A molecular marker for the risk of endometriosis recurrence can help to customize a post-operative treatment plan for an individual patient. The aim of this study was to compare the expression of genes coding for estradiol (mER, ERα, ERβ) and progesterone (PGRmc, mPR, PR-A, PR-B) receptors in first-time and relapsing patients with ovarian endometriosis. Our study included 94 women of reproductive age with ovarian endometriosis: 82 first-time and 12 relapsing patients. The expression of genes coding for steroid receptors was measured using reverse transcription polymerase chain reaction. Recurrent conditions were characterized by a change in the expression of estrogen receptors and unchanged expression of progesterone receptors. Expression of mER in the tissue of patients with first-time endometriosis was 15.09 ± 1.18. Patients undergoing recurrence demonstrated a 3-fold increase in mER expression (from 15.09 ± 1.18 to 44.45 ± 9.1). Also, in such patients ERα expression was 5 times higher increasing from 11.71 ± 0.22, which is an average value for first-time patients, to 10.02 ± 3.81, while ERβ expression surged 7-fold from 10.47 ± 1.05 to 1.68 ± 0.55 (p < 0.05). Transcription of the studied receptors in the pathological tissue depended on the stage of the disease: in relapsing patients expression of estradiol receptors underwent some changes, while expression profile of progesterone receptors remained unchanged. Sensitivity of endometrial tissue to gestogens is clinically important and serves as a basis for a successful hormone-based relapse prevention.

Keywords: ovarian endometriosis, PCO, estradiol receptors, progesterone receptors

Correspondence should be addressed: Lolita Bulatova

Ostrovtianova 1, Moscow, 117997; lolita.bulatova.87@mail.ru

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EXPRESSION OF STEROID HORMONE RECEPTORS IN THE TISSUE OF ENDOMETRIOMAS IN FIRST-TIME AND RELAPSING PATIENTS

Bulatova LS1, Solomatina AA1, Kareva EN2, Kotsyubinskaya NA2

1 Department of Obstetrics and Gynecology, Faculty of Pediatrics, Pirogov Russian National Research Medical University, Moscow
2 Department of Molecular Pharmacology and Radiobiology, Biomedical Faculty, Pirogov Russian National Research Medical University, Moscow

A molecular marker for the risk of endometriosis recurrence can help to customize a post-operative treatment plan for an individual patient. The aim of this study was to compare the expression of genes coding for estradiol (mER, ERα, ERβ) and progesterone (PGRmc, mPR, PR-A, PR-B) receptors in first-time and relapsing patients with ovarian endometriosis. Our study included 94 women of reproductive age with ovarian endometriosis: 82 first-time and 12 relapsing patients. The expression of genes coding for steroid receptors was measured using reverse transcription polymerase chain reaction. Recurrent conditions were characterized by a change in the expression of estrogen receptors and unchanged expression of progesterone receptors. Expression of mER in the tissue of patients with first-time endometriosis was 15.09 ± 1.18. Patients undergoing recurrence demonstrated a 3-fold increase in mER expression (from 15.09 ± 1.18 to 44.45 ± 9.1). Also, in such patients ERα expression was 5 times higher increasing from 11.71 ± 0.22, which is an average value for first-time patients, to 10.02 ± 3.81, while ERβ expression surged 7-fold from 10.47 ± 1.05 to 1.68 ± 0.55 (p < 0.05). Transcription of the studied receptors in the pathological tissue depended on the stage of the disease: in relapsing patients expression of estradiol receptors underwent some changes, while expression profile of progesterone receptors remained unchanged. Sensitivity of endometrial tissue to gestogens is clinically important and serves as a basis for a successful hormone-based relapse prevention.

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OSOBNENOSTI EKSPRESSII GENOV RECEPTOROV STEROIDNYX GORMONOV V TKANAYakh Pervichnykh i Rezidiviruyushikh Endometriodnykh Obrazovaniiy Yachnikov

L. S. Bulatova1, A. A. Solomatina1, E. N. Kareva2, N. A. Kotsyubinskaya2

1 Кафедра ангиологии и лимфологии, педиатрический факультет,
Российский национальный исследовательский медицинский университет имени Н. И. Пирогова,
2 Кафедра молекулярной фармакологии и радиобиологии, медико-биологический факультет,
Российский национальный исследовательский медицинский университет имени Н. И. Пирогова

