bowel obstruction caused by endometriosis: A case report and review of the literature. World Journal of Gastroenterology. 2008;**14**(21):3430-3434

[2] Sampson JA. Metastatic or embolic endometriosis, due to the menstrual dissemination of endometrial tissue into the venous circulation. The American Journal of Pathology. 1927 Mar;**3**(2): 93-110.43

[3] Kao AP, Wang KH, Chang CC, et al. Comparative study of human eutopic and ectopic endometrial mesenchymal stem cells and the development of an in vivo endometriotic invasion. model. Fertility and Sterility. 2011;**95**(4): 1308-13015.e1. DOI: 10.1016/j. fertnstert.2010.09.064

[4] Painter JN, Anderson CA, Nyholt DR, et al. Genome-wide association study identifies locus at 7p15.2 associated with endometriosis. Nature Genetics. 2011;**43**(1):51-54. DOI: 10.1038/ng.731

[5] Koninckx PR, Braet P, Kennedy SH, et al. Dioxin pollution and endometriosis in Belgium. Human Reproduction. 1994;**9**(6):1001-1002

[6] Revised American Fertility Society classification of endometriosis: 1985. Fertility and Sterility. 1985;**43**(3):351-352

[7] Maheux-Lacroix S, Nesbitt-Hawes E, Deans R, et al. Endometriosis fertility index predicts live births following surgical resection of moderate and severe endometriosis. Human Reproduction. 2017;**32**(11):2243-2249. DOI: 10.1093/humrep/dex291

[8] Vercellini P, Vendola N, Bocciolone L, et al. Laparoscopic aspiration of ovarian endometriomas. Effect with postoperative gonadotropin releasing hormone agonist treatment. The Journal of Reproductive Medicine. 1992;**37**(7):577-580

[9] Bulun SE. Endometriosis. The New England Journal of Medicine. 2009;**360**:268-279

[10] Sinaii N, Cleary SD, Ballweg ML, et al. High rates of autoimmune and endocrine disorders, fibromyalgia, chronic fatigue syndrome and atopic diseases among women with endometriosis: A survey analysis. Human Reproduction. 2002;**17**:2715-2724

[11] Braun DP, Ding J, Shen J, et al. Relationship between apoptosis and the number of macrophages in eutopic endometrium from women with and without endometriosis. Fertility and Sterility. 2002;**78**(4):830-835

[12] Braun DP, Ding J, Dmowski WP. Peritoneal fluid-mediated enhancement of eutopic and ectopic endometrial cell proliferation is dependent on tumor necrosis factor-alpha in women with endometriosis. Fertility and Sterility. 2002;**78**(4):727-732

[13] Gaetje R, Kotzian S, Herrmann G, et al. Invasiveness of endometriotic cells in vitro. Lancet. 1995;**346**(8988): 1463-1464

[14] Li Y, Adur MK, Kannan A, et al. Progesterone Alleviates Endometriosis via Inhibition of Uterine Cell Proliferation, Inflammation and Angiogenesis in an Immunocompetent Mouse Model. PLoS One. 2016;**11**(10):e0165347. DOI: 10.1371/ journal.pone.0165347 eCollection 2016

[15] Xue Q, Lin Z, Cheng YH, et al. Promoter methylation regulates estrogen receptor 2 in human endometrium and endometriosis. Biology of Reproduction. 2007;**77**(4):681-687

[16] Meyer JL, Zimbardi D, Podgaec S, et al. DNA methylation patterns

of steroid receptor genes ESR1, ESR2and PGR in deep endometriosis compromising the rectum. International Journal of Molecular Medicine. 2014;**33**(4):897-904. DOI: 10.3892/ijmm.2014.1637

[17] Han SJ, Jung SY, Wu SP, et al. Estrogen receptor β modulates apoptosis complexes and the inflammasome to drive the pathogenesis of endometriosis. Cell. 2015;**163**(4):960-974. DOI: 10.1016/j.cell.2015.10.034

[18] Carli C, Metz CN, Al-Abed Y, et al. Up-regulation of cyclooxygenase-2 expression and prostaglandin E2 production in human endometriotic cells by macrophage migration inhibitory factor: involvement of novel kinase signaling pathways. Endocrinology. 2009;**150**(7):3128-3137. DOI: 10.1210/en.2008-1088

[19] Rocha AL, Carrarelli P, Novembri R, et al. Activin A stimulates interleukin 8 and vascular endothelial growth factor release from cultured human endometrial stromal cells: possible implications for the pathogenesis of endometriosis. Reproductive Sciences. 2012;**19**(8):832-838. DOI: 10.1177/1933719111434542

[20] Piva M, Sharpe-Timms KL. Peritoneal endometriotic lesions differentially express a haptoglobin-like gene. Molecular Human Reproduction. 1999;**5**(1):71-78

[21] Suzumori N, XXi Z, Suzumori K. Elevated angiogenin levels in the peritoneal fluid of women with endometriosis correlate with the extent of the disorder. Fertility and Sterility. 2004;**82**(1):93-96

[22] Chung HW, Wen Y, Choi EA, et al. Pleiotrophin (PTN) and midkine (MK) mRNA expression in eutopic and ectopic endometrium in advanced stage endometriosis. Molecular Human Reproduction. 2002;**8**(4):350-355

[23] Zucchini C, De Sanctis P, Facchini C, et al. Performance of circulating placental growth factor as a screening marker for diagnosis of ovarian endometriosis: A pilot study. Int J Fertil Steril. 2016;**9**(4):483-489

[24] Hur SE, Lee JY, Moon HS, et al. Angiopoietin-1, angiopoietin-2 and tie-2 expression in eutopic endometrium in advanced endometriosis. Molecular Human Reproduction. 2006;**12**(7):421-426

[25] Drosdzol-Cop A, Skrzypulec-Plinta V. Selected cytokines and glycodelin A levels in serum and peritoneal fluid in girls with endometriosis. The Journal of Obstetrics and Gynaecology Research. 2012;**38**(10):1245-1253. DOI: 10.1111/j.1447-0756.2012.01860.x

[26] Kocbek V, Vouk K, Mueller MD, et al. Elevated glycodelin-A concentrations in serum and peritoneal fluid of women with ovarian endometriosis. Gynecological Endocrinology. 2013;**29**(5):455-459. DOI: 10.3109/09513590.2013.769516

[27] García-Manero M, Santana GT, Alcázar JL. Relationship between microvascular density and expression of vascular endothelial growth factor in patients with ovarian endometriosis. Journal of Women's Health. 2008;**17**(5):777-782. DOI: 10.1089/jwh.2007.0695

[28] Alcázar JL, García-Manero M. Ovarian endometrioma vascularization in women with pelvic pain. Fertility and Sterility. 2007;**87**(6):1271-1276

[29] Takehara M, Ueda M, Yamashita Y, et al. Vascular endothelial growth factor A and C gene expression in endometriosis. Human Pathology. 2004;**35**(11):1369-1375

[30] Hulboy DL, Rudolph LA, Matrisian LM. Matrix metalloproteinases as mediators of reproductive function.

Molecular Human Reproduction. 1997;**3**(1):27-45

[31] Chung HW, Lee JY, Moon HS, et al. Matrix metalloproteinase-2, membranous type 1 matrix metalloproteinase, and tissue inhibitor of metalloproteinase-2 expression in ectopic and eutopic endometrium. Fertility and Sterility. 2002;**78**(4):787-795

[32] Kang YJ, Jeung IC, Park A, et al. An increased level of IL-6 suppresses NK cell activity in peritoneal fluid of patients with endometriosis via regulation of SHP-2 expression. Human Reproduction. 2014;**29**(10):2176-2189. DOI: 10.1093/humrep/deu172

[33] Szamatowicz J, Laudański P, Tomaszewska I. Matrix metalloproteinase-9 and tissue inhibitor of matrix metalloproteinase-1: A possible role in the pathogenesis of endometriosis. Human Reproduction. 2002;**17**(2):284-288

[34] Ahn SH, Edwards AK, Singh SS, et al. IL-17A contributes to the pathogenesis of endometriosis by triggering proinflammatory cytokines and angiogenic growth factors. Journal of Immunology. 2015;**195**(6):2591-2600. DOI: 10.4049/jimmunol.1501138

[35] Ramón L, Gilabert-Estellés J, Castelló R, et al. mRNA analysis of several components of the plasminogen activator and matrix metalloproteinase systems in endometriosis using a real-time quantitative RT-PCR assay. Human Reproduction. 2005;**20**(1):272-278

[36] Gilabert-Estellés J, Ramón LA, España F, et al. Expression of angiogenic factors in endometriosis: relationship to fibrinolytic and metalloproteinase systems. Human Reproduction. 2007;**22**(8):2120-2127

[37] HOk K, Yang KM, Kang IS, et al. Expression of CD44s, vascular endothelial growth factor, matrix metalloproteinase-2 and Ki-67 in peritoneal, rectovaginal and ovarian endometriosis. The Journal of Reproductive Medicine. 2007;**52**(3):207-213

[38] Gilabert-Estelles J, Ramon LA, España F, et al. Expression of the Fibrinolytic Components in Endometriosis. Pathophysiology of Haemostasis and Thrombosis. 2006;**35**(1-2):136-140

[39] Hudelist G, Lass H, Keckstein J, et al. Interleukin 1alpha and tissue-lytic matrix metalloproteinase-1 are elevated in ectopic endometrium of patients with endometriosis. Human Reproduction. 2005;**20**(6):1695-1701

[40] Maeda N, Izumiya C, Taniguchi K, et al. Role of NK cells and HLA-G in endometriosis. Frontiers in Bioscience (Scholar Edition). 2012;**4**:1568-1581

[41] Sato N, Tsunoda H, Nishida M, et al. Loss of heterozygosity on 10q23.3 and mutation of the tumor suppressor gene PTEN in benign endometrial cyst of the ovary: possible sequence progression from benign endometrial cyst to endometrioid carcinoma and clear cell carcinoma of the ovary. Cancer Research. 2000;**60**(24):7052-7056

[42] Dinulescu DM, Ince TA, Quade BJ, et al. Role of K-ras and Pten in the development of mouse models of endometriosis and endometrioid ovarian cancer. Nature Medicine. 2005;**11**(1):63-70

[43] Govatati S, Kodati VL, Deenadayal M, et al. Mutations in the PTEN tumor gene and risk of endometriosis: a case-control study. Human Reproduction. 2014;**29**(2):324-336. DOI: 10.1093/humrep/det387

[44] Nakayama K, Toki T, Nikaido T, et al. Genetic alterations in microsatellite marker sites among tumor suppressor genes in endometriosis.

Gynecologic and Obstetric Investigation. 2001;**51**(4):240-242

[45] Hsieh YY, Chang CC, Tsai FJ, et al. Glutathione S-transferase M1*null genotype but not myeloperoxidase promoter G-463A polymorphism is associated with higher susceptibility to endometriosis. Molecular Human Reproduction. 2004;**10**(10):713-717

[46] Kang YJ, Jeung IC, Park A, et al. An increased level of IL-6 suppresses NK cell activity in peritoneal fluid of patients with endometriosis via regulation of SHP-2 expression. Human Reproduction. 2014;**29**(10):2176-2189. DOI: 10.1093/humrep/deu172

[47] Takamura M, Osuga Y, Izumi G, et al. Interleukin-17A is present in neutrophils in endometrioma and stimulates the secretion of growth-regulated oncogene–a (Gro-a) from endometrioma stromal cells. Fertility and Sterility. 2012;**98**(5):1218-1224.e1-2. DOI: 10.1016/j.fertnstert.2012.07.1117

[48] Salmeri FM, Laganà AS, Sofo V, et al. Behavior of tumor necrosis factor-a and tumor necrosis factor receptor 1/tumor necrosis factor receptor 2 system in mononuclear cells recovered from peritoneal fluid of women with endometriosis at different stages. Reproductive Sciences. 2015;**22**(2):165-172. DOI: 10.1177/1933719114536472

[49] Dawood MY, Khan-Dawood FS. Differential suppression of menstrual fluid prostaglandin F2a, prostaglandin E2, 6-keto prostaglandin F1a and thromboxane B2 by suprofen in women with primary dysmenorrhea. Prostaglandins & Other Lipid Mediators. 2007;**83**(1-2):146-153

[50] Akoum A, Kong J, Metz C, et al. Spontaneous and stimulated secretion of monocyte chemotactic protein-1 and macrophage migration inhibitory factor by peritoneal macrophages in women with and without endometriosis. Fertility and Sterility. 2002;**77**(5):989-994

[51] Yang Y, Zhang X, Zhou C, et al. Elevated immunoreactivity of RANTES and CCR1 correlate with the severity of stages and dysmenorrhea in women with deep infiltrating endometriosis. Acta Histochemica. 2013;**115**(5):434-439. DOI: 10.1016/j.acthis.2012.10.006

[52] Ouyang Z, Osuga Y, Hirota Y, et al. Interleukin-4 induces expression of eotaxin in endometriotic stromal cells. Fertility and Sterility. 2010;**94**(1):58-62. DOI: 10.1016/j.fertnstert.2009.01.129

[53] Leconte M, Chouzenoux S, Nicco C, et al. Role of the CXCL12-CXCR4 axis in the development of deep rectal endometriosis. Journal of Reproductive Immunology. 2014;**103**:45-52. DOI: 10.1016/j.jri.2013.12.121

[54] Morin M, Bellehumeur C, Therriault MJ, et al. Elevated levels of macrophage migration inhibitory factor in the peripheral blood of women with endometriosis. Fertility and Sterility. 2005;**83**(4):865-872

[55] Attar E, Tokunaga H, Imir G, et al. Prostaglandin E2 via steroidogenic factor-1 coordinately regulates transcription of steroidogenic genes necessary for estrogen synthesis in endometriosis. The Journal of Clinical Endocrinology and Metabolism. 2009;**94**(2):623-631. DOI: 10.1210/jc.2008-1180

[56] Carli C, Metz CN, Al-Abed Y, et al. Up-regulation of cyclooxygenase-2 expression and prostaglandin E2 production in human endometriotic cells by macrophage migration inhibitory factor: involvement of novel kinase signaling pathways. Endocrinology. 2009;**150**(7):3128-3137. DOI: 10.1210/en.2008-1088

[57] Jana S, Chatterjee K, Ray AK, et al. Regulation of matrix metalloproteinase-2 activity by COX-2-PGE2-pAKT axis promotes angiogenesis in endometriosis. PLoS One. 2016;**11**(10):e0163540. DOI: 10.1371/journal.pone.0163540 eCollection 2016

[58] Chuang PC, Sun HS, Chen TM, et al. Prostaglandin E2 induces fibroblast growth factor 9 via EP3-dependent protein kinase Cdelta and Elk-1 signaling. Molecular and Cellular Biology. 2006;**26**(22):8281-8292

[59] Viganó P, Pardi R, Magri B, et al. Expression of intercellular adhesion molecule-1 (ICAM-1) on cultured human endometrial stromal cells and its role in the interaction with natural killers. American Journal of Reproductive Immunology. 1994;**32**(3):139-145

[60] Maeda N, Izumiya C, Oguri H, et al. Aberrant expression of intercellular adhesion molecule-1 and killer inhibitory receptors induces immune tolerance in women with pelvic endometriosis. Fertility and Sterility. 2002;**77**(4):679-683

[61] Sipak-Szmigiel O, Włodarski P, Ronin-Walknowska E, et al. Serum and peritoneal fluid concentrations of soluble human leukocyte antigen, tumor necrosis factor alpha and interleukin 10 in patients with selected ovarian pathologies. Journal of Ovarian Research. 2017;**10**(1):25. DOI: 10.1186/s13048-017-0320-9

[62] Drosdzol-Cop A, Skrzypulec-Plinta V. Selected cytokines and glycodelin A levels in serum and peritoneal fluid in girls with endometriosis. The Journal of Obstetrics and Gynaecology Research. 2012;**38**(10):1245-1253. DOI: 10.1111/j.1447-0756.2012.01860.x

[63] Kocbek V, Grandi G, Blank F, et al. TNFa-induced IKKb complex activation influences epithelial, but not stromal cell survival in endometriosis. Molecular Human Reproduction. 2016;**22**(11):768-777

[64] Rakhila H, Girard K, Leboeuf M, et al. Macrophage migration inhibitory factor is involved in ectopic endometrial tissue growth and peritoneal-endometrial tissue interaction in vivo: a plausible link to endometriosis development. PLoS One. 2014;**9**(10):e110434. DOI: 10.1371/journal.pone.0110434 eCollection 2014

[65] Khoufache K, Bazin S, Girard K, et al. Macrophage migration inhibitory factor antagonist blocks the development of endometriosis in vivo. PLoS One. 2012;**7**(5):e37264. DOI: 10.1371/journal.pone.0037264

[66] Hamburg MA, Collins FS. The Path to Personalized Medicine. The New England Journal of Medicine. 2010;**363**(4):301-304. DOI: 10.1056/NEJMp1006304

[67] Kingsmore SF. Multiplexed protein measurement: technologies and applications of protein and antibody arrays. Nature Reviews. Drug Discovery. 2006;**5**(4):310-320

[68] Mitchell P. A perspective on protein microarrays. Nature Biotechnology. 2002;**20**(3):225-229