Для разработки индивидуального плана послеоперационного ведения пациенток необходим поиск молекулярно-фармакологического маркера принадлежности пациенток к группе риска рецидивирования эндометриоза яичников. Целью исследования было проведение сравнительный анализ экспрессии генов рецепторов эстрadiола (mER, ERα, ERβ) и прогестерона (PGRmc, mPR, PR-A, PR-B) в ткани первичного эндометриоидного образования яичников и при рецидиве заболевания. В исследование вошли 94 пациентки репродуктивного возраста с эндометриоидными образованиями яичников: 82 пациентки с первичным эндометриозом и 12 с рецидивом заболевания. Для определения экспрессии генов стероидных рецепторов использовали метод полимеразной цепной реакции с обратной транскрипцией (ОТПЦР). При рецидиве заболевания в эндо-метриоидной ткани яичника выявлен сдвиг спектра эстрогеннойых рецепторов, а уровень рецепторов прогестерона оставался неизменным. Уровень экспрессии mER в ткани первичного эндометриоза составил 15.09 ± 1.18. Обнаружено увеличение экспрессии mER в 3 раза (c 15.09 ± 1.18 в первичной эндометриоидной ткани до 44.45 ± 9.1 в ткани с рецидивом эндометриоза), ERα в 5 раз (c 11.71 ± 2.22 до 10.02 ± 3.81), а также снижение экспрессии рецептора ERβ в 7 раз (c 10.47 ± 1.05 до 1.68 ± 0.55) по сравнению с эндометриоидной тканью яичника при первичном заболевании (p < 0.05). Транскрипция рецепторов эндометриоидной ткани яичника зависит от стадии заболевания: при рецидивировании наблюдается изменение спектра рецепторов эстрadiола на фоне интактного рецепторного аппарата для прогестерона. Клиническое значение имеет сохранность чувствительности ткани к гестагенам как основа успешной противорецедивной гормональной терапии.

Ключевые слова: эндометриоз яичников, ПЦР, рецепторы эстрadiола, рецепторы прогестерона

Для корреспонденции: Bulatova Lolita Sainyavlina
ул. Островтитенова, д. 1, г. Москва, 117997; lolita.bulatova.87@mail.ru

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Endometriosis is a chronic benign estrogen-dependent condition [1, 2]. A tendency to recur and a negative impact on a woman’s general health, quality of life and ability to work make endometriosis a clinically and socially significant disease. In patients receiving surgical treatment for endometriosis, cumulative recurrence rates are 6.4%, 10%, 19.9%, and 30.9% at 2, 3, 5, and 6 years after the surgery, respectively [3]. There is no consensus in the medical community on what triggers the recurrence of the disease, which complicates the search for biochemical markers that could be used to monitor the effect of treatment in first-time and relapsing patients [4].

Among the possible causes of the recurrence proposed in the literature is incomplete excision of endometriotic lesions during the surgery; it is still debatable, though, whether it is a true recurrence or a new primary disease. Hormone prevention therapy can alter the status of steroid receptors in the endometrium and affect the sensitivity of lesions to further treatment. Therefore, transcription profiles of steroid receptors can differ in first-time and relapsing patients. The aim of this work was to compare the expression levels of genes coding for membrane and nuclear estrogen (mER, ERα, ERβ) and progesterone (PGRMC1, mPR, PR-A, PR-B) receptors in the endometriotic tissue of first-time patients with ovarian endometriomas and patients with recurrences. This will help us understand whether progestins are a rational treatment choice for recurrent endometriosis.

METHODS

The study included 94 women of reproductive age diagnosed with endometriosis: 82 were first-time patients and 12 had a relapse. All patients gave informed consent to participate.

The patients diagnosed with endometriosis for the first time were included in the study if they were of reproductive age, their ultrasound scans suggested ovarian endometriomas, and the diagnosis was confirmed laparoscopically and histologically. For relapsing patients, inclusion criteria were as follows: reproductive age, prior surgery for endometriosis, ultrasound scans suggestive of endometriomas that were later confirmed laparoscopically and histologically.

Patients with extragenital and genital comorbidities (uterine myoma, adenomyosis, ovarian tumors) and those who had undergone a hormone therapy before were excluded from the study. All tests were carried out in the proliferative phase of the menstrual cycle.

Based on the histologically verified diagnosis, the women were divided into 2 groups: group I included 82 patients with ovarian endometriomas, group II consisted of 12 relapsing patients. The age of the participants varied from 18 to 44 years, averaging 31.4 ± 5.2 years. The age of the patients included in group I varied from 22 to 41 years, the mean age was 34.2 ± 5.3 years. In group II the mean age was 33.4 ± 6.9 years, implying that the groups were comparable in terms of age. Disease duration ranged from 1.5 to 5 years in group I and from 2 months to 1.5 years in group II. The most common complaint in both groups was pelvic pain (35 cases or 53% in group I and 6 cases or 67.4% in group II).