[69] Uetz P, Giot L, Cagney G, et al. A comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae. Nature. 2000;**403**(6770):623-627

[70] Ito T, Chiba T, Ozawa R, et al. A comprehensive two-hybrid analysis to explore the yeast protein interactome. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**(8):4569-4574

[71] Hanash S. Disease proteomics. Nature. 2003;**422**(6928):226-232

[72] Keshishian H, Addona T, Burgess M, et al. Quantification of cardiovascular biomarkers patient plasma by targeted mass spectrometry and stable isotope dilution. Molecular & Cellular Proteomics. 2009;**8**(10):2339-2349. DOI: 10.1074/mcp.M900140-MCP200

[73] Robinson WH, DiGennaro C, Hueber W, et al. Autoantigen microarrays for multiplex characterization of autoantibody responses. Nature Medicine. 2002;**8**(3):295-301

[74] Belov L, de la Vega O, dos Remedios CG, et al. Immunophenotyping of leukemias using a cluster of differentiation antibody microarray. Cancer Research. 2001;**61**(11):4483-4489

[75] Lin Y, Huang R, Chen LP, et al. Profiling of cytokine expression by biotin-labeled-based protein arrays. Proteomics. 2003;**3**(9):1750-1757

[76] Gaster RS, Hall DA, Nielsen CH, et al. Matrix-insensitive protein assays push the limits of biosensors in medicine. Nature Medicine. 2009;**15**(11):1327-1332. DOI: 10.1038/nm.2032

[77] Ramachandran N, Hainsworth E, Bhullar B, et al. Self-assembling protein microarrays. Science. 2004;**305**(5680):86-90

[78] Tjalsma H, Schaeps RMJ, Swinkels DW. Immunoproteomics: From biomarker discovery to diagnostic applications. Proteomics. Clinical Applications. 2008;**2**(2):167-180. DOI: 10.1002/prca.200780012

[79] Gygi SP, Rist B, Griffin TJ, Eng J, Aebersold R. Proteome analysis of low-abundance proteins using multidimensional chromatography and isotope-coded affinity tags. Journal of Proteome Research. 2002;**1**(1):47-54

[80] Kubota K, Wakabayashi K, Matsuoka T. Proteome analysis of secreted proteins during osteoclast differentiation using two different methods: two-dimensional electrophoresis and isotope-coded affinity tags analysis with two-dimensional chromatography. Proteomics. 2003;**3**(5):616-626

[81] Yates JR 3rd, McCormack AL, Schieltz D, et al. Direct analysis of protein mixtures by tandem mass spectrometry. Journal of Protein Chemistry. 1997;**16**(5):495-497

[82] McCormack AL, Schieltz DM, Goode B, et al. Direct analysis and identification of proteins in mixtures by LC/MS/MS and database searching at the low-femtomole level. Analytical Chemistry. 1997;**69**(4):767-776

[83] Hess JL, Blazer L, Romer T, et al. Immunoproteomics. Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences. 2005;**815**(1-2):65-75

[84] Bruenner BA, Yip TT, Hutchens TW. Quantitative analysis of oligonucleotides by matrix-assisted laser desorption/ionization mass spectrometry. Rapid Communications in Mass Spectrometry. 1996;**10**(14):1797-1801

[85] Issaq HJ, Conrads TP, Prieto DA, et al. SELDI-TOF MS for diagnostic proteomics. Analytical Chemistry. 2003;**75**(7):148A-155A

[86] Boyle MDP, Hess JL, Nuara AA, et al. Application of immunoproteomics to rapid cytokine detection. Methods. 2006;**38**(4):342-350

[87] Kroot JJC, Laarakkers CMM, Geurts-Moespot AJ, et al. Immunochemical and mass-spectrometry-based serum hepcidin assays for iron metabolism

disorders. Clinical Chemistry. 2010;**56**(10):1570-1579. DOI: 10.1373/clinchem.2010.149187

[88] Tabibzadeh S, Becker JL, Parsons AK. Endometriosis is associated with alterations in the relative abundance of proteins and IL-10 in the peritoneal fluid. Frontiers in Bioscience. 2003;**8**:A70-A78

[89] Ferrero S, Gillott DJ, Remorgida V, et al. Haptoglobin beta chain isoforms in the plasma and peritoneal fluid of women with endometriosis. Fertility and Sterility. 2005;**83**(5):1536-1543

[90] Liu H, Lang J, Zhou Q, Shan D, Li Q. Detection of endometriosis with the use of plasma protein profiling by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry. Fertility and Sterility. 2007;**87**(4):988-990

[91] Ferrero S, Gillott DJ, Remorgida V, et al. Proteomic analysis of peritoneal fluid in women with endometriosis. Journal of Proteome Research. 2007;**6**(9):3402-3411

[92] Zhang H, Niu Y, Feng J, et al. Use of proteomic analysis of endometriosis to identify different protein expression in patients with endometriosis versus normal controls. Fertility and Sterility. 2006;**86**(2):274-282

[93] Wang L, Zheng W, Mu L, et al. Identifying biomarkers of endometriosis using serum protein fingerprinting and artificial neural networks. International Journal of Gynaecology and Obstetrics. 2008;**101**(3):253-258. DOI: 10.1016/j.ijgo.2008.01.018

[94] Fowler PA, Tattum J, Bhattacharya S, et al. An investigation of the effects of endometriosis on the proteome of human eutopic endometrium: a heterogeneous tissue with a complex disease. Proteomics. 2007 Jan;**7**(1):130-142

[95] Wang N, Zhang Y, Liu B. Demographic and Clinical Features of Endometrial Polyps in Patients with Endometriosis. BioMed Research International. 2016;**2016**:1460793. DOI: 10.1155/2016/1460793

[96] Ferrero S, Gillott DJ, Remorgida V, et al. Proteomic analysis of peritoneal fluid in fertile and infertile women with endometriosis. The Journal of Reproductive Medicine. 2009;**54**(1):32-40

[97] Sharpe KL, Bertero MC, Vernon MW. Detection of a progesterone-induced secretory protein synthesized by the uteri but not the endometriotic implants of rats with induced endometriosis. Fertility and Sterility. 1991;**55**(2):403-410

[98] Sharpe KL, Vernon MW. Polypeptides synthesized and released by rat ectopic uterine implants differ from those of the uterus in culture. Biology of Reproduction. 1993;**48**(6):1334-1340

Chapter 4

Progesterone Resistance and Adult Stem Cells' Genomic and Epigenetic Changes in the Puzzle of Endometriosis

Manuela Cristina Russu

Abstract

Endometriosis is a chronic inflammatory disease under hormonal/non-hormonal regulation, and microenvironment influences, originating in adult stem cells (mainly of bone marrow/endometrial progenitor mesenchymal type), and their exosomes, with special migratory and adhesion capacities. The postmenstrual repair with regeneration of eutopic and ectopic endometrium has similar genetic and epigenetic changes versus disease-free women. The competition between ectopic and eutopic endometrium for a limited supply of stem cells, and the depletion of normal stem cells flux to the uterus is considered the novel mechanism through which endometriosis interferes with endometrial functions and fertility. The gene expression DNA/RNA or microRNA changes/dysregulation of estrogen and progesterone receptors represent a possible explanation of progesterone resistance or loss of progesterone signalling in ectopic, and eutopic endometrium versus normal. The genes' changes involved in hormonal/non-hormonal pathways control of eutopic/ectopic endometrial cells, and of invaded tissues/organs may explain the disease persistency, progression and severity. Deficient DNA methylation of ERβ, the initial genomic event is followed by pathologic over-expressed ERβ in ectopic stromal cells, and it dictates the decline of PR isoforms, PRB being significantly lower in ectopic and eutopic endometrium. Altered expression of ERα, ERβ, and PRs accompanies the conversion of resident normal endometrial cells to ectopic lesions.

Keywords: endometriosis, progesterone resistance, stem cells, steroid receptors, genomic, epigenetics

1. Introduction

Endometriosis is a chronic inflammatory disease, estrogen-dependent and progesterone-resistant, with a multifactorial etiology, which can appear from intrauterine life [1, 2] and can progress and aggravate as a hidden disease in adolescence [3], being more common during reproductive years, when it is more studied. It is an invasive and metastatic disease, being different from malignancies by missing nuclear atypia [4]. Endometriosis is characterized and diagnosed by

the presence and growth of endometrial-like glands and stroma outside the uterine cavity and musculature, which undergoes cyclic proliferation and breakdown similar to the eutopic endometrium, with peculiar symptoms in most cases, and non-specific also in others. The genetic predisposition, and the epigenetic changes, the systemic, and local environmental factors, and the dysfunctions in endocrine and immune systems, which make possible the new cellular connections induced by exosomes between original tissues and ectopically attached adult stem cells, with bone marrow or endometrial source, are believed to a play significant role in the establishment, maintenance, and progression of endometriosis [5, 6] and its consequences on women's fertility and quality of life. The human endometrium is an angiogenic tissue, with enormous waves of regenerative capacities through cell proliferation, differentiation, and recruitment of inflammatory cells, with episodes of apoptosis and events of breakdown and regeneration without scar, but in ectopic endometrium progression to deeply infiltrative lesions, the scar is present in the form of important fibrosis. The ovarian hormones, estrogens, and progesterone through their receptors—genetic and epigenetic regulated—play an important role in physiology and pathophysiology of eutopic and ectopic endometrium. Excess estradiol and progesterone resistance are documented in eutopic and ectopic endometrium of ill women.

2. Origin and qualities of ectopic endometrium

2.1 Hypotheses and theories on the mechanisms of ectopic endometrium development in different stages

Endometriosis is a multifactors disease, a disease of hypothesis and theories, and many concepts are elaborated in order to elucidate the diagnosis and treatment, to stop the chronic pains and the progression which is like that of a cancer, and the possible malignant transformation in 1% cases [7, 8]. There were explored multiple hypotheses and theories, being reloaded of some old ones, in the conditions of the modern technological possible pathological assessments of eutopic and ectopic endometrium (flow cytometry, immunohistochemistry, genetic analysis), in association with evidences from animal models as mice, rats, baboons, and marmosets [9].

The most referred hypothesis was based on the retrograde deposits of viable endometrial fragments refluxed through the fallopian tubes during the menstruation into the peritoneal cavity, where they attach and invade the peritoneal mesothelium or pelvic organs to establish ectopic growth of endometrial tissue. It is known as J. Samson's theory—or the "transplantation" theory—which is the most widely accepted [10].

The retrograde menstrual blood flow is present in nearly 90% of women [11], but women who develop endometriosis have larger volumes of retrograde menstrual flow than women without disease [12]. The retrograde blood flow is considered to be at the origin of ectopic endometrium in early onset endometriosis (EOE), starting around thelarche/menarche or early adolescence, and EOE may have an origin different from the adult variant, originating from neonatal uterine bleeding [13].

Besides the transportation of endometrial debris from uterine cavity through the fallopian tubes to the peritoneal and organs' surface, the rare condition of the dissemination through regional lymphatic circulation and lymph nodes [14, 15] to extrapelvic, distant organs, with no direct connection to the uterus- thorax, brain, and its structures, is discussed [16]. Since many years have passed, other factors associated with retrograde menstrual blood flow, such as the uterine contractions

during menstruation [17, 18] and/or the involvement of peritoneal fluid [19, 20] as favorable factors for the transportation and spread of retrograde endometrial debris in the peritoneal cavity, are discussed. The retrograde endometrial debris are associated to the adhesive, proliferative, and invasive properties of endometrial cells, mainly of the stromal/mesenchymal ones [21], and to the chemokines (E-cadherin, N-cadherin) elaborated by endometrial cells [22, 23], and in connection to leukocyte actions [24, 25] and to the adhesion properties of the mesothelium are explained the ectopic endometriosis lesions. It is considered that in patients with endometriosis it is a real cross talk between endometrial stromal cells and mesothelial cells, which have a common embryological origin, as it will be discussed later. The new tissue injury and repair (TIAR) concept is comparable [26, 27]: uterine dysperistalsis and hyperperistalsis may induce more trauma, with dislocation of more basal endometrium, and a greater number of stemlike cells present in the retrograde refluxed menstrual blood.

Other two theories are partially connected to one another and to embryologic development. The "coelomic metaplasia" theory—according to the same cell lineage origin of the thoracic, abdominal, and pelvic peritoneum, the Müllerian ducts, and the ovarian germinal epithelium, all being derived from the coelomic wall epithelium of developing embryo, and through metaplasia they generate endometriosis [28]. The second "embryological theory" is very old [29, 30], and it considers the embryonic rests, considering that the presence of cells with the Müllerian origin within the peritoneal cavity are induced to form endometrial tissue when subjected to the appropriate stimuli. von Recklinghausen [29] from Austria was the first who described this disease at the level of the uterus (adenomyomas and cystadenomas) and the tube, and in his opinion their origins were remnants of the Wolffian body. The embryological theory has been recently re-proposed by the scientists from the Italian Foundation of Endometriosis (Rome) (Signorile PG, Baldi A) [8] and the lympho-vascular metastasis or the iatrogenic direct graft implant or the "implantation" theory. Their concept is based on the stem cells as origin of ectopic endometrium.

Hypotheses and theories are actually combined, being considered that endometriosis is the final stage of the combination of some aberrant biological processes, some starting from the intrauterine life, like the "Müllerianosis": the endometrial cells are dislocated outside the uterus during organogenesis, at the moment of mesoderm differentiation from the Müllerian ducts [2, 31]. "Müllerianosis" was revealed by immunohistochemistry in human female fetuses at different gestational ages by the scientists from the Italian Foundation of Endometriosis, from Rome [1, 2, 8]. The Italian scientists have discovered ectopic endometrial cells in five different ectopic sites, outside the uterine cavity, being cited the rectovaginal septum, the proximity of the Douglas pouch, the mesenchymal tissue close to the posterior wall of the uterus, and the rectal tube at the level of muscularis propria and in the wall of the uterus. All these areas are common places of endometriosis in adult women.

Another theory explains that the retrograde menstrual blood flow is possible in women with a genetic predisposition and with an improper innate immune answer, which is conditioned by the exposure to some environmental factors [5]. Recently, the local microenvironment or the peritoneal involvement in the defense against uterine menstrual debris [endometrial cells with plasticity and in terminal differentiation state, plus microvesicles (composed of diverse types of membranes of plasma and endosomal membrane origin) and exosomes released from the endometrial cells] is considered to play a crucial role in the development of endometriosis [6]. The human endometrial exosomes and nanoparticles (~100 nm diameter) released from endometrial cells are mediating the

intercellular connection/communication, and they can transfer small RNAs and mRNA via the extracellular environment to cells at distant sites [32, 33], especially in the stroma as happens in cancer [34], a fact that will be discussed in the next subchapters. The peritoneal clearance through apoptosis of the implanted ectopic endometrial islands from the uterine debris is under genetic, immune system and sexual steroid hormone control. The nature of predisposing individual genetic, biochemical, and hormonal factors or inherent defects of the endometrium, peritoneal cavity, or immune system of patients with endometriosis is unclear [35]. There are controversies if endometriosis is an autoimmune disease due to anti-endometrial antibodies, which were detected in the serum of women with endometriosis and on the increase/decrease/balance in the activity of T-helper1 (Th-1)/T-helper 2 (Th-2) cells in women with endometriosis [36], associated with anti-inflammatory cytokines as IL-4, IL-6, and IL-10, which primarily regulate the intensity and duration of the inflammatory response by suppressing the effects of pro-inflammatory cytokines [interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF), although some have also inflammatory roles [37]. The pro-inflammatory cytokines primarily initiate and amplify the inflammatory response to endometrial debris by signaling the recruit of additional immune cells and pro-inflammatory mediators to the site of endometrial cell adherence. IL-6 is most studied, being with prominent inflammatory and anti-inflammatory functions, which challenge the understanding of its full role in endometriosis. IL-6 is mainly produced by macrophages, and by Th-1 cells and B cells, fibroblasts and endothelial cells may produce IL-6 specially in women with endometriosis, who have higher levels of this cytokine in ectopic and eutopic endometrium and much higher with the severity stage of disease than normal women [38]. All these cytokines and chemokines discovered in endometriosis development are similarly involved in pelvic inflammatory disease and are under genomic and hormonal control through their microRNA receptors, inclusive for progesterone, with well-known anti-inflammatory actions.

Three different forms of endometriosis are described in the pelvic cavity: peritoneal, ovarian, and deeply infiltrating lesions. The development of endometrial ectopic island on the parietal peritoneal surface or different organs' surfaces is an end point of many molecular mechanisms necessary for the establishment and survival of endometrial implants that may have common origin to the peritoneum. The steps described in endometriotic lesion development are analyzed since many years. After the shedding of viable endometrial cells during menstruation, the retrograde transport of exosomes (vesicles released by the endometrial cells in the terminal state of differentiation and with plasticity) and menstrual cells, with their attachment to the peritoneum and formation of temporary ectopic lesion, under the control of immune system is necessary. The invasion into the mesothelium, survival, and proliferation of the ectopic endometrial cells with endometriotic lesion formation is the final step [5, 6]. In healthy women, the immune system removes the temporary ectopic lesions through apoptosis induction. The released exosomes of ectopic endometrial cells could facilitate immune evasion; enhance proliferation, invasion, and angiogenesis in the lesion, and subsequently progress into a persistent endometriotic lesions. Exosomes could therefore be one important factor to enable a temporary endometriotic lesion to establish a sufficient blood supply in order to grow and survive at the ectopic site, as they act in an autocrine, paracrine, and endocrine manner in intercellular communication. The current knowledge suggests different roles of endometrial fragments outside the uterine cavity: the endometrial stromal cells are involved in the attachment to the

peritoneum, whereas endometrial glandular epithelial cells primarily play a role in the invasion and growth of the lesion. These events are possibly related to a defect in the ability of peritoneal natural killer (NK) cells to eliminate the endometrial fragments regurgitated with menstrual debris by lysis and by the release of soluble non-specific factor(s) which interfere with NK cells from human endometrial stromal cells [39], as well as higher levels of IL-6, which suppress them [40]. The human endometrial mesenchymal stem cells (HMSCs) have another capacity, that of transdifferentiation or metaplasia, which permits them to differentiate in cells without any embryological connection, or to complete differentiated cells, with the change of phenotype [41], a biologic event supposed to be during the dormant phase from fetal life to adolescence [42]in association to some external triggers as transient hypoxia, chronic inflammation.

Endometrial cells are able to exploit the promotion of vasculogenesis and angiogenesis mediated by the inflammatory response that they trigger in cooperation with both immune cells and local tissue, usually the peritoneum. In advanced stages of illness, the ectopic endometrium islands have an enormous development of blood vessels, a high degree of fibrosis, and a degree of neurogenesis. Blood vessel development depends on two processes induced by ectopic endometrial islands—vasculogenesis and angiogenesis—triggered by the endothelial progenitor cells (EPCs) with bone marrow stem cell origin or endometrial progenitor/regenerative/stem cell origin. The high levels of angiogenetic factors as vascular endothelial growth factor (VEGF) and other angiogenic factors including IL-6, IL-8, and TNF-α mediate the process of angiogenesis by activating angiogenic switch of endothelial cells. Lesion local production of estradiol maintains the expression of VEGF and promotes macrophages to produce VEGF and monocyte chemoattractant protein (MCP)-1. Thereby, intercellular communication mediated *via* exosomes could represent a missing link between the different theories on the pathogenesis of endometriosis. Exosomes released by eutopic or ectopic endometrium or shedded endometrial cells could induce metaplasia of cells at ectopic sites ("coelomic metaplasia" and "induction" theories) or aid in tissue remodeling after injury (TIAR concept) (**Figure 1**) [26].

In 2018, a team from Saint Petersburg [43] published a hypothesis on the existence of a special endometriosis development program (EMDP) which switches on in the progenitor/stem cells (SCs) of the endometrium in SCs descended from the Müllerian duct; EMDP suggests that the cells are prone to give rise to endometriosis partly through endometrial-mesenchymal transition, their invasivity into the peritoneum lining, and differentiation and growth into endometriotic lesions. The EMDP was subdivided into three parts: the first one in intrauterine period and the last two in the postnatal life. (1) Transition of mesodermal embryonic cells into cells of the endometrium within Muller duct rudiments, (2) acquisition of endometrial cell abnormalities and cell transition into endometriotic SCs, and (3) invasion of the SCs into the peritoneum lining and their differentiation into endometriotic lesions (**Figure 2**).

2.1.1 The new concept of stem cells for the development of endometriosis

Stem cells are undifferentiated cells with the capacity to remain in this stage for some generations, after cell proliferations. Maintenance of the stem cell population requires cellular self-renewal, i.e., the capacity to generate identical daughter cells. Alternatively, stem cells can undergo asymmetric division, producing an identical daughter cell and a more differentiated daughter, or symmetric division producing two daughter stem cells or two transit-amplifying progenitors. Although neither

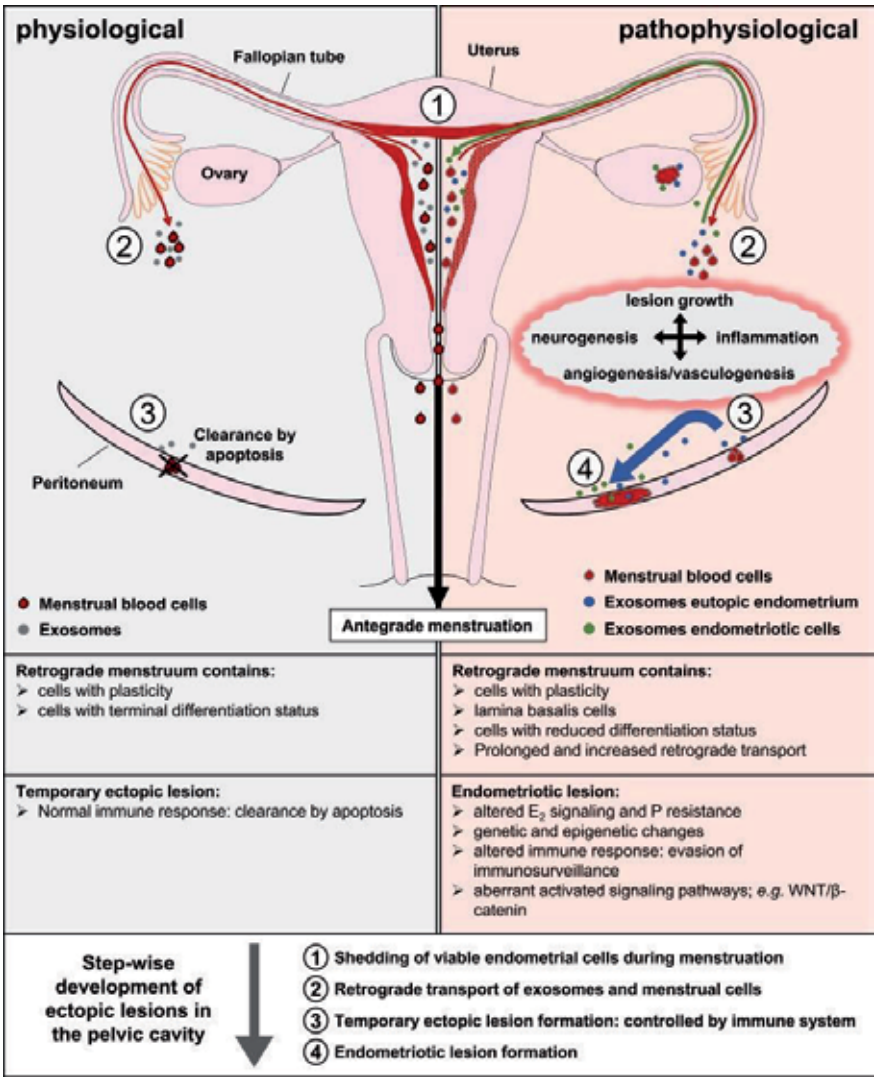

Figure 1.
Molecular and cellular pathways in endometriosis. Adapted from Klemmt et al. [6].

progenitors nor precursors are called stem cells, it is often practically difficult to distinguish adult stem cells from their progenitor/precursor cells. Indeed, for instance, a small subset of hematopoietic progenitor cells have been termed hematopoietic stem cells (HSCs).

The stem cells have in vivo the capacity to participate to natural phenomenon as the repair and regeneration of damaged tissues, as is the endometrium during and after menstruation.

Since then, in many populations worldwide, adult stem cells (ASCs)—known as somatic stem cells or tissue-specific stem cells, such as the blood [44], intestines [45], muscles [46], skin [47], nervous system [48–50], heart, liver [51, 52], dental pulp, adipose tissue, synovial membrane, umbilical cord blood, amniotic fluid [53, 54], and the endometrium [55–58], are found.

The human endometrium is an extraordinary model for controlled tissue remodeling. The endometrial tissue renewal at each menstrual cycle during reproductive years (about 7 mm within 1 week in every menstrual cycle, and 500 menstrual cycles during a woman's lifetime) [44], and in postpartum, with conservation of

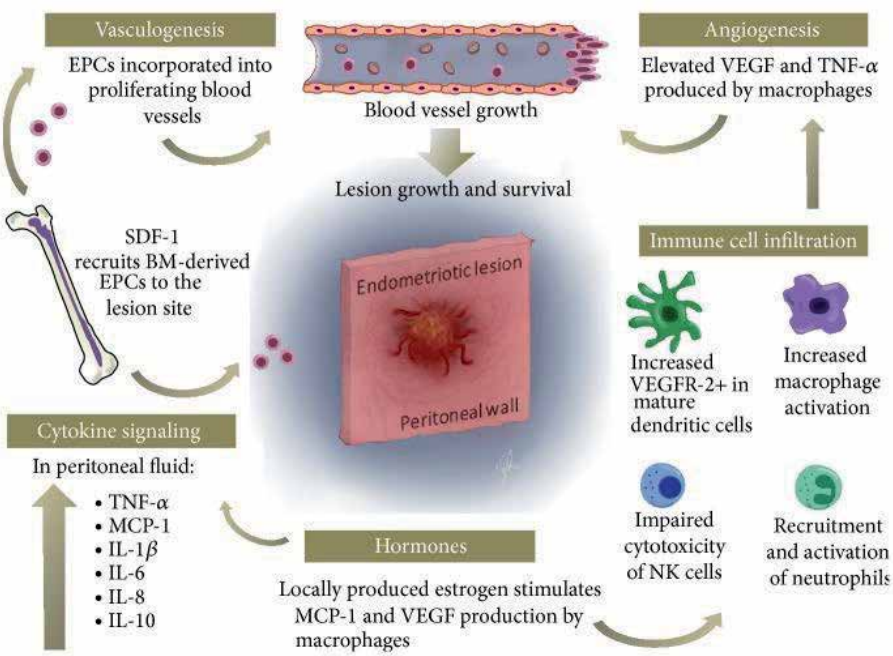

Figure 2.
Neovascularization and ectopic endometrium growth on peritoneal surface. Adopted from Ahn et al. [35]. This is an open-access article distributed under the Creative Commons Attribution Noncommercial License, which permits unrestricted use, distribution, and reproduction in any medium, provided that the original work is properly cited.

this capacity even in postmenopausal years during hormone therapy, is actually explained by the presence of adult stem cells; there are controversies on these cells origin—bone marrow stem cells or endometrial stem cells—epithelial, stromal/mesenchymal, endothelial, niche and side population stem cells [55, 59–61]. There are studies on these types of cells in many research laboratories and medical centers from Australia, Brazil, the USA, Italy, France, Romania, Spain, and the UK, as well as there are controversies if endometrial epithelial cells are derived from the bone marrow [62, 63]. Independent of their origin, the endometrial stem cells are developing the ectopic island of endometriosis from the intrauterine life in female gender [59, 61, 64], and after a dormant period of life, at the menarche they restart their aggressive actions, modulated by sexual steroid hormones.

The concept of endometrial origin of stem cell populations was first proposed by Prianishnikov VA in 1978 [65] due to the highly regenerative nature of the endometrium, and actually the medical communities are waiting to produce novel therapies with these progenitor cells in regenerative medicine, even in infertility from endometriosis [55, 61, 66].

2.2 Adult bone marrow stem cells as the origin of ectopic endometrium

It was postulated that stem cells (SCs) that originated from bone marrow could be attracted in the human endometrium, with generation of endometrial epithelial and stromal cells [61, 67], from the fetal life, but their participation in endometriosis should be proven [68, 69], and mesenchymal bone marrow SCs (bmSCs) in inflammation sites in the peritoneum were found [70]. An Australian team who analyzed the endometrial stem cells for 10 years after their first published paper [61] considers the stem cells originating in the bone marrow to be fibroblasts with

clonogenicity, plastic adherence properties, and multilineage differentiation into bone and marrow lineages in cultures, and they renamed these cells as multipotent mesenchymal stromal cells maintaining the acronym MSCs, for bmMSCs. These cells have comparable properties to stromal endometrial stem subpopulation cells, which will be discussed in the next subchapter.

2.3 Endometrial stem/regenerative/progenitor cells as the origin for ectopic endometrium: the novel concept on HESCs and their progenitor cell capacities

After the preliminary hypothesis and discussion regarding the recovering capacities from the basalis of the primate endometrium, as a bifunctional germinal compartment [65, 71, 72], and the discovery of endometrial regenerative/progenitor/stem cells [55, 56, 73–75], with their presence in the menstrual blood [76–78], a new hypothesis/concept which tries to clarify the underlying pathophysiologic mechanisms of endometriosis, besides the proposed conventional theories explaining the three different aspects of endometriosis, was elaborated. In the concept of Padykula et al. [72] after the menstrual shedding of endometrial functionalis layer, the remaining basal layer was believed to behave as a germinal compartment from which various types of endometrial cells proliferate and differentiate, suggesting that putative endometrial stem cells reside in the basalis. Endometrial stem cells have the characteristics of adult stem cells (ASCs), namely, the clonogenicity and high ability to proliferate, differentiate, and induce rapid angiogenesis, a fact that may contribute to consider their binary qualities in natural functions in the normal endometrium and in endometriosis [5, 79]. Their involvement in endometriosis is from intrauterine life [64], with reactivation after a dormant period in adolescence years in the form of so-called early-onset endometriosis (**Figure 3**) [80].

There are described different types of human endometrial stem cells (HESCs): epithelial—with confirmed location in the basalis of endometrial epithelium, as was postulated [72] stromal/mesenchymal, endothelial, side population—which can differentiate into epithelial and stromal endometrial cells [81], a niche for human epithelial stem cells, and label-retaining cells (LRCs) [82]. All these stem cells can be actually identified with specific markers for immunohistochemistry in endometrial biopsy and/or can be isolated from menstrual blood, being available some devices, as menstrual cups for collection [83]. After repeated examinations of endometrium specimens in all phases of the menstrual cycle, it was concluded that endometrial regenerative cells are located in the basalis, the functionalis being shed at every menstruation, and the basalis which remains intact is the origin of each new cycling endometrium [73]. At 10 years after their first published paper on human and mouse endometrial stem cells, isolated from uterine tissue at hysterectomy, Gargett et al. [61]—from Monash University and Monash Medical Centre (Australia)—are discussing HESC involvement in uterine pathology (endometriosis, adenomyosis, Asherman syndrome, endometrial cancer). The mesenchymal/stromal/endometrial stem cells (eMSCs) or fibroblasts are the most studied. When compared to mesenchymal bone marrow stem cells, there are some differences regarding eMCS abilities to generate a vascularized stroma, with the capacity to differentiate into decidualized stroma when transplanted into an animal at the single-cell level. The eMCSs produced endometrial stroma and incorporated into renal parenchymal blood vessels when xenografted under the kidney capsule of immunocompromised NSG mice [84].

Human endometrial mesenchymal stem cells (HMSCs) were identified with specific markers used for their enrichment [CD146(+)PDGFRβ(+) (platelet-derived

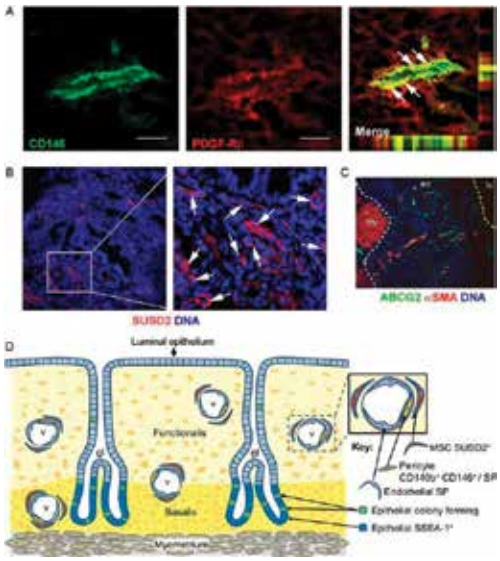

Figure 3.
Localization of human endometrial mesenchymal stem cells. (A–C) Immunofluorescence images of human endometrium showing perivascular identity of human eMSCs. (A) Co-localization (white arrows) of CD146 and platelet-derived growth factor receptor beta (PDGF-Rβ) in pericytes of venules and possibly capillaries in the functionalis stroma. (B) Perivascular SUSD2 expression (white arrows). (C) ATP-binding cassette, subfamily G member 2 (ABCG2) and αSMA co-staining showing perivascular and endothelial identity of SP cells. The white dotted lines indicate the junction between the endometrium (en) and myometrium (my) and yellow dotted line indicates the luminal surface (lu) of the uterine epithelium. (D) Schematic showing location of stem/progenitor cells identified in the human endometrium. Epithelial progenitor cells are postulated to be a subpopulation of cells located in the basalis, in the base of the glands, by SSEA-1 marker. Sushi domain containing-2+ (SUSD2+) eMSCs are perivascular cells. eMSC co-expressing CD146 and PDGFRβ/CD140b are most likely pericytes, as they are located adjacent to endothelial cells in vessels (v) in both the basalis and the functionalis. SP cells are a heterogeneous population comprising CD31+ endothelial cells and CD140b+CD146+ pericytes. Scale bar in (A) = 50 μm. (A) Adopted from Gargett et al. [61]. This is an open-access article distributed under the terms of the Creative Commons Attribution Noncommercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits noncommercial reuse, distribution, and reproduction in any medium, provided that the original work is properly cited. For commercial reuse, please contact journals.permissions@oup.com.

growth factor receptor β) and SUSD2(+) (sushi domain containing-2), and they were distinguished from stromal fibroblasts. HMSCs were located perivascular, and like the pericyte, they were identified in endometrial basalis and functionalis vessels [78]. These Australian researchers presented the similarities and differences between eMSCs and endometrial stromal fibroblasts and the identity of bone marrow-derived cells involved in endometrial function.