The frequency of menstrual disorders was significantly lower in group I than in group II: 25.6% vs. 14.7%, respectively. In group I, 35 (52.4%) women had a history of pregnancy; in group II such women made 45.5% (4 individuals). Infertility was diagnosed in 8 (65.7%) relapsing and 32 (25.9%) first-time patients. The percentage of women with somatic pathology in both groups was almost equal: 35.5% and 34.9% respectively.

Apart from general and pelvic examinations, the patients underwent a 2D Doppler ultrasound scan on VOLUSON-730 Expert (GEKretz, Zipf, Austria). The procedure was performed using the standard technique and an endovaginal probe (3.5–10.0 MHz). All patients underwent a laparoscopic surgery (equipment by Karl Storz, Germany). The excised tissue was subjected to the histological analysis. Expression of steroid receptors was also analyzed.

The endometriotic tissue samples were tested for the expression of genes coding for membrane (mER) and nuclear (ERα and ERβ) estrogen receptors and membrane (mPR and PGRMC-1) and nuclear (PR-A and PR-B) progesterone receptors. Briefly, mRNA was extracted from the tissue samples using the RIBO-prep kit (AmpliSens, Russia) according to the manufacturer’s protocol. cDNA was synthesized from an mRNA template through reverse transcription using the set of reagents REVERTA-L (AmpliSens, Russia). Real-time PCR was performed in iCycler IQ5 (BioRad, Germany) using the reagent kit 2.5x for RT-PCR with SYBR Green I detection. Genes coding for GAPDH (glyceraldehyde-3-phosphate-dehydrogenase) were used as control (see the Table).

Gene expression was measured using the values of 0.2ΔΔCt (to assess the differences between the groups) and 2−ΔΔCt (to calculate relative quantities), where ΔΔCt = Ct (target gene) — Ct (GAPDH) and ΔΔCt = ΔCt (first-time patients) — ΔCt (relapsing patients).

All data were processed in GraphPadPrism 5.0. Normality of distribution was tested using the Kolmogorov-Smirnov test. Independent variables were compared using the nonparametric Mann-Whitney U test. Differences were considered significant at p ≤ 0.05.

RESULTS

Estrogen and progesterone are major regulators of proliferation of the endometrium or tissues related to it in the reproductive female tract. Each steroid hormone regulates expression of hundreds of genes at different stages of the menstrual cycle [5]. Relative mRNA quantities for estrogen (mER, ERα, ERβ) and progesterone (PGRMC1, mPR, PR-A, PR-B) receptors expressed in the endometriotic ovarian tissue obtained from first-time and relapsing patients are shown in Fig. 1.

The comparative analysis demonstrates that in relapsing patients estrogen expression levels in the endometriotic tissue are different from those in first-time patients: a 3-fold increase is observed for mER (p = 0.0016), a 5-fold increase — for ERβ (p = 0.0024), and a 7-fold drop — for ERα (p = 0.0001).

Table. The sequence of synthetic oligonucleotides used to study expression of steroid receptors and GAPDH by RT-PCR [http://www.ncbi.nlm.nih.gov/protein].
DISCUSSION

Estrogen and progesterone effects are largely mediated by the nuclear receptors of these hormones (ER and PR). Estrogen and progesterone interact with their receptors and activate them. Unlike ER and PR, membrane receptors of steroid hormones are understudied but still known to have an auxiliary role in modulating the expression of nuclear receptors [6].

Estradiol is a key regulator of endometrial tissue growth and survival; it also has a crucial role in inflammation and endometrial pain. Estradiol exerts its steroid growth-promoting effect on the endometrium when produced locally or transported to the endometrial tissue with blood. ER subtypes (ERα and ERβ) are proteins with high affinity to estradiol and are coded for by separate genes. Although ERα and ERβ are both present in the endometrium, ERα seems to be the major mediator of estrogen effect [7]. Elevated levels of ERα are associated with the activation of target tissue proliferation, whereas ERβ limits transcriptional activity of ERα, exerting an antiproliferative effect [8, 9].