It is a novel concept regarding the migratory and invasive abilities of HESCs and their progenitor cells, being increasingly recognized their contribution to the intense tissue remodeling associated with embryo implantation, trophoblast invasion, endometrial regeneration, and endometriosis/adenomyosis progress [85]. Numerous reports indicate that endometriosis and adenomyosis are associated with increased basal and stimulated invasiveness of HESCs and their progenitor cells, suggesting a link between a heightened menstrual repair response and the formation of ectopic implants. The abilities of migration and invasiveness of HESCs are controlled by a complex array of hormones—estradiol and progesterone, growth factors, chemokines, and inflammatory mediators—and involve signaling through Rho GTPases, phosphatidylinositol-3-kinase, and mitogen-activated protein kinase pathways, studied in laboratories from Western Europe (Germany, The Netherlands, Italy), first in early pregnancy and later in endometrial pathology [86, 87].

The clonogenic qualities of epithelial and mainly of stromal/mesenchymal stem cells, in association with the migratory and invasive qualities of the last ones from the eutopic endometrium of women with endometriosis, may explain the development of endometriosis. It can be discussed if stem/progenitor cells which develop the implants of endometriosis are abnormal by the increase of their capabilities to stabilize and implant in ectopic islands, through the lack of the tumor suppressor molecule E-cadherin [22], or if normal stem/progenitor cells are implanting on abnormal peritoneum [88].

In other studies, the answers to these issues were to discuss a combination of the genetic predisposition or the special immunohistochemical pattern of mesenchymal or stromal stem cells from the retrograde menstrual blood flow, with high expression of CD9, CD10, and CD29– [89], and/or with DNA/RNA changes, and/or with an improper immune response possible at exposure at some environmental factors [5].

2.4 Comparison of eutopic and ectopic endometrium with normal cycling endometrium

Normal endometrium is containing large quantities of distinct stromal cells with abundant PRs, which influence epithelial cell proliferation and differentiation and protect against carcinogenic transformation. In endometriosis, eutopic and ectopic tissues do not respond sufficiently to progesterone, being considered to be progesterone-resistant, a fact that contributes to proliferation and survival of ectopic endometrium. The ectopic endometrium is similar to eutopic endometrium but with some peculiarities. The restoration of eutopic endometrium after each menstruation is without scar, a fact that is not present in ectopic endometrium, which undergoes cyclic proliferation and breakdown similar to the endometrium, but with a fibrotic progressive scar. On the other hand, between the two endometrial areas are very strong connections, considered to be bidirectional, and a competition between ectopic and eutopic endometria for a limited supply of stem cells, with different origins, that contribute to restoration/repair of eutopic endometrium after menstruation or other pathological conditions, and the depletion of normal stem cells flux to the uterus is a novel mechanism by which endometriosis interferes with endometrial function and fertility [90].

The phenotype, proliferative, and differentiation in vitro capacities of stromal cells from ectopic lesion from peritoneum, ovarian and deeply infiltrating endometriosis in comparison to the same structures of normal women, were analyzed using the contrast microscopy, immunocytochemistry, and functional bioassays [21]. The doubling time of stromal cells from the deeply infiltrating lesions is lower than that of endometrial stromal cells, and the levels of prolactin and insulin-like growth factor-binding protein-1 (synthesized by the stromal cells) are reduced in supernatants from stromal cells derived from the three types of lesions and from the endometrium of ill women. The conclusion of the British doctors was that endometriotic cell lines and endometrial cells from women with endometriosis are losing the capacity of differentiation, which can explain the cells' capacities for proliferation and survival in the ectopic environment.

When treating the complications of endometriosis, it is recommended to evaluate not only the effect on the endometriotic lesion itself, but it is also essential to consider the effect on normal uterine endometrium in the categories of reproductive age population, including stem cell recruitment as an essential means of uterine repair [90].

The molecular analyses of eutopic and ectopic endometrium versus endometrium of disease-free women are bringing us some news for endometrial pathologies—endometriosis and endometrioid endometrial cancer. The activation of AKT

pathway in endometriosis is reported in a relatively small number of studies. The study of p(Ser473)-AKT in ectopic and eutopic tissue in different stages of the menstrual cycle depicted a higher level of the AKT in endometriotic tissues [91], mainly from ovarian endometriomas, a fact confirmed by other studies, which have revealed in examined endometrioma tissues an association of lower levels of PTEN in epithelial cells of both endometrial types [92–94].

3. Progesterone resistance in endometriosis

3.1 Short history of progesterone resistance/pseudocorpus luteum insufficiency

Progesterone resistance (PR) was first described and nominated as pseudocorpus luteum insufficiency [95], being considered as a local defect of progesterone (P4) action on endometrial stroma. The patient with this disorder was an infertile woman with normality regarding menstrual cycle, duration of luteal phase, and plasma immunoreactive LH and P4 concentrations but with an immature endometrium histologically. These clinicians were the first who assessed the progesterone receptors in the endometrium, as it was recognized 5 years later by Chrousos et al. [96]. PR is discussed together with other hormones' resistances, and it is a clinical consequence of many gynecological disorders regarding the ovaries—PCOS, the endometrium—abnormal uterine bleeding associated with endometrial hyperplasia, the eutopic endometrium in endometriosis, the decidual causes of infertility or recurrent pregnancy loss, or the myometrium—for miscarriage and preterm birth, when it is lost the uterine quiescence. The fetal endometrium remains progesterone-resistant, except when fetal distress causes decidualization and menstruation at birth [80], or the "neonatal menstruation," which is a biomarker reflecting a stage of endometrium development that may subsequently have an impact on the reproductive life of the adolescent and the young adult.

PR is discussed as the decreased sensitivity and responsiveness of the target tissue to bioavailable P4 [96]. In the medical literature, PR is limited to endometriosis, but more and more papers do not agree to this [97]. At the first glance, the terms "estrogen dependence" and "progesterone resistance" appear to describe opposite sides of the same coin. Actually, there are emerging evidences suggesting that PR in endometriosis is not just a consequence of perturbed progesterone signal transduction caused by chronic inflammation, but it is associated with epigenetic chromatin changes that determine the intrinsic responsiveness of endometrial cells to differentiation cues [98]. The concept of "progesterone resistance," and its clinical relevance, is far from being well established and it is in need for redefinition [98].

3.2 Estrogen and progesterone action in eutopic and ectopic endometrium

The uterus is one of the most important organs with different cell types differently influenced by sexual steroid hormones, and the relative balance of progesterone and estrogen steroidal activity governs the function of normal endometrium throughout the menstrual cycle. The growth-promoting effects of estrogen during the proliferative phase of the cycle are countered by progesterone antiproliferative actions at the postovulatory onset of the secretory phase, with decidualizing the endometrial stroma later in the secretory phase. The steroid hormones linked to specific nuclear receptors which are able to bind to the promoter of target genes and then regulate proliferation and/or differentiation processes, in order to prepare the stroma/decidua for embryo's implantation [99].

Endometriosis is appreciated as an ultimate hormonal disease, owing much to its estrogen dependency and aberrations in estrogen production and metabolism and progesterone resistance. Several lines of evidence have linked endometriosis with excessive estradiol (E2) signaling in the ectopic tissues [100]. The high ectopic endometrium levels of E2 are associated with its local biosynthesis through the presence of very well-known enzymes (17β-hydroxysteroid dehydrogenase-1 and aromatase) [101, 102] and with the activation of estrogen receptors and stimulation of mitotic activity and inflammatory response. The proliferative, pro-inflammatory, and antiapoptotic effects of E2 appear to be exacerbated in women with endometriosis; physiological E2 concentrations are able to induce an enhanced inflammatory response mediated by local chemokine production and to reinforce the mechanisms of cell survival mediated by extracellular signal-regulated kinases and Bcl-2 [103]. Progesterone controls endometrial proliferation and differentiation, which are important cellular events in uterine function—normal menstruation, embryo implantation, and protection against the development of estrogen-driven endometrial cancer. The postovulatory surge in P4 triggers a highly coordinated and sequential response as arrest of estrogen-dependent epithelial cell proliferation, followed by the secretory transformation of the glands, recruitment of various bone marrow-derived immune cells, and angiogenesis [104]. P4 acts on stromal cells of the normal endometrium and is inducing the secretion of paracrine factor(s), which are inducing the expression of the enzyme 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD-2) which metabolizes the biologically active estrogen E_2 to estrone (E_1), in the neighboring epithelial cells, and the epithelial cell proliferation is arrested.

Since then it is known that progesterone reduces natural killer (NK) cell activity [105], increases suppressor cell levels [106], inhibits cytotoxic T-cell activity [107], induces the production of lymphocyte-blocking proteins [108], and modifies the cytokine response from the Th-1 to the pre-pregnancy Th-2 pattern [109]. All these cytokines are involved actually in the defense to the ectopic retrograde endometrial debris, exosomes, and endometrial stromal stem cells.

Recently in the USA, in Michigan State University—in the Departments of Comparative Biosciences, of Animal Science, and of Molecular & Integrative Physiology, the effects of progesterone to alleviate endometriosis, induced in the peritoneal cavities of immunocompetent female mouse and maintained with exogenous estrogen, if the administration is before the induced endometriotic lesions are discussed (**Table 1**) [110].

P4 is regulating multiple events on reproductive tissues, the responses to P4 are vastly different in normal and ill target tissues and cells, the mechanisms responsible for this striking contrast in progesterone's effects in normal versus diseased tissues are largely unknown, and one plausible explanation being the specific microenvironment within target tissues—including locally secreted factors, expressed receptors, and paracrine and autocrine communication—determines the overall effect of P4.

- P4 restricts expansion of the ectopic lesions by inhibiting endometrial cell proliferation and neovascularization
- P4 suppresses E_2-dependent inflammatory responses in the ectopic lesions
- P4 maintains ER-α-/PR-mediated signaling; their loss in the ectopic lesions leads to resistance to P4 therapy if the treatment is postinduction of lesion

Table 1.
Progesterone alleviates endometriosis, induced in the peritoneal cavities of female immunocompetent mouse and maintained with estrogen (after Li et al. [110]).

3.3 Estrogens' and progesterone receptors in eutopic and ectopic endometrium

The ovarian hormones, estrogen and progesterone, modulate uterine events in a spatiotemporal manner, from the midsecretory phase, and prepare the endometrium to become receptive to the blastocyst signals, and decidualized stromal cells are seen from the late secretory phase of the menstrual cycle.

Being an estrogen-dependent illness, aberrant levels of estrogen receptors (ER-α and ER-β, with their genes *ESR1* and *ESR2*) are observed in women with endometriosis. Both ERs play essential roles in the establishment and development of ectopic lesions [111], and ample evidence indicates that ER-β is excessively expressed in the ectopic lesions, when compared with normal endometrium [112], being described a positive ratio of miRNA *ESR2* to *ESR1 in endometriomas in comparison to endometriotic implants and eutopic endometrium* [113]. The studies on knockout mouse have shown that the attachment of ectopic endometrium, the sizes, and the proliferation with endometriosis progression are associated with the presence of *ESR2 and absence of ESR1* [114].

P4 and its receptors PR-A and PR-B have an important role in endometriosis. The eutopic endometrium of ill women has an attenuate answer to P4; the PR isoform B is not expressed in their endometrium, being only the isoform PR-A, because progesterone-responsive genes are not deleted in eutopic endometrium of ill women in comparison with normal women in the early secretory phase of the cycle, a fact that suggests that these women have a progesterone-resistant phenotype [114, 115].

Recent studies have confirmed isoform PR-A predominance in endometriotic ill women and have shown its presence in all menstrual cycle phases [116], as it is similar to the condition of endometrioid endometrial cancer, with overexpression of PR-A isoform. Another aspect is the higher level of PR-A in ovarian endometriosis comparative to peritoneal form [116].

Gargett et al. [61] are discussing the endometrial gland estrogen receptors [ER-α (ESR1) and ER-β (ESR2)] and their genes *ESR1* and *ESR2* and PR. Using special markers as stage-specific embryonic antigen 1 (SSEA-1 or CD15) to localize the endometrial epithelial stem/progenitor cells in the basalis of endometrial glands of cycling women, the SSEA-1^+ endometrial epithelial cells in culture had greater telomerase activity, and longer telomeres, and were more quiescent with lower proliferation rates than SSEA-1^- epithelial cells and features of progenitor cell populations [117]. It was revealed that in polarized epithelium, SSEA-1+ cells expressed lower levels of ER-α (ESR1) and PR when compared with the SSEA-1– cells [117], suggesting a less differentiated cell phenotype and reliance on growth factors released from ESR1-expressing niche cells to mediate estrogen-induced proliferative signals. In contrast, earlier studies [118] have shown that ESR1 is detected in basalis glands of the normal endometrium throughout the menstrual cycle, whereas functionalis expression is restricted to the proliferative stage, a fact that suggested to the Australian researchers that human endometrial epithelial progenitor cells are a subset of the SSEA-1+ population that may reside in the functionalis abutting the basalis.

3.4 Progesterone resistance in endometriosis: mechanisms of progesterone resistance in endometriosis

Endometriosis is a chronic inflammatory estrogen-dependent and progesterone-resistant disease, because of missing or blunted progesterone-induced

molecular changes or inadequate response to progesterone of both the eutopic and ectopic endometrial cells and tissue [119, 120], facts that are present all duration of the menstrual cycle. Several mechanisms were described, being discussed whether progesterone resistance is innate, acquired, or present in eutopic and ectopic tissue. Many concepts try to cover the disorder, with clinical relevance; the mechanisms of progesterone responsiveness converge to the reduction of the nuclear PRs, steroid receptor coactivators, or downstream or degradation of other molecular effectors (TGFβ, Dickkopf-1, retinoic acid, c-*myc*, etc.). The lack of the PR-B isoform is associated with very low levels of PR-A isoform; and in the conditions of stromal cell defect, P4 does not induce epithelial 17β-HSD-2 expression, and this is the cause of continuing epithelial mitosis, by the failure of E2 metabolism to E1, mainly in women with moderate/severe disease, compared with the normal ones [119].

The altered ratio between PR isoforms is associated with an altered complex network of interactions with signaling pathways, downstream effectors, transcription factors, coregulators, chromatin-remodeling factors, and DNA [120]. Studies have shown the role of non-genomic overactive pathways of transcription factors as AKT pathways [with three isoforms (Akt1, Akt2, Akt3) differently involved in endometriosis initiation and progression and endometrial cancer] and MAPK pathway (only in the stromal cells) [121]. Rapid activation of AKT by estradiol and progestins promotes survival of endometriotic stromal cells, by downregulation of PRs, with proliferation and migration of endometrial stromal cells [122]. Experiments on xenograft mice have proven a decreased cell viability and increased apoptosis to cells in culture after AKT inhibitor administration [92, 123]. The overactive AKT pathway is associated with the inflammatory and hormonal nature of endometriosis [81, 123]. Overactive AKT from endometriotic stromal cells attenuates also decidualization through its downstream target FOXO1 [124].

3.5 Progesterone receptors in eutopic and ectopic endometrium

Progesterone action has been primarily ascribed to the well-characterized classical signaling pathway involving ligand binding, activation of nuclear progesterone receptors (PRs), and subsequent activation of genes containing progesterone response elements (PREs). Immunohistochemistry identifies during the mid to late luteal phase of menstrual cycle five progesterone nuclear receptors or classic PRs (PR-A, PR-B) and the truncated isoforms (PR-C, PR-M, and PR-S; the PR-C is generated by the initiation of translation from further downstream, not recognized usually), and the two types of cell surface-associated proteins [membrane progesterone receptors (mPRs) and the progesterone membrane receptor component (PGMRC)]. PR-A and PR-B belong to a family of ligand-activated transcription factors and share common structural and functional elements (i.e., regulatory region). The P4 genomic mechanism of action is exerted through specific progesterone response elements (PREs) within the promoter region of target genes to regulate transcription of the genes. These PRs induce classic regulation of gene expression while also transducing signaling cascades that originate at the cell membrane and ultimately activate transcription factors. As for estrogens the genomic and non-genomic mechanisms of P4 are coupled, and the nuclear PRs are upregulated by E2 in endometrial cells, implicating crucial progenitor cells, as preferential targets of P4.

A functional feedback interaction between the P4 and estrogen hormonal systems is crucial for normal endometrial differentiation/decidualization—a key

step toward the establishment of pregnancy, and for balancing the often-opposing actions of the progesterone/PR and estrogen/ER systems [125].

Recently, a new paradigm of the direct effect of P4 on the cells' chromatin, with chromatin remodeling and gene regulation in stem/progenitor cells, is discussed. The interaction of nuclear receptors and other transcription factors with the chromatin is considered to be a highly dynamic process, characterized by rapid cycles, measured in seconds of transient association and dissociation with the chromatin [126]. The classic description was a static one, considering a slow process during hours and sometimes days and during which PRs are activated, then bind to the promoter of target genes, recruit coregulators, and assemble them in a multimeric complex that has the right enzymatic activity to modify the local chromatin structure, which in turn will lead to changes in the transcriptional machinery, efficacy of RNA synthesis, translation, and, ultimately, protein levels. The "non-genomic" mechanisms explain the rapid activation of cytoplasmic kinase signaling that can result in both transcription-independent and transcription-dependent effects. These "non-genomic" actions can be partially explained by membrane transport *via* nuclear receptor. The mPRs (molecular mass of approximately 40 kDa) had thought to be composed of three subtypes, mPR-α, mPR-β, and mPR-γ, which belong to the seven-transmembrane domain adiponectin Q receptor (PAQR) family, plus two new discovered subtypes (mPRδ and mPRε). Progesterone receptor membrane component-1 (PGRMC-1) and PGRMC-2, with a single-transmembrane domain protein, are mediating the rapid non-genomic effects of E2 and P4, such as the activation of MAPK signaling and intracellular Ca^{2+} increase [127, 128]. mPRβ activates also MAPK cascade, without GPCR signaling, and progesterone-stimulated mPRβ activation did not exhibit the elevation of [Ca^{2+}] [129]. In comparison to the mPRs, the single-transmembrane protein Pgrmc1 (molecular mass 25–28 kDa) and the related Pgrmc2 are a part of a multi-protein complex that binds to P4, to other steroids, and to pharmaceutical compounds [127].

Actually, the novel techniques allow genome-wide mapping of binding nuclear receptors to DNA and real-time monitoring of transcription. *PR* expression in uterine cells is stimulated by estrogens *via* ER-α, and consequently progesterone responsiveness is dependent on the presence of an estrogenic drive [130]; low levels of estrogen are required for progesterone responsiveness throughout the luteal phase, and, conversely, ER-α expression in uterine cells is inhibited by progesterone *via* PRs [131]. It is known that continuous exposure of the endometrium to progesterone downregulates PR expression in the endometrial epithelium [132], the PR being detected only in stroma and myometrium throughout most of gestation in the ovine uterus [133].

Endometrial biopsies from luteal phase (days 15 ± 31) of normal, fertile subjects detected a steady decrease in expression of stromal cell PRs (both isoforms A and B) immunostaining from a mid-cycle maximum and a more rapid decrease in expression of PR in epithelial cell, particularly between days 22 and 24, from the mid-cycle maximum also [134, 135].

The human nuclear PRs are encoded by a single gene located on chromosome 11 (11q22–11q23). Expression of *PR* is controlled by two promoters to produce two major mRNA transcripts that encode two proteins: the full-length *PR-B* (116 kDa) controlled by the distal *PR-B* promoter region and initiated from the first AUG translational start codon and *PR-A* (94 kDa) controlled by the proximal PR-A promoter region and initiated from the second AUG (492 bases upstream) translational start codon. It is now generally accepted that response to P4 is determined by the combined actions of PR-A and PR-B, which upon ligand binding form

homodimers or heterodimers that have distinct transcriptional activities at specific sets of gene promoters.

The PR initiates paracrine signaling within the uterine microenvironment during the preimplantation period and of extrauterine microenvironment in cases of ectopic endometrium. P4 facilitates the subsequent downstream expression of PR targets within the epithelium and stroma. The potential molecular pathways which are involved in the pathological mechanisms and in endometriosis were assessed and determined: c-Jun, CREB-binding protein, protein kinase B (AKT), and cyclin D1 (CCND1) signaling [136].

The dysregulation of the PR isoforms has been shown to occur in women with endometriosis, demonstrating the integral requirement for proper balance of these isoforms in hormone-responsive tissues [114].

PRs are differentially expressed in the endometrial structures—glands and stroma, and during menstrual cycle. The transformation of endometrial stromal fibroblasts (ESC) into specialized secretory cells (decidualization) is fundamental for the establishment of a receptive endometrial microenvironment which can support and maintain pregnancy.

PR-A is exerting a negative control on PR-B-mediated transcription and the mediated transcription of the ER and glucocorticoid receptors [137], a fact that may underlie, at least in part, the mechanism by which progesterone functionally antagonizes the effects of estrogen. PR-A and PR-B can interact as dimers with DNA progesterone-responsive element and with signaling proteins of the Src/Ras/Erk pathway outside the nucleus [138].

De novo motif analysis indicated that, although the two isoforms bind to the same DNA sequence motif, they are both common and unique neighboring motifs where other transcription factors, such as FOSL1/FOSL2, JUN, C/EBPβ, and STAT3, bind to and dictate the transcriptional activities of these isoforms. Chromatin immunoprecipitation sequencing in combination with gene expression profiling revealed that PR-B controls a substantially larger cistrome and transcriptome than PR-A during human endometrial stem cell differentiation (**Figure 4**).

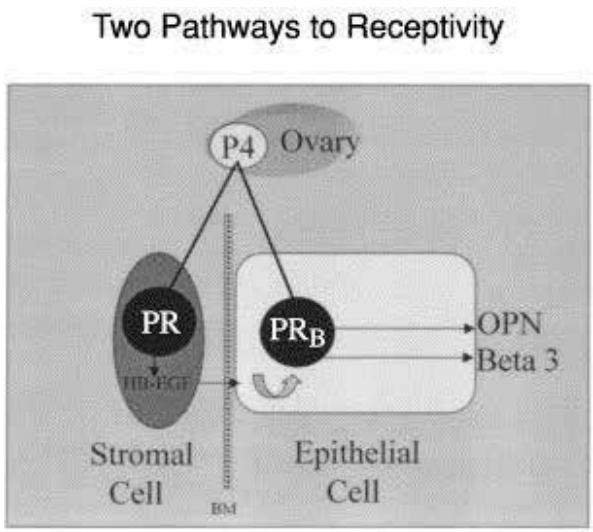

Figure 4.
Progesterone action on the epithelial and stromal cells of the endometrium to prepare it for embryo receptivity. Progesterone has both a direct influence on endometrial epithelium via PR-B, and an indirect influence via the endometrial stromal cells. Adapted from Groll and Lessey [196].

4. Genomic and epigenetic changes of adult stem cells in ectopic and eutopic endometrium: heredity in endometriosis

Women suffering from endometriosis were observed since then to have peculiarities including genetic predisposition, aberrant immunological response, and altered peritoneal environment that make them susceptible to attachment and growth of ectopic endometrial cells, with their exosomes, and that the ectopic endometriotic lesions are histologically similar to their putative eutopic precursors, yet significant biochemical differences exist between these two tissues. The findings provide a framework for causality and mechanisms underlying attenuate progesterone response in endometriosis, especially in the deeply infiltrating groups, but there are still unclear or controverted aspects in the genetics and consequences of progesterone resistance in endometriosis [139].

4.1 Genomic regulation in endometriosis

The genetic aspects in endometriosis must be referred to the complex pathogenomic architecture and multiple interactions of the genetic qualities of the migratory and adhesive mesenchymal/stromal cells, to the qualities of the tissues/organs where the ectopic endometrial cells arrive, and to other factors involved in the development of the disease, as the complex combinations of functional protein modules, and alteration of different metabolic pathways by sexual steroid hormones, their receptors and co-regulators, and immune systems. The genes of endometrial stem cells, such as *OCT4*, *SOX2*, *SOX15*, *NOTCH1*, and *TWIST1*, which can be deregulated in endometriosis, leading to altered SC behavior of migration and adhesivity in ectopic location, which is associated with miRNA of angiogenic factors [42]. The transcriptome analysis of eutopic and ectopic endometrium shows genetic peculiarities in women with endometriosis versus disease-free women [140–142], with relevant dysregulation of the proliferative-to-secretory transition in the endometrium in cases with endometriosis, PR gene polymorphism, and epigenetic alterations in stem cell populations, and their resulting exosomes in endometriosis, including imbalance of miRNA expression of PRs, histone and DNA modifications, and chromosomal aberrations, are some possible answers to the unclear pathophysiologic aspects and to some clinical relevance complications—as chronic severe pelvic pain, dyspareunia, infertility, miscarriages, recurrent pregnancy loss, preterm birth, and progression to malignancy, specially of the ovary, which were assessed by recent, large international collaborative studies [143, 144]. There are questions about the relationships between eutopic and ectopic endometria, regarding the trigger of progesterone-attenuated response and infertility [145]. It is unknown if the defective endometrium gives rise to a predisposition toward endometriosis and infertility or, alternatively, if endometriosis causes the altered endometrial receptivity, in cases with failure of ART. In experiments on mice, the methylation of *Hoxa 10* and *Hoxa 11* (known as a potential mechanism responsible for altered gene expression) decreased the endometrial receptivity in the endometriosis group.

The eutopic endometrium of ill women has an attenuate response to P4 because estrogen-responsive genes are nor suppressed in their stromal cells comparative to normal women in early secretory phase of menstrual cycle, a fact that suggests a resistant phenotype to progesterone [111, 114, 115].

4.2 Heredity in endometriosis

Since the 1990s, there is increasing evidence of a germline predisposition to endometriosis. A familial clustering of endometriosis in humans [146] and rhesus

monkeys [147] as well as increased prevalence among first-degree relatives of women with all disease severities compared to the general population [148] has been reported. The age at onset of symptoms is similar in affected, non-twin sisters [149], and there is concordance in monozygotic twins [147]. The complex gynecologic disorder of endometriosis was since then recognized as showing heritable tendencies, with recurrent risks of 5–7% for first-degree relatives [150]. Familial and epidemiologic studies support that this disease is a genetic disorder of polygenic/multifactorial inheritance, being determined the number and locations of causative genes. Using microarray for real-time PCR validation for the genome-wide linkage analysis, in a study of 1176 families with affected sibling pairs versus disease-free women, Treloar et al. [151] identified a region of significant linkage to endometriosis on chromosome 10q26, and four genes [a disintegrin and metalloproteinase domain 12 (ADAM12; 10q26.3; 2.29 ESE), arginyltransferase 1 (ATE1; 10q26.13; 1.61 PE, 1.57 ESE), and fibronectin type III and ankyrin repeat domains 1 (FANK1; 10q26.2; −1.85 ESE)] which have statistical significant changes, but the gene CYP26A1 had a fold change in the real-time PCR analysis that did not reach statistical significance. After the publication of this study, there are debates on the familial aggregation [152, 153].

4.3 Progesterone receptor polymorphism

Endometriosis is considered a polygenic disorder [154] requiring alterations in multiple biological pathways for the establishment and proliferation of ectopic endometrial cells, a fact documented in a meta-analysis of European and Japanese cohorts [155]. The human progesterone receptor gene is located on chromosomes 11q22–11q23 [156].

Multiple genes are expressed differentially in the eutopic endometrium of ill patients vs. disease-free women; and the most studied gene expression, the HOX gene, was proven to be altered in stromal stem cells from the ectopic endometrium [157]. To date, many deregulated genes have been identified in endometriotic cells with a wide variety of functions, including apoptosis, vascularization, cell cycle regulation, DNA repair, encoding detoxification enzymes, immune system regulation, and cell adhesion. PR polymorphism is controverted in literature [152]; some authors consider to have little or no relevance in endometriosis development. The multinational study [144] of a total of 45,923 cis-eQTLs for 417 unique genes and 2968 trans-eQTLs affecting 82 unique genes showed dynamic changes in expression of individual genes along the cycle, which include alteration in both mean expression and transcriptional silencing. The genetic polymorphisms predispose women to endometriosis [158], a fact that was not sustained by the results of three meta-analyses on association of endometriosis and some genetic polymorphisms coding for dioxin detoxification enzymes, sex steroid biosynthesis, and their receptors [159–161], even though meta-analyses is known to have upward biases in risk estimates. A quite old review on this topic found "a strikingly large amount of conflicting results" and concluded that "polymorphisms may have a limited value in assessing possible development of endometriosis" [152].

The endometrial specimens in different phases of the menstrual cycle, mainly in the secretory phase, were analyzed and there was demonstrated a signature of enhanced cellular survival, persistent expression of genes involved in DNA synthesis, and cellular mitosis in the setting of endometriosis, were analyzed, and, on another hand, the genes for susceptibility of attenuated progesterone response when in endometriosis from the families *FOXO1A*, *MIG6*, and *CYP26A1* (151), *HOX* [142], *WNT* [68], and 12 single nucleotide polymorphisms at 10 independent genetic loci associated with endometriosis have also been identified were identified [162].

Comparative gene expression analysis of progesterone-regulated genes in secretory-phase endometrium confirmed the observation of attenuated progesterone

response, which is the main issue of this chapter. The genetic polymorphism of PRs was studied also on *PROGINS* receptor gene [163], recently on the PROGINS allele [143], and on *+331G/A* genes (especially for comparison between superficial and deeply infiltrating endometrioses [86]). The +331C/T has been shown to influence the transcription of PR-B relative to PR-A with the T allele favoring PR-B [137, 164]. PR-B acts as a classic hormone receptor, mediating the effects of progesterone, whereas PR-A acts as a repressor of PR-B, and as a result, the presence of the +331T allele is hypothesized to lead to increase the effect of P4.

In the recent published multinational and multicenter collaborative study focused on the genotypes for the +331C/T SNP (associated with low risk of endometriosis, because of increased synthesis of PR-B) and PROGINS allele (equivocal from other analyses) in cases with a history of endometriosis [144]. The occurrence of endometriosis was reduced in women carrying one or more copies of the +331T allele, whereas there was no association between the PROGINS allele and endometriosis (**Table 2**). The conclusion regarding PRs was that the +331T allele drives to a reduced PR-A to PR-B ratio, and if the observed association with endometriosis is confirmed, it would suggest that this ratio is important for endometriosis; more than this the authors' conclusion was that a reduced risk is biologically plausible since endometriosis is responsive to progesterone.

4.4 Progesterone target genes

In the last 15–20 years, the relationship between PR-A and PR B is more and more understood. The exclusive expression of PR-B in the luminal epithelium may act as a reservoir of PR-A heterodimerizing partners, or PR-B may homodimerize and regulate epithelial target genes. The expression ratio of the PR isoforms within the uterine compartments could potentially regulate the gene expression profile; it was observed in ill women. The expression of progesterone target genes is blunted, and decidualization is inadequate [115]. The prototype progesterone-responsive gene, glycodelin, was discovered to be strikingly downregulated in the endometrium of women with endometriosis compared with disease-free women [165], and recently glycodelin is the third biomarker for early diagnosis of endometriosis, besides zinc-α and brain-derived neurotrophic factor (BDNF).

Other earlier studies were considering the additional effect of environmental factors as dioxin to genes [166] or the interaction between multiple genes and/or the interaction between genes to other factors to produce the disease phenotype [167].

The location of endometrial stem cells outside the uterus may be another explanation of their different actions in ectopic and eutopic endometrium in ill women in comparison to disease-free women [78].

Single nucleotide polymorphism (SNP)	Cases with endometriosis (n)	Cases without endometriosis (n)	OR (95% CI)	p-Value	p^{het}
+331 C/T (rs10895068)	345	5369	0.65 (0.43, 0.98)	0.042	0.46
PROGINS C/A (rs1042838)	343	5339	0.94 (0.76, 1.16)	0.56	0.24

OR = odds ratio, compares heterozygotes and rare homozygotes to common homozygotes for +331C/T, per copy of the allele carried for PROGINS, stratified on study site and age group; p-value for heterogeneity across study sites.

Table 2.
Association between the two PRs SNPs and endometriosis in the multinational study population (adapted from Fung et al. [144]).

4.5 MicroRNA (miRNA) dysregulation of steroid hormone receptor expression in endometriosis

The receptors of sexual steroid hormones (ERs, PRs) are involved in endometriosis, and correlated to them is the exploration of the correlation between microRNA (miRNA) and ER/PR in eutopic and ectopic endometrium. There are recently discovered stranded noncoding RNAs (ncRNAs) [168]. MicroRNAs (miRNAs) are single-stranded noncoding RNA molecules with approximately 22 nucleotides in length, and they control posttranscriptional gene regulation, which were proposed to contribute to human reproductive physiology; their abnormal expression was involved in the pathogenesis of many diseases of female reproductive tract, including endometriosis. While the majority of the literature supports the notion that miRNAs inhibit translation, there is some evidence that miRNAs can actually enhance translation in certain biological scenarios.

miRNAs are postulated to play a role in normal biological processes, to be critical regulators of cellular development and physiology, while their mis-expression has been associated with numerous diseases [169]. A recently published paper [170] at the Department of Molecular and Integrative Physiology, at the University of Kansas Medical Center (USA) tries to determine if the "mis-expressed" endometriotic tissue is a cause (driver of the disease) or a result of endometriosis (passenger).

More than 10 years ago, there are comparative microanalyses of genetic expressions—the miRNAs in women with ectopic endometrial cells and eutopic endometrium—that have proven an alternative pattern in the two groups [171–173], as well as the differences of ill women versus disease-free women [172–175]. miRNA regarding ER shows a ratio of 100:1 in *ESR2* (RE-β) to *ESR1* (RE-α) in endometriomas vs. superficial endometriotic lesions and eutopic endometrium [112, 113].