Endometriotic tissue differs from the eutopic endometrium in terms of expression of steroid receptors. The literature reports (though scarcely) higher levels of ERα, lower levels of ERα, and very low levels of both PR isoforms, in particular PR-B, in the endometriotic tissue in comparison with the eutopic endometrium [10, 11]. In this study we employed RT-PC to quantify mRNA of nuclear receptors in primary endometrial and endometriotic stromal cells. For ERα, mRNA levels were 7 times lower in the endometriotic stroma than in the endometrial stromal cells. For ERα, mRNA levels were dramatically higher (~34 times) in the endometriotic stromal cells in comparison with the normal endometrial stroma where ERα demonstrated a very low expression or the absence of it. For PR and PR-B, total mRNA levels were significantly lower in the endometriotic stroma than in the endometrial stromal cells [12]. Recently, there have been reports of elevated PR-A in patients with endometriosis regardless of the menstrual cycle phase [13]. It is known that ERα activation leads to the transcription of a number of genes in the stromal cells of endometriotic lesions, such as the SGK1 kinase gene which maintains viability of stromal cells by inhibiting proapoptotic factors and through phosphorylation and inactivation of FOXO3a [14].

It our previous study we have demonstrated that endometriotic lesions are characterized by the increased expression of nuclear ERα, in comparison with healthy ovarian tissue. Those findings are consistent with the literature [14]. Elevated ERα levels inhibit ERα expression in the endometriotic lesions, and the increased ERα to ERα ratio observed in

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**Fig 1.** Expression of sex steroid receptors in the endometriotic tissue of patients with first-time diagnosed and recurrent endometriomas

**Note:** p — significance of differences (Mann-Whitney U); [mRNA] — expression of the target gene relative to GAPDH (0.5^ΔΔCt×100)
endometriotic stromal cells is proportionate to the degree of inhibition of progesterone receptor expression and the increase in cyclooxygenase-2 mRNA levels.

In the present study, we do not discriminate between different histological subtypes of the endometriotic tissue in relapsing patients. These conditions can lead to local inflammation and resistance to progestins [15]. We have not found any changes in the expression of genes coding for membrane and nuclear progesterone receptors that could accompany changes in the expression profiles of estrogen receptors.

Our study demonstrates that ERα mRNA levels (perhaps owing to the abnormal hypomethylation of its promoter) are significantly higher in the endometriotic tissue in relapsing than in first-time patients. At the same time, ERβ mRNA concentrations are lower in relapsing patients. No reliable reduction in the expression of progesterone receptor genes has been observed in our study accompanying the changing estrogen receptor profiles, therefore, sensitivity of the endometriotic tissue to exogenous progestins is retained in relapsing patients.

Considering the above, changes in the expression profiles of estrogen receptors in patients with recurrent endometriosis can be reflective of the aggravating estrogen-dependent processes in the endometrium. It is known that the endometriotic stromal cell contains a full pack of genes involved in a steroidogenic cascade sufficient for converting cholesterol to estradiol [16]. Besides, the endometriotic tissue is characterized by the aberrant expression of aromatase, which stimulates local synthesis of estrogens, some cytokines and metalloproteinasises. Moreover, deficiency of type 2 17β-hydroxysteroid dehydrogenases converting 17β-estradiol to a not so active estrone contributes to the accumulation of active estrogens in the tissue [17].

In our previous work we have demonstrated the differences in the expression of steroid receptors between the healthy ovarian tissue and the endometriotic tissue. In endometriosis, ERα expression (2.3 ± 0.6) was 2.4 times higher (p = 0.022) than the healthy tissue (0.8 ± 0.3); PR-A expression in the endometriotic tissue (5.5 ± 2.0) was 9.7 times higher (p = 0.008) than in the healthy tissue (1.3 ± 1.3); PR-B expression in the endometriotic tissue (0.7 ± 0.2) was 3.5 times higher (p = 0.005) than in the controls (0.08 ± 0.03) [18].

We conclude that in patients with recurrent endometriosis the expression of progesterone receptors remains unchanged against the background of changing estrogen expression profiles, providing a theoretical support for a progestin-based prevention therapy for patients with endometriosis in the post-operative period.

CONCLUSIONS

Our findings demonstrate that in relapsing patients estrogen expression levels in the endometriotic tissue are different from those in patients with first-time diagnosis: a 3-fold increase is observed for (p = 0.0016), a 5-fold increase — for ERα (p = 0.0024), and a 7-fold drop — for ERβ (p = 0.0001). In other words, the receptor status of the endometriotic tissue is different between first-time and relapsing patients in terms of mRNA levels of estrogen receptors. This confirms the involvement of estrogen receptors in promoting proliferation of hormone-dependent tissues of the female reproductive tract. The absence of differences in the expression of progesterone receptors indicates retained sensitivity of the tissue to progestins in recurrent endometriosis.

References

Литература

2. Баскаков В. П. Клиника и лечение эндометриоза. М.: Медицина; 1990. 238 с.
6. Карева Е. Н., Шимановский Н. Л. Молекулярные механизмы действия гестагенов. Экспериментальная и клиническая фармакология. 2011; 74 (4): 36–42.