Different miRNAs are identified by microarray with real-time reverse transcription-polymerase chain reaction (real-time RT-PCR), and they were done in paired eutopic/ectopic endometrium from the same patients. In the published papers, there are identified upregulated (over expressed) and downregulated (underexpressed) miRNA expressions in eutopic and different ectopic lesion locations (peritoneal, ovarian), and some are "mis-expressed." Using the Ingenuity Pathway Analysis (IPA) software, potential molecular pathways were assessed and determined to involve c-Jun, CREB-binding protein, protein kinase B (AKT), and cyclin D1 (CCND1) signaling, all of which have previously been associated with endometriosis pathogenesis [136]. According to the involvement in endometriosis, the molecules directly involved in endometriosis (cytokines, enzymes, growth factors, kinases, ion channels, ligand-dependent nuclear receptors, peptidases, phosphatases) are assessed.

There are differences between authors; there were conflicting reports on whether or not miRNA expression was influenced by the stage of the menstrual cycle (proliferative [136] or secretory [176]), the type of endometrial cell (miRNA of endometrial stromal cells was for the first time assessed by Hawkins et al. [177]), the type of miRNA, and the level in eutopic and ectopic tissue [miRNA from *miR-29* family (was evaluated using primary human endometrial stromal cells in vitro)], which had different levels—(high for [136] and [176] and decreased for [178], who compared it to disease-free women). The role of *miR-29c* in endometrial cell proliferation, invasion, and apoptosis in vitro was examined. *miR-29c* suppressed endometrial cell proliferation and invasion, promoting cell apoptosis. The conclusion was that *miR-29c* exhibits inhibitory action on endometrial cell proliferation and invasion by inhibiting the expression of *c-Jun*. Another studied miRNA—the stromal cell *miR-183*, was examined in response to ovarian steroids (17β-estradiol and P4), and inflammatory cytokines (TNF-α) were concluded from this study that repressed levels of *miR-183* may modulate the growth and invasive potential of

endometriotic endometrial stromal cells contributing to the development and progression of endometriosis [179]. The microarray to identify differentially expressed miRNAs between endometriotic lesion tissues from women with stage III/IV disease compared with eutopic endometrium from women without endometriosis has shown that lesion tissue expressed significantly higher levels of expression of seven miRNAs and significantly lower levels of expression of ten miRNAs [180]; some miRNAs were predictors of angiogenesis in endometriotic lesions. Expression of VEGFA was significantly upregulated, whereas EGFR2, PTEN, and CXCR4 were markedly downregulated in lesion tissue compared with the endometrium from women without endometriosis. The authors concluded that the differentially expressed miRNAs could modulate VEGFA, EGFR2, PTEN, and/or CXCR4 expression and contribute to the pathogenesis of endometriosis. Their conclusion is quite similar to other researchers from Spain [181]: the expression levels of miRNAs related to angiogenesis (the pro-angiogenic factors (VEGF-A) and the angiogenesis inhibitor thrombospondin-1 (TSP-I) are different in eutopic endometrium from that observed in ovarian endometrioma, rectovaginal nodule. Ovarian endometriomas exhibited significantly lower levels of pro-angiogenic VEGF-A mRNA and protein and higher levels of *miR-125a* and *miR-222* than the corresponding eutopic endometrium. In contrast, levels of the angiogenesis inhibitor were significantly higher in endometriomas, and this was associated with reduced levels of *miR-17-5p*.

According to the results of the last mentioned study [170], it was considered that from the initial generation of miRNA profiles, investigations were focused on specific miRNAs and putative targets, which can become relevant to the pathophysiology of endometriosis and may aid in determining whether these miRNAs function as drivers of the disease.

miR-126 was proposed to be analyzed as a regulator of angiogenesis, growth, adhesion, and invasion in ectopic endometriotic lesions versus eutopic endometrium of disease-free women, and the results of this parameter are discordant—higher in earlier studies [136], and reduced in the more recent one, the level of reduction being parallel to the severity of endometrioma [182].

4.6 Epigenetics in endometriosis: methylation pattern of sexual steroid hormone receptors

There is accumulating evidence that various epigenetic aberrations exist in endometriosis. In the last 10 years, evidence from reviewed and retrieved studies has emerged that endometriosis may be an epigenetic disease [153], epigenetics appearing to have a better explanatory power than genetics, and to be a common denominator for hormonal and immunological aberrations in the puzzle of endometriosis. Genomic imprinting; DNA methylation; histone modifications with different nominations (acetylation/ histone phosphorylation/histone ubiquitylation/ histone sumoylation); microRNAs [183] and recently discovered, stranded, noncoding RNAs (ncRNAs) [168]; transcription factor network; and chromatin remodeling are known to regulate transcription of target genes. Genomic imprinting is an epigenetic phenomenon known to regulate DNA methylation of either maternal or paternal alleles [184], and the male and female germ lines guide the allele-specific DNA methylation marking and histone modification onto specific gene regions of parental alleles [185]. The epigenome, the collection of DNA methylation, and the histone modifications can be influenced by environmental factors. Exposure to xenobiotics, chronic inflammation, and transient hypoxia are associated with DNA hypomethylation of stromal endometrial stem cells through the destabilization of *DNMT1* mRNA; *DNMT1*, *DNMT3A*, and *DNMT3B* are overexpressed in the epithelial component of endometriotic implants as compared to normal controls or in the

eutopic endometrium of women with endometriosis [186], but fetal programming postulates that chronic adult-onset diseases with an epigenetic component originate in utero when the early embryo is exposed to factors that permanently shape its epigenetic mark, a fact presented in the previous subchapter.

Aberrant DNA methylation represents a possible mechanism, linking gene expression alterations observed in endometriosis with hormonal and environmental factors. Methylation is one of the most important epigenetic functions that implies the addition of methyl group at the DNA dinucleotides in the position 5 of the cytosine of the "promoter" zone and induces silencing of the gene under DNA methyltransferase action [68, 187].

Endometriotic lesions have altered methylation patterns of ER-β, and the ER may mediate regulation of one another [188]. The authors consider that aberrant DNA hypomethylation of ER may favor the progression to cancer of old ectopic endometrial lesions.

DNA methylation makes possible the process "epithelial to mesenchymal transition" (EMT), in which the epithelial cells lose polarized organization of the cytoskeleton and cell-to-cell contacts, acquiring the high motility of mesenchymal cells. It is supposed that two stimulating signals, hypoxia and estrogen, can activate the EMT process in endometriosis through different pathways. The pathways involve many cellular factors such as TGF-β and Wnt, ultimately leading to cell proliferation and migration, and the changes of epithelial cells are thought to be prerequisites for the original establishment of ectopic endometriosis lesions [189].

The reasons for non-response to progestins in endometriosis are not entirely clear, studies point the possible epigenetic silencing of the PR gene, without knowledge of causes for the epigenetic silencing of PRs. DNA methylation of the ER and PR promoter has been demonstrated in endometriosis; inflammation and oxidative stress can be involved in epigenetic changes in DNA and chromatin remodeling proteins [190–192]. ER-β promoter is hypomethylated in endometriotic cells, which accounts for its overexpression [193]. The promoter of PR-B hypermethylation is concomitant to reduction of PR-B, a fact explaining progesterone resistance in endometriosis [11, 114].

Ectopic endometrial stromal cells are hypomethylated, and are different from normal endometrial stromal cells which are hypermethylated [194].

The hypermethylation of *HOXA 10* promoter reduces HOXA 10 expression in induced endometriosis of stromal endometrial cells of mice [195].

Author details

Manuela Cristina Russu
"Carol Davila" University of Medicine and Pharmacy, "Dr I Cantacuzino" Discipline of Obstetrics and Gynecology, Bucharest, Romania

*Address all correspondence to: manuela_russu@yahoo.com

References

[1] Signorile PG, Baldi F, Bussani R, Baldi A, et al. Embryologic origin of endometriosis: Analysis of 101 human female fetuses. Journal of Cellular Physiology. 2012;**227**(4):1653-1656. DOI: 10.1002/jcp.22888

[2] Crispi S, Piccolo MT, D'Avino A, Signorile PG, et al. Transcriptional profiling of endometriosis tissues identifies genes related to organogenesis defects. Journal of Cellular Physiology. 2013;**226**(9):1927-1937. DOI: 10.1002/jcp.24358

[3] Brosens I, Gordts S, Benagiano G. Endometriosis in adolescents is a hidden, progressive and severe disease that deserves attention, not just compassion. Human Reproduction. 2013;**28**:2026-2031

[4] Starzinski-Powitz A, Zeitvogel A, Schreiner A, Baumann R. In search of pathogenic mechanisms in endometriosis: The challenge for molecular cell biology. Current Molecular Medicine. 2001;**1**(6):655-664

[5] Sasson IE, Taylor HS. Stem cells and the pathogenesis of endometriosis. Annals of the New York Academy of Sciences. 2008;**1127**:106-115

[6] Klemmt PAB, Starzinski-Powitz A. Molecular and cellular pathogenesis of endometriosis. Current Women's Health Reviews. 2018;**14**(2):106-116. DOI: 10.2174/1573404813666170306163448

[7] Forte A, Cipollaro M, Galderisi U. Genetic, epigenetic and stem cell alterations in endometriosis: New insights and potential therapeutic perspectives. Clinical Science (London). 2014;**126**(2):123-138. DOI: 10.1042/CS20130099

[8] Signorile PG, Baldi F, Bussani R, Baldi A, et al. Ectopic endometrium in human foetuses is a common event and sustains the theory of müllerianosis in the pathogenesis of endometriosis, a disease that predisposes to cancer. Journal of Experimental & Clinical Cancer Research. 2009;**28**:49. DOI: 10.1186/1756-9966-28-49

[9] Grümmer R. Animal models in endometriosis research. Human Reproduction Update. 2006;**12**:641-649

[10] Sampson JA. Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. American Journal of Obstetrics and Gynecology. 1927;**14**:469. Cited by Signorile PG, et al. (2012)

[11] Giudice LC, Kao LC. Endometriosis. Lancet. 2004;**364**:1789-1799

[12] Halme J et al. Retrograde menstruation in healthy women and in patients with endometriosis. Obstetrics and Gynecology. 1984;**64**:154

[13] Benagiano G, Guo SW, Putterman P, Gordts S, Brosens I. Progress in the diagnosis and management of adolescent endometriosis: An opinion. Reproductive Biomedicine Online. 2018;**36**(1):102-114. DOI: 10.1016/j.rbmo.2017.09.015

[14] Tempfer CB, Wenzl R, Horvat R, et al. Lymphatic spread of endometriosis to pelvic sentinel lymph nodes: A prospective clinical study. Fertility and Sterility. 2011;**96**(3):692-696

[15] Gong Y, Tempfer CB. Regional lymphatic spread in women with pelvic endometriosis. Medical Hypotheses. 2011;**76**(4):560-563

[16] Machairiotis N, Stylianaki A, Dryllis G, et al. Extrapelvic endometriosis: A rare entity or an under diagnosed condition? Diagnostic Pathology. 2013;**8**(1):1-12

[17] Bulletti C, De Ziegler D, Polli V, Flamigni C, et al. Characteristics of uterine contractility during menses in women with mild to moderate endometriosis. Fertility and Sterility. 2002;**77**(6):1156-1116

[18] Leyendecker G, Kunz G, Herbertz M, et al. Uterine peristaltic activity and the development of endometriosis. Annals of the New York Academy of Sciences. 2004;**1034**:338-355

[19] Surrey ES, Halme J. Effect of peritoneal fluid from endometriosis patients on endometrial stromal cell proliferation in vitro. Obstetrics & Gynecology. 1990;**76**(5 Pt 1):792-797

[20] Koninckx PR, Kennedy SH, Barlow DH. Pathogenesis of endometriosis: The role of peritoneal fluid. Gynecologic and Obstetric Investigation. 1999;**47**(Suppl. 1):23-33

[21] Klemmt PA, Carver JG, Koninckx P, Mardon HJ, et al. Endometrial cells from women with endometriosis have increased adhesion and proliferative capacity in response to extracellular matrix components: Towards a mechanistic model for endometriosis progression. Human Reproduction. 2007;**22**(12):3139-3147. DOI: 10.1210/jc.2009-0207

[22] Gaetje R, Kotzian S, Starzinski-Powitz A, et al. Nonmalignant epithelial cells, potentially invasive in human endometriosis, lack the tumor suppressor molecule E-cadherin. The American Journal of Pathology. 1997;**150**(2):461-467

[23] Zeitvogel A, Baumann R, Starzinski-Powitz A. Identification of an invasive, N-cadherin-expressing epithelial cell type in endometriosis using a new cell culture model. The American Journal of Pathology. 2001;**159**(5):1839-1852

[24] Jones RK, Bulmer JN, Searle RF. Immunohistochemical characterization of proliferation, oestrogen receptor and progesterone receptor expression in endometriosis: Comparison of eutopic and ectopic endometrium with normal cycling endometrium. Human Reproduction. 1995;**10**(12):3272-3279

[25] Jones RK, Bulmer JN, Searle RF. Phenotypic and functional studies of leukocytes in human endometrium and endometriosis. Human Reproduction Update. 1998;**4**(5):702-709

[26] Leyendecker G, Wildt L, Mall G. The pathophysiology of endometriosis and adenomyosis: Tissue injury and repair. Archives of Gynecology and Obstetrics. 2009;**280**(4):529-538

[27] Leyendecker G, Wildt L. A new concept of endometriosis and adenomyosis: Tissue injury and repair (TIAR). Hormone Molecular Biology and Clinical Investigation. 2011;**5**(2):125-142

[28] Gruenwald P. Origin of endometriosis from the mesenchyme of the coelomic walls. American Journal of Obstetrics and Gynecology. 1942;**44**:474. Cited by Sasson IE, Taylor HS (2008)

[29] Von Recklinghausen F. Adenomyomas and cystadenomas of the wall of the uterus and tube: Their origin as remnants of the wolffian body. Wiener klinische Wochenschrift. 1896;**8**:530. Cited by Sasson IE, Taylor HS (2008)

[30] Russell WW. Aberrant portions of the Mullerian duct found in an ovary. Bulletin of the Johns Hopkins Hospital. 1899;**10**:10. Cited by Sasson IE, Taylor HS (2008)

[31] Laganà AS, Sturlese E, Retto G, Sofo V, Triolo O. Interplay between misplaced Müllerian-derived stem cells

and peritoneal immune dysregulation in the pathogenesis of endometriosis. Obstetrics and Gynecology International. 2013;**2013**:527041

[32] Ng YH, Rome S, Jalabert A, Salamonsen LA, et al. Endometrial exosomes/microvesicles in the uterine microenvironment: A new paradigm for embryo-endometrial cross talk at implantation. PLoS ONE. 2013;**8**(3):e58502. DOI: 10.1371/journal.pone.0058502

[33] Nguyen HP, Simpson RJ, Salamonsen LA, Greening DW. Extracellular vesicles in the intrauterine environment: Challenges and potential functions. Biology of Reproduction. 2016;**95**(5):109

[34] Braicu C, Tomuleasa C, Monroig P, CaWangn GA, et al. Exosomes as divine messengers: Are they the Hermes of modern molecular oncology. Cell Death and Differentiation. 2015;**22**(1):34-45

[35] Ahn SH, Monsanto SP, Miller C, Tayade C, et al. Pathophysiology and immune dysfunction in endometriosis. BioMed Research International. 2015;**2015**:795976. DOI: 10.1155/2015/795976

[36] Gorai I, Ishikawa M, Minaguchi H, et al. Antiendometrial autoantibodies are generated in patients with endometriosis. American Journal of Reproductive Immunology. 1993;**29**(2):116-123. DOI: 10.1111/j.1600-0897.1993.tb00575.x

[37] Cameron MJ, Kelvin DJ. Cytokines and chemokines—Their receptors and their genes: An overview. Advances in Experimental Medicine and Biology. 2003;**520**:8-32. DOI: 10.1007/978-1-4615-0171-82

[38] Bergqvist A, Bruse C, Carlberg M, Carlström K. Interleukin 1β, nterleukin-6, and tumor necrosis factor-β in endometriotic tissue and in endometrium. Fertility and Sterility. 2001;**75**(3):489-495. DOI: 10.1016/S0015-0282(00)01752-0

[39] Somigliana E, Viganò P, Gaffuri B, et al. Modulation of NK cell lytic function by endometrial secretory factors: Potential role in endometriosis. American Journal of Reproductive Immunology. 1996;**36**(5):295-300. DOI: 10.1111/j.1600-0897.1996.tb00179

[40] Kang YJ, Jeung IC, Park A, Yoon SR, et al. An increased level of IL-6 suppresses NK cell activity in peritoneal fluid of patients with endometriosis via regulation of SHP-2 expression. Human Reproduction. 2014;**29**:2176-2189. DOI: 10.1093/humrep/deu172

[41] Tosh D, Slack JMW. How cells change their phenotype. Nature Reviews. Molecular Cell Biology. 2002;**3**:187-194. DOI: 10.1038/nrm761

[42] Proestling K, Birner P, Streubel B, et al. Enhanced expression of the stemness-related factors OCT4, OX15 and TWIST1 in ectopic endometrium of endometriosis patients. Reproductive Biology and Endocrinology. 2016;**14**:1-11. DOI: 10.1186/s12958-016-0215-4

[43] Baranov V, Malysheva O, Yarmolinskaya Y. Pathogenomics of endometriosis development. International Journal of Molecular Sciences. Jul 2018;**19**(7):1852. DOI: 10.3390%2Fijms19071852

[44] Spangrude GJ, Smith L, Uchida N, Weissman I, et al. Mouse hematopoietic stem cells. Blood. 1991;**8**(6):1395-1402

[45] Bjerknes M, Cheng H. Clonal analysis of mouse intestinal epithelial progenitors. Gastroenterology. 1999;**8**(1):7-14

[46] Jankowski R, Deasy B, Huard J. Muscle-derived stem cells. Gene Therapy. 2002;**8**(10):642-647

[47] Alonso L, Fuchs E. Stem cells of the skin epithelium. Proceedings of the National Academy of Sciences of the United States of America. 2003;**8** (Suppl 1):11830-11835

[48] Morrison SJ, White PM, Zock C, Anderson DJ. Prospective identification, isolation by flow cytometry, and in vivo self-renewal of multipotent mammalian neural crest stem cells. Cell. 1999;**8**(5):737-749

[49] Uchida N, Buck DW, He D, Weissman IL, et al. Direct isolation of human central nervous system stem cells. Proceedings of the National Academy of Sciences of the United States of America. 2000;**8**(26):14720-14725

[50] Rietze RL, Valcanis H, Bartlett PF, et al. Purification of a pluripotent neural stem cell from the adult mouse brain. Nature. 2001;**8**(6848):736-739

[51] Beltrami AP, Cesselli D, Pignatelli A, et al. Multipotent cells can be generated in vitro from several adult human organs (heart, liver, and bone marrow). Blood. 2007;**8**(9):3438-3446

[52] Crisan M, Yap S, Zhang L, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell. 2008;**8**(3):301-313

[53] Kalervo Väänänen H. Mesenchymal stem cells. Annals of Medicine. 2005;**8**(7):469-479

[54] da Silva ML, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. Journal of Cell Science. 2006;**8**(11):2204-2213

[55] Gargett CE. Stem cells in gynaecology. The Australian & New Zealand Journal of Obstetrics & Gynaecology. 2004;**44**:380-386

[56] Gargett CE. Identification and characterisation of human endometrial stem/progenitor cells. The Australian & New Zealand Journal of Obstetrics & Gynaecology. 2006;**46**:250-253

[57] Chan RW, Gargett CE. Identification of label retaining cells in mouse endometrium. Stem Cells. 2006;**8**(6):1529-1538

[58] Gargett CE, Schwab KE, Zillwood RM, Nguyen HP, Wu D. Isolation and culture of epithelial progenitors and mesenchymal stem cells from human endometrium. Biology of Reproduction. 2009;**8**(6):1136-1145

[59] Gargett CE, Chan RW, Schwab KE. Hormone and growth factor signaling in endometrial renewal: Role of stem/progenitor cells. Molecular and Cellular Endocrinology. 2008;**288**(1-2):22-29

[60] Masuda H, Anwar SS, Bühring HJ, Rao JR, Gargett CE. A novel marker of human endometrial mesenchymal stem-like cells. Cell Transplantation. 2012;**21**:2201-2214

[61] Gargett CE, Schwab KE, Deane JA. Endometrial stem/progenitor cells: The first 10 years. Human Reproduction Update. 2016;**22**(2):137-163

[62] Mints M, Jansson M, Palmblad J, et al. Endometrial endothelial cells are derived from donor stem cells in a bone marrow transplant recipient. Human Reproduction. 2008;**23**:139-143

[63] Morelli SS, Rameshwar P, Goldsmith LT. Experimental evidence for bone marrow as a source of nonhematopoietic endometrial stromal and epithelial compartment cells in a murine model. Biology of Reproduction. 2013;**89**:7

[64] Gargett CE, Schwab KE, Brosens JJ, Puttemans P, Benagiano G, Brosens I. Potential role of endometrial stem/progenitor cells in the pathogenesis of early-onset endometriosis.

Molecular Human Reproduction. 2014;**20**(7):591-598

[65] Prianishnikov VA. On the concept of stem cell and a model of functional-morphological structure of the endometrium. Contraception. 1978;**18**(3):213-223

[66] Yang J, Huang F. Stem cell and endometriosis: New knowledge may be producing novel therapies. International Journal of Clinical and Experimental Medicine. 2014;**7**(11):3853-3858

[67] Taylor HS. Endometrial cells derived from donor stem cells in bone marrow transplant recipients. Journal of the American Medical Association. 2004;**292**:85

[68] Borghese B, Zondervan KT, Abrao MS, Chapron C, Vaiman D. Recent insights on the genetics and epigenetics of endometriosis. Clinical Genetics. 2017;**91**:254-264. DOI: 10.1111/cge.12897

[69] Lagana AS, Salmeri FM, Vitale SG, Triolo O, Gotte M. Stem cell trafficking during endometriosis: May epigenetics play a pivotal role? Reproductive Sciences. Jul 2018;**25**(7):978-979. [Epub 2017 Jan 19] DOI: 10.1177/1933719116687661

[70] Grimstad FW, Decherney A. A review of the epigenetic contributions to endometriosis. Clinical Obstetrics and Gynecology. 2017;**60**:467-476. DOI: 10.1097/GRF.0000000000000298

[71] Padykula HA. Cellular and tissue mechanisms of primate uterine endometrial renewal: An hypothesis. Advances in Experimental Medicine and Biology. 1987;**230**:1-12

[72] Padykula HA, Coles LG, Kaiserman-Abramof IR, et al. The basalis of the primate endometrium: A bifunctional germinal compartment. Biology of Reproduction. 1989;**40**:681-690

[73] Schwab KE, Chan RW, Gargett CE. Putative stem cell activity of human endometrial epithelial and stromal cells during the menstrual cycle. Fertility and Sterility. 2005;**84**(Suppl. 2):1124-1130

[74] Gargett BE, Chan RW. Endometrial stem/progenitor cells and proliferative disorders of the endometrium. Minerva Ginecologica. 2006;**58**:511-526

[75] Maruyama T, Masuda H, Ono M, Kajitani T, Yoshimura Y. Human uterine stem/progenitor cells: Their possible role in uterine physiology and pathology. Reproduction. 2010;**140**:11-22

[76] Meng X, Ichim ET, Zhong J, Riordan HN, et al. Endometrial regenerative cells: A novel stem cell population. Journal of Translational Medicine. 2007;**5**:57

[77] Patel AN, Park E, Kuzman M, Allickson JG, et al. Multipotent menstrual blood stromal stem cells: Isolation, characterization, and differentiation. Cell Transplantation. 2008;**17**:303-311

[78] Deane JA, Gualano RC, Gargett CE. Regenerating endometrium from stem/progenitor cells: Is it abnormal in endometriosis, Asherman's syndrome and infertility? Current Opinion in Gynecology and Obstetrics. 2013;**25**(3):193-200. DOI: 10.1097/GCO.0b013e32836024e7

[79] Figueira PG, Abrão MS, Krikun G, Taylor HS. Stem cells in endometrium and their role in the pathogenesis of endometriosis. Annals of the New York Academy of Sciences. 2011;**1221**:10-17

[80] Putterman P, Benagiano G, Gargett CE, Romero R, Guo SW, Brosens I. Neonatal uterine bleeding as a biomarker for reproductive disorders during adolescence: A worldwide call for systematic registration by nurse midwife. Journal of

Maternal-Fetal and Neonatal Medicine. 2017;**30**(12):1434-1436

[81] Kato K, Yoshimoto M, Wake N, et al. Characterization of side-population cells in human normal endometrium. Human Reproduction. 2007;**22**:1214-1223

[82] Gurung S, Deane JA, Masuda H, Maruyama T, Gargett CE. Stem cells in endometrial physiology. Seminars in Reproductive Medicine. 2015;**33**(5):326-332. DOI: 10.1055/s-0035-1558405

[83] Warren LA, Shih A, Renteira SM, Gregersen PK, et al. Analysis of menstrual effluent: Diagnostic potential for endometriosis. Molecular Medicine. 2018;**24**(1):1. DOI: 10.1186/s10020-018-0009-6

[84] Masuda H, Matsuzaki Y, Hiratsu E, Asada H, et al. Stem cell-like properties of the endometrial side population: Implication in endometrial regeneration. PLoS ONE. 2010;**5**:e10387

[85] Weimar CH, Macklon NS, PostUiterweer ED, Brosens JJ, Gellersen B. The motile and invasive capacity of human endometrial stromal cells: Implications for normal and impaired reproductive function. Human Reproduction Update. 2013;**19**(5):542-557. DOI: 10.1093/humupd/dmt025

[86] Gentilini D, Vigano P, Carmignani L, Spinelli M, Busacca M, Di Blasio AM. Progesterone receptor +331G/a polymorphism in endometriosis and deep-infiltrating endometriosis. Fertility and Sterility. 2008;**90**:1243-1245

[87] Gellersen B, Reinmann K, Samalecos A, Aupers S, Bamberger AM. Invasiveness of human endometrial stromal cells is promoted by decidualization and by trophoblast-derived signals. Human Reproduction. 2010;**25**(4):862-873. DOI: 10.1093/humrep/dep468

[88] Hull M, Escareno CR, Godsland JM, et al. Endometrial-peritoneal interactions during endometriotic lesion establishment. American Journal of Pathology. 2008;**173**(3):700-715

[89] Nikoo S, Ebtekar M, Zarnani AH, et al. Menstrual blood-derived stromal stem cells from women with and without endometriosis reveal different phenotypic and functional characteristics. Molecular Human Reproduction. Sep 2014;**20**(9):905-918. [Epub 2014 Jun 16]. DOI: 10.1093/molehr/gau044

[90] Sakr S, Naqvi H, Komm B, Taylor SH. Endometriosis impairs bone marrow-derived stem cell recruitment to the uterus whereas Bazedoxifene treatment leads to endometriosis regression and improved uterine stem cell engraftment. Endocrinology. 2014;**155**(4):1489-1497. DOI: 10.1210/en.2013-1977

[91] Cinar O, Seval Y, Arici A, et al. Differential regulation of Akt phosphorylation in endometriosis. Reproductive BioMedicine Online. 2009;**19**:864-871

[92] Leconte M, Nicco C, Batteux F, et al. The mTOR/AKT inhibitor temsirolimus prevents deep infiltrating endometriosis in mice. The American Journal of Pathology. 2011;**179**:880-889

[93] Zhang H, Zhao X, Liu S, Li J, Wen Z, Li M. 17betaE2 promotes cell proliferation in endometriosis by decreasing PTEN via NFkappaB-dependent pathway. Molecular and Cellular Endocrinology. 2010;**317**:31-43

[94] Lee II, Kim JJ. Influence of AKT on progesterone action in endometrial diseases. Biology of Reproduction. 2014;**91**(3):63

[95] Keller DW, Wiest WG, Strckler RC, et al. *Pseudocorpus luteum* insufficiency: A local defect of progesterone action on endometrial stroma. Journal of

Clinical Endocrinology and Metabolism. 1979;**48**(1):127-132

[96] Chrousos GP, MacLusky NJ, Brandon DD, Lipsett MB, et al. Progesterone resistance. Advances in Experimental Medicine and Biology. 1986;**196**:317-328. DOI: 10.1007/978-1-4684-5101-6_21

[97] Li X, Feng Y, Lin J-F, Billing H, Shao R. Endometrial progesterone resistance and PCOS. Journal of Biomedical Science. 2014;**21**(1):2. DOI: 10.1186/1423-0127-21-2

[98] Al-Sabbagh M, Lam EW, Brosens JJ. Mechanisms of endometrial progesterone resistance. Molecular and Cellular Endocrinology. 2012;**358**:208-215. DOI: 10.1016/j.mce.2011.10.035

[99] Groothuis PG, Dassen HH, Romano A. Estrogen and the endometrium lessons learned from gene expression profiling in rodents and human. Human Reproduction Update. 2007;**13**:405-417

[100] Kitawaki J, Kado N, Ishihara H, Honjo H, et al. Endometriosis: The pathophysiology as an estrogen-dependent disease. The Journal of Steroid Biochemistry and Molecular Biology. 2002;**83**(1-5):149-155

[101] Acien P, Velasco I, Gutierrez M, Martinez-Beltran M. Aromatase expression in endometriotic tissues and its relationship to clinical and analytical findings. Fertility and Sterility. 2007;**88**(1):32-38

[102] Ferrero S, Remorgida V, Maganza C, Venturini P, et al. Aromatase and endometriosis: Estrogens play a role. Annals of the New York Academy of Sciences. 2014;**1317**:17-23

[103] Reis FM, Petraglia F, Taylor RN. Endometriosis: Hormone regulation and clinical consequences of chemotaxis and apoptosis. Human Reproduction Update. 2013;**19**(4):406-418. DOI: 10.1093/humupd/dmt010

[104] Gellersen B, Brosens IA, Brosens JJ. Decidualization of the human endometrium: Mechanisms, functions, and clinical perspectives. Seminars in Reproductive Medicine. 2007;**25**:445-453

[105] Hansen KA, Opsahl MS, Nieman LK, Baker JR Jr, Klein TA. Natural killer cell activity from pregnant subjects is modulated by RU 486. American Journal of Obstetrics and Gynecology. 1992;**166**:87-90

[106] Brierley J, Clark DA. Characterization of hormone-dependent suppressor cells in the uterus of mated and pseudopregnant mice. Journal of Reproductive Immunology. 1987;**10**:201-217

[107] Mannel DN, Falk W, Yron I. Inhibition of murine cytotoxic T cell responses by progesterone. Immunology Letters. 1990;**26**:89-94

[108] Barakonyi A, Polgar B, Szekeres-Bartho J. The role of gamma/delta T-cell receptor-positive cells in pregnancy: Part II. American Journal of Reproductive Immunology. 1999;**42**:83-87

[109] Choi BC, Polgar K, Xiao L, Hill JA. Progesterone inhibits in-vitro embryotoxic Th1 cytokine production to trophoblast in women with recurrent pregnancy loss. Human Reproduction. 2000;**15**(Suppl 1):46-59

[110] Li Y, Adur KM, Kannan A, Davila J, Li Q , et al. Progesterone alleviates endometriosis via inhibition of uterine cell proliferation, inflammation and angiogenesis in an immunocompetent mouse model. PLoS One. 2016;**11**(10):e0165347

[111] Burns KA, Rodriguez KF, Hewitt SC, Korach KS, et al. Role of estrogen

receptor signaling required for endometriosis-like lesion establishment in a mouse model. Endocrinology. 2012;**153**:3960-3971. DOI: 10.1210/en.2012-1294

[112] Bulun SE, Monsavais D, Pavone ME, et al. Role of estrogen receptor-beta in endometriosis. Seminars in Reproductive Medicine. 2012;**30**(1): 39-45. DOI: 10.1055/s-0031-1299596

[113] Bukulmez O, Hardy DB, Carr BR, Word RA, Mendelson CR. Inflammatory status influences aromatase and steroid receptor expression in endometriosis. Endocrinology. 2008;**149**:1190-1204. DOI: 10.1210/en.2007-0665

[114] Attia GR, Zeitoun K, Bulun SE, et al. Progesterone receptor isoform a but not B is expressed in endometriosis. The Journal of Clinical Endocrinology and Metabolism. 2000;**85**:2897-2902

[115] Burney OR, Talbi S, Hamilton EA, Giudice CL, et al. Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. Endocrinology. 2007;**148**(8):3814-3826. DOI: 10.1210/en.2006-1692

[116] Bedaiwy MA, Dahoud W, Mesiano S, et al. Abundance and localization of progesterone receptor isoforms in endometrium in women with and without endometriosis and in peritoneal and ovarian endometriotic implants. Reproductive Sciences. 2015;**22**:1153-1161. DOI: 10.1177/1933719115585145

[117] Valentijn AJ, Palial K, Gargett CE, Hapangama DK, et al. SSEA-1 isolates human endometrial basal glandular epithelial cells: Phenotypic and functional characterization and implications in the pathogenesis of endometriosis. Human Reproduction. 2013;**28**:2695-2708

[118] Leyendecker G, Herbertz M, Kunz G, Mall G. Endometriosis results from the dislocation of basal endometrium. Human Reproduction. 2002;**17**:2725-2736

[119] Bulun SE, Cheng YH, Yin P, Kim JJ, et al. Progesterone resistance in endometriosis: Link to failure to metabolize estradiol. Molecular and Cellular Endocrinology. 2006;**248**(1-2):94-103. DOI: 10.1016/j.mce.2005.11.041

[120] Kim JJ, Sefton EC, Bulun SE. Progesterone receptor action in leiomyoma and endometrial cancer. Progress in Molecular Biology and Translational Science. 2009;**87**:53-85

[121] Yin X, Pavone ME, Lu Z, Wei J, Kim JJ. Increased activation of the PI3K/AKT pathway compromises decidualization of stromal cells from endometriosis. The Journal of Clinical Endocrinology and Metabolism. 2012;**97**:E35-E43

[122] Gentilini D, Busacca M, Di Francesco S, Di Blasio AM, et al. PI3K/Akt and ERK1/2 signalling pathways are involved in endometrial cell migration induced by 17beta-estradiol and growth factors. Molecular Human Reproduction. 2007;**13**:317-322

[123] Eaton JL, Unno K, Caraveo M, Lu Z, Kim JJ. Increased AKT or MEK1/2 activity influences progesterone receptor levels and localization in endometriosis. The Journal of Clinical Endocrinology and Metabolism. 2013;**98**:E1871-E1879

[124] Klemmt PA, Carver JG, Kennedy SH, Koninckx P, Mardon HJ. Stromal cells from endometriotic lesions and endometrium from women with endometriosis have reduced decidualization capacity. Fertility and Sterility. 2006;**85**(3):564-572

[125] Kaya HS, Hantak AM, Bagchi MK, et al. Roles of progesterone receptor

A and B isoforms during human endometrial decidualization. Molecular Endocrinology. 2015;**29**(6):882-895. DOI: 10.1210/me.2014-1363

[126] McNally JG, Muller WG, Hager GL, et al. The glucocorticoid receptor: Rapid exchange with regulatory sites in living cells. Science. 2000;**287**:1262-1265

[127] Thomas P. Characteristics of membrane progestin receptor alpha (mPRalpha) and progesterone membrane receptor component 1 (PGMRC1) and their roles in mediating rapid progestin actions. Frontiers in Neuroendocrinology. 2008;**29**:292-312

[128] Singh M. Ovarian hormones elicit phosphorylation of Akt and extracellular-signal regulated kinase in explants of the cerebral cortex. Endocrine. 2001;**14**:407-415

[129] Thomas P, Pang Y, Tubbs C, et al. Steroid and G protein binding characteristics of the seatrout and human progestin membrane receptor alpha subtypes and their evolutionary origins. Endocrinology. 2007;**148**(2):705-718

[130] Tsai M-J, Clark JH, Schrader WT, O'Malley BW. Mechanisms of action of hormones that act as transcription-regulatory factors. In: Wilson J, Foster D, Kronenberg H, Larsen P, editors. Williams Textbook of Endocrinology. Philadelphia: W.B. Saunders Company; 1998. pp. 55-94

[131] Khan JA, Bellance C, Loosfelt H, et al. Differential regulation of breast cancer-associated genes by progesterone receptor isoforms PRA and PRB in a new bi-inducible breast cancer cell line. PLoS ONE. 2012;**7**:e45993

[132] Spencer TE, Becker WC, Bazer FW, et al. Ovine interferon-tau regulates expression of endometrial receptors for estrogen and oxytocin but not progesterone. Biology of Reproduction. 1995;**53**:732-745

[133] Spencer TE, Bazer FW. Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. Frontiers in Bioscience. 2002;**7**:d1879-d1898

[134] Lessey BA, Killam AP, Metzger DA, McCarty KS Jr, et al. Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. The Journal of Clinical Endocrinology and Metabolism. 1988;**67**:334-340

[135] Press MF, Udove JA, Greene GL. Progesterone receptor distribution in the human endometrium. Analysis using monoclonal antibodies to the human progesterone receptor. American Journal of Pathology. 1988;**131**:112-124

[136] Ohlsson Teague EM, Van der Hoek KH, Van der Hoek MB, et al. MicroRNA-regulated pathways associated with endometriosis. Molecular Endocrinology. 2009;**23**(2):265-275

[137] Vegeto E, Shahbaz MM, Wen DX, McDonnell DP, et al. Human progesterone receptor a form is a cell- and promoter-specific repressor of human progesterone receptor B function. Molecular Endocrinology. 1993;**7**:1244-1255. DOI: 10.1210/me.7.10.1244

[138] Boonyaratanakornkit V, McGowan E, Sherman L, Cheskis BJ, et al. The role of extranuclear signaling actions of progesterone receptor in mediating progesterone regulation of gene expression and the cell cycle. Molecular Endocrinology. 2007;**21**:359-375

[139] Aghajanova L, Velarde MC, Giudice LC. Altered gene expression profiling in endometrium: Evidence for progesterone resistance. Seminars in Reproductive

Medicine. 2010;**28**(1):51-58. DOI: 10.1055/s-0029-1242994

[140] Sharpe-Timms KL. Endometrial anomalies in women with endometriosis. Annals of the New York Academy of Sciences. 2001;**943**:131-147

[141] Burney OR, Talbi S, Hamilton EA, Giudice CL, et al. Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. Endocrinology. 2007;**148**(8):3814-3826. DOI: 10.1210/en.2006-1692

[142] Bondza PJ, Maheux R, Akoun M. Insights into endometriosis-associated endometrial dysfunctions: A review. Frontiers in Bioscience (Elite Edition). 2009;**1**:415-428

[143] Near MA, Wu HA, Templeman C, Pearce C, et al. Progesterone receptor gene polymorphisms and risk of endometriosis: Results from an international collaborative effort. Fertility and Sterility. 2011;**95**(1):40-45

[144] Fung JN, Mortlock S, Girling JE, Montgomery GW, et al. Genetic regulation of disease risk and endometrial gene expression highlights potential target genes for endometriosis and polycystic ovarian syndrome. Scientific Reports. 2018;**8**(1):11424. DOI: 10.1038/s41598-018-29462-y

[145] Lee B, Du H, Taylor HS. Experimental murine endometriosis induces DNA methylation and altered gene expression in eutopic endometrium. Biology of Reproduction. 2009;**80**(1):79-85. DOI: 10.1095/biolreprod.108.070391

[146] Kennedy S, Mardon H, Barlow D. Familial endometriosis. Journal of Assisted Reproduction and Genetics. 1995;**12**(1):32-34

[147] Hadfield RM, Yudkin PL, Coe C, et al. Risk factors for endometriosis in the rhesus monkey (Macaca mulatta): A case-control study. Human Reproduction Update. 1997;**3**(2):109-115

[148] Moen MH, Magnus P. The familial risk of endometriosis. Acta Obstetricia et Gynecologica Scandinavica. 1993;**72**(7):560-564

[149] Kennedy S, Hadfield R, Mardon H, Barlow D. Age of onset of pain symptoms in non-twin sisters concordant for endometriosis. Human Reproduction. 1996;**11**(2):403-405

[150] Bischoff F, Simpson JL. Genetic basis of endometriosis. Annals of the New York Academy of Sciences. 2004;**1034**:284-299

[151] Treloar SA, Zhao ZZ, Armitage T, Duffy DL, Wicks J, O'Connor DT, et al. Association between polymorphisms in the progesterone receptor gene and endometriosis. Molecular Human Reproduction. 2005;**11**:641-647

[152] Falconer H, D'Hooghe T, Fried G. Endometriosis and genetic polymorphisms. Obstetrical & Gynecological Survey. 2007;**62**:616-628

[153] Guo SW. MHR basic science of reproductive medicine. Epigenetics in Endometriosis. 2009;**15**(10):587-607. DOI: 10.1093/molehr/gap064

[154] Grechukhina O, Petracco R, Popkhadze S, et al. A polymorphism in a let-7 microRNA binding site of KRAS in women with endometriosis. EMBO Molecular Medicine. 2012;**4**(3):206-217

[155] Nyholt DR, Low SK, Anderson CA, et al. Genome-wide association meta-analysis identifies new endometriosis risk loci. Nature Genetics. 2012;**44**(12):1355-1359

[156] Rousseau-Merck MF, Misrahi M, Loosfelt H, Milgrom E, Berger R.

Localization of the human progesterone receptor gene to chromosome 11q22-q23. Human Genetics. 1987;**77**:280-282

[157] Taylor HS, Bagot C, Kardana A, Olive D, Arici A. HOX gene expression is altered in the endometrium of women with endometriosis. Human Reproduction. 1999;**14**(5):1328-1331

[158] Barlow DH, Kennedy S. Endometriosis: New genetic approaches and therapy. Annual Review of Medicine. 2005;**56**:345-356

[159] Guo SW. Association of endometriosis risk and genetic polymorphisms involving sex steroid biosynthesis and their receptors: A meta-analysis. Gynecologic and Obstetric Investigation. 2005;**61**:90-105

[160] Guo SW. Glutathione S-transferases M1 (GSTM1)/T1 (GSTT1)gene polymorphisms and endometriosis: A meta-analysis of genetic association studies. Molecular Human Reproduction. 2005;**11**:729-743

[161] Guo SW. The association of endometriosis risk and genetic polymorphisms involving dioxin detoxification enzymes: A systematic review. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2006;**124**:134-143

[162] Matalliotakis M, Zervon MI, Eliopoulos Zondervan KT, Goulichnos GN, et al. The role of IL-16 gene polymorphisms in endometriosis. International Journal of Molecular Medicine. 2018;**41**(3):1469-1476. DOI: 10.3892/ijmm.2018.3368

[163] Wieser F, Schneeberger C, Tong D, Wenzl R, et al. PROGINS receptor gene polymorphism is associated with endometriosis. Fertility and Sterility. 2002;**77**:309-312

[164] Berchuck A, Schildkraut JM, Henriott A, et al. Progesterone receptor promoter +331A polymorphism is associated with a reduced risk of endometrioid and clear cell ovarian cancers. Cancer Epidemiology, Biomarkers & Prevention. 2004;**13**:2141-2147

[165] Meola J, Blassioli Dentillo D, Martelli L, et al. Glycodelin expression in the endometrium of healthy women and in the eutopic and ectopic endometrium of women with endometriosis. Fertility and Sterility. 2000;**51**(5):1676-1168

[166] Koninckx PR, Braet P, Kennedy SH, Barlow DH. Dioxin pollution and endometriosis in Belgium. Human Reproduction. 1994;**9**(6):1001-1002

[167] Koninckx PR, Barlow D, Kennedy S. Implantation versus infiltration: The Sampson versus the endometriotic disease theory. Gynecologic and Obstetric Investigation. 1999;**47** (Suppl. 1):3-9

[168] Kurokawa R, Rosenfeld MG, Glass CK. Transcriptional regulation through noncoding RNAs and epigenetic modifications. RNA Biology. 2009;**6**: 233-236

[169] Mendell JT. MicroRNAs critical regulators of development, cellular physiology and malignancy. Cell Cycle. 2005;**4**(9):1179-1184

[170] Nothnick WB. MicroRNAs and endometriosis: Distinguishing drivers from passengers in disease Pathogenesis. Seminars in Reproductive Medicine. 2017;**35**(2):173-180. DOI: 10.1055/s-0037-1599089

[171] Taylor RN, Lundeen SG, et al. Emerging role of genomics in endometriosis research. Fertility and Sterility. 2002;**78**(4):694-698

[172] Giudice LC. Genomics' role in understanding the pathogenesis

of endometriosis. Seminars in Reproductive Medicine. 2003;**21**:119-124

[173] Giudice LC. Application of functional genomics to primate endometrium: Insights into biological processes. Reproductive Biology and Endocrinology. 2006;**4**(Suppl 1):S4

[174] Gogusev J, de Joliniere, et al. Detection of DNA copy number changes in human endometriosis by comparative genomic hybridization. Human Genetics. 1999;**105**:451

[175] Wu Y, Strawn E, et al. Genomic alterations in ectopic and eutopic endometria of women with endometriosis. Gynecologic and Obstetric Investigation. 2006;**62**:148-159

[176] Filigheddu N, Gregnanin I, Porporato PE, et al. Differential expression of microRNAs between eutopic and ectopic endometrium in ovarian endometriosis. Journal of Biomedicine and Biotechnology. 2010;**2010**:369549

[177] Hawkins SM, Creighton CJ, Han DY, et al. Functional microRNA involved in endometriosis. Molecular Endocrinology. 2011;**25**(5):821-832

[178] Long M, Wan X, La X, Gong X, Cai X. miR-29c is downregulated in the ectopic endometrium and exerts its effects on endometrial cell proliferation, apoptosis and invasion by targeting c-Jun. International Journal of Molecular Medicine. 2015;**35**(4):1119-1125

[179] Shi XY, Gu L, Chen J, Guo XR, Shi YL. Downregulation of miR-183 inhibits apoptosis and enhances the invasive potential of endometrial stromal cells in endometriosis. International Journal of Molecular Medicine. 2014;**33**(1):59-67

[180] Yang RQ , Teng H, Xu XH, Liu XJ, et al. Microarray analysis of microRNA deregulation and angiogenesis-related proteins in endometriosis. Genetics and Molecular Research. 2016;**15**(2):gmr7826. DOI: 10.4238gmr.15027820

[181] Ramón LA, Braza-Boïls A, Gilabert-Estellés J, Estelles A, et al. Micro RNAs expression in endometriosis and their relation to angiogenic factors. Human Reproduction. 2011;**26**(5):1082-1090

[182] Liu S, Gao S, Wang XY, Wang DB. Expression of miR-126 and Crk in endometriosis: miR-126 may affect the progression of endometriosis by regulating Crk expression. Archives of Gynecology and Obstetrics. 2012;**285**(4):1065-1072

[183] Ambros V. The functions of animal microRNAs. Nature. 2004;**2004**(431):350-355

[184] Kobayashi H, Higaskiura Y, Koike N, Yamada Y, et al. Genes downregulated in endometriosis are located Near the known imprinting genes. Reproductive Sciences. 2014;**21**(8):966-972

[185] Swaney WT. Genomic imprinting and mammalian reproduction. Hormones and Behavior. 2011;**59**(3):369-374

[186] Hsiao KY, Wu MH, Chang N, Tsai SJ, et al. Coordination of AUF1 and miR-148a destabilizes DNA methyltransferase 1 mRNA under hypoxia in endometriosis. Molecular Human Reproduction. 2015;**21**:894-904. DOI: 10.1093/molehr/gav054

[187] Kouroura O, Sifakis S, Spandidos AD. DNA methylation in endometriosis. Molecular Medicine Reports. 2016;**13**(4):2939-2948

[188] Xue Q , Lin Z, Yin P, Bulun S, et al. Transcriptional activation of steroidogenic factor-1 by

hypomethylation of the 5? CpG island in endometriosis. Journal of Clinical Endocrinology and Metabolism. 2007;**92**:3261-3267

[189] Yang YM, Yang WX. Epithelial-to-mesenchymal transition in the development of endometriosis. Oncotarget. 2017;**8**(25):41679-41689. DOI: 10.18632

[190] Wu Y, Strawn E, Basir Z, Halverson G, Guo SW. Promoter hypermethylation of progesterone receptor isoform B (PR-B) in endometriosis. Epigenetics. 2006;**1**:106-111

[191] Meyer JL, Zimbardi D, Rainho CA, et al. DNA methylation patterns of steroid receptor genes ESR1, ESR2 and PGR in deep endometriosis compromising the rectum. International Journal of Molecular Medicine. 2014;**33**:897-904

[192] Dyson MT, Roqueiro D, Monsivais Bulun SE, et al. Genome-wide DNA methylation analysis predicts an epigenetic switch for GATA factor expression in endometriosis. PLOS Genetics. 2014;**10**:e1004158

[193] Wang H, Dey SK. Roadmap to embryo implantation: Clues from mouse models. Nature Reviews. Genetics. 2006;**7**:185-119

[194] Xu J, Li Q. Review of the in vivo functions of the p160 steroid receptor coactivator family. Molecular Endocrinology. 2003;**17**:1681-1692

[195] Li X, O'Malley BW. Unfolding the action of progesterone receptors. The Journal of Biological Chemistry. 2003;**278**:39261-39264

[196] Groll J, Lessey B. For glob. Libr. women's med- implantation. GLOWM—Global Library of Women Medicine FIGO. 2009. ISSN: 1756-2228; DOI: 10.3843/GLOWM.10318

Edited by Giovana Aparecida Gonçalves

What are the objectives of the genetic study of individuals? There is great interest from the medical community and also much concern from the lay press about the potential benefits and harms of genetic screening, gene therapy, and even the possibility of cloning individuals. The current use of genetic tests for the detection and treatment of endometriosis is still at an early stage but very important. The determination of susceptibility markers will be increasingly explored in clinical studies and their uses will be much more defined. Still, it seems increasingly likely that major changes will occur over the next decade in how we evaluate and treat our patients. In particular, surgeons and clinicians will have the opportunity to use a number of new tests to predict the future appearance of endometriosis in patients still free of the disease. They may have the power to explore the best therapeutic modality for a particular patient according to his/her genetic makeup. And they will be able to more specifically target prevention measures for family members of people already affected by the disease. It should be understood that molecular diagnosis, especially in asymptomatic individuals, does not mean disease but an increased risk of developing a disease. Ethical implications exist and should not be underestimated. Patients should be advised about the likely implications of such tests, not only after but especially before the achievement of these. A major step has already been overcome and we currently have the basic tools for a new leap in understanding human pathologies responsible for much of the world's mortality. Bridging the great barrier that still separates this basic knowledge and clinical practice is quite a significant challenge.

Published in London, UK

IntechOpen

ISBN 978-1-78985-136-6
9 781789 851366