EPIGENETIC PROFILE OF ENDOMETRIAL PROLIFERATION IN THE DIFFERENT MORPHOTYPES OF ENDOMETRIAL HYPERPLASIA

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INTRODUCTION

The problem of combating endometrial cancer remains in the center of attention of gynecologists and oncologists because of the high incidence and mortality of this malignant neoplasm. Carcinogenesis is a large-scale process during which epigenetic changes take place leading to the transformation of normal endometrial cells through a series of intermittent pre-tumor cells into endometrial cancer [15, 16, 20]. Endometrial hyperplasia can be a cause of uterine bleeding and make worse women's life quality [5]. According to the data of the WHO, most cases of endometrial cancer can be prevented by early detection of pre-tumor pathology of the endometrium, identification of its carcinogenic risk and rational treatment [3, 9, 10, 12, 18, 30].

Nowadays, current genetic and molecular technologies allow us to obtain valuable information about the so-called epigenetic character of the endometrium in different morphotypes of hyperplasia, sometimes individual for each patient, come closer to understanding the biology of tumor growth and its predictors, identify biomarkers that contribute to early detection of carcinogenesis on the body of hyperplastic processes, to predict the progression of the disease and optimize approaches to its treatment and prevention based on targeted impact on specific epigenetic targets [1, 8, 11, 22].

Research aim was to determine the proliferative activity of the endometrium in different morphotypes of its hyperplasia based on the study of the main molecular markers of the cell cycle.

MATERIALS AND METHODS

The key molecular antigens of the cell cycle were examined by sampling 137 morphological specimens of endometrium, including 61 with endometrial hyperplasia without atypia (HE), 36 with atypical endometrial hyperplasia (AHE), as well as 40 samples with normal morphology in accordance with phase I or II of the menstrual cycle. The material was obtained from 137 premenopausal women during a diagnostic endometrial biopsy for abnormal uterine bleeding or increased endometrial thickness above the normative indexes during ultrasound examination.

It is worth mentioning that out of 97 women with endometrial hyperplasia, the disease was isolated in 83 (85.6%) cases, and in 14 (14.4%) cases it was comorbid by uterine leiomyoma. Nineteen (19.5%) women had previously been taking various types of combined oral contraceptives for contraception and had stopped at least 2 years before being included in the study.

The age of the women in the study sample was 41 to 50 years old, and their average value did not vary significantly between the groups of healthy patients and those suffering from HE or AHE.

Endometrial biopsy samples were examined at the pathomorphological and immunohistochemistry laboratory of the Diagnostic Center of the Dnipropetrovsk Medical Academy of the Ministry of Health of Ukraine certified according to the ISO 9001–2000 standard. Immunohistochemical (IHC) examination of nuclear and cytosolic antigens was performed in paraffinic sections of biologic specimens of endometrium using monoclonal or polyclonal antibodies produced by DakoCytomation (Denmark), Termo Scientific (USA) and systems of visualization UltraVision LP (Lab Vision) and LSAB2, EnVision (Dako).

The state of proliferation in the studied cells was assessed by the expression of cyclin D1 gene and nuclear antigen Ki-67. Cyclin D1 induces the cell to leave the stationary G0 phase of the cell cycle and enter its synthetic S phase (DNA replication), i.e. the phase of preparation for mitosis.

Nuclear antigen Ki-67 belongs to the regulatory proteins of the G1-M phase of the cell cycle. The proliferation index based on the cyclin D1 and Ki-67 expression was calculated as the ratio of intranuclear reaction to the total number of cells in the endometrial samples, regardless of the intensity of reaction [21]. The cytomembrane glycoproteins E-cadherin and β-catenin were tested as additional markers of proliferation and differentiation of endometrial cells. Positive immunohistochemical reaction using monoclonal antibodies to these antigens allows determining the number of cells in the state of preparation for mitosis in tissues, that is, their proliferative efficiency, as well as the state of completed differentiation.

The activity of hormonal ways of proliferation was determined by the distribution of estradiol receptors (ER) and progesterone receptors (PGR). Therefore, ER and PGR are exclusively nuclear antigens, only nuclear reactions (at least 10 in sight

at ×400 magnification) were taken into account in the evaluation of immunobarburation according to the following criteria: absence of expression, weak expression, moderate and intensive expression. Taking into account that cells with positive reaction to ER and PGR have significant differences in the intensity of IHC coloring, we used the average value or H-index, which was calculated according to the formula: H = (% of cells with weak reaction x 1) + (% of cells with moderate reaction x 2) + (% of cells with intense reaction x 3).

Values from 0 to 50 on the H-index were considered for the absence of ER and PGR expression, from 50 to 100 – expression of antigens to receptors was considered weakly positive; 100 points or more – positive [21, 22].

Statistical evaluation of the results was performed by using the licensed statistical software Statistica version 6.1 (StatSoft Inc., USA) [4].

RESEARCH RESULTS

It is known that cell proliferation in reproductive organs is mainly related to the influence of sex hormones on the genome, mediated through interaction with nuclear ER and PGR, where the state of their expression determines the formation of a signal response in the form of activation of the cell cycle [16, 22, 29, 28, 31].

The results of the examination of ER and PGR expression are demonstrated in Fig. 1, which shows that the index of ER expression behind the H-index in the epithelium of the glands did not vary in the normal endometrium in the I (proliferative) phase of the cycle (180 ± 8,3 and 193.1 ± 12.2 respectively), but 1.43 times exceeded this indication of the second (secretory) phase of the cycle (180 ± 8.3 and 125.4 ± 5.7 respectively, p <0,05). In stromal cells of the endometrium a similar pattern of ER expression was observed, where the H-index in the HE samples was practically identical in the endometrium of the proliferative phase of the cycle (170.5 ± 4.1 vs. 166 ± 9.7 respectively), but 1.39 times higher than the H-index in stromal cells of normal endometrium in the secretory phase (respectively, 170.5 ± 4.1 and 122.4 ± 4.8; p <0,05).

The H-index PGR value in the glandular cells of the HE samples (201.7 ± 11.5) did not vary significantly in the normal endometrium of healthy women in the first phase of the cycle (193.2 ± 12.2) and slightly exceeded the value of H-index PGR in the glandular cells of the endometrium of the second phase of the cycle (178.7 ± 6.3). It is noteworthy that the PGR H-index in the stromal cells of the HE samples was 1.4 times greater than in the normal endometrium in the first phase of the cycle (respectively, 197.5 ± 9.3 and 140.2 ± 4.4; p <0.05), and 1.69 times more than in the stromal cells of the normal endometrium in the second phase of the cycle (197.5 ± 9.3 and 116.6 ± 3.1 respectively; p <0.05). Thus, with HE ER expression, both in the glandular and stromal cells, was at the level of receptor activity in normal endometrium during the proliferative phase of the cycle, but significantly exceeded these indicators in normal endometrium of the second phase of the menstrual cycle. The same direction of PGR expression was observed in the glandular cells of HE, but H-index PGR was significantly higher in the stromal cells of HE in comparison to normal endometrium in both phases of menstrual cycle.

Expression of ER and PGR decreased significantly in women with AHE (Fig. 1, 2). Thus, in the glandular epithelium, the H-index ER was lower in 2.6 times of this index in the normal endometrium of the first phase (respectively, 74.6 ± 3.9 and 193.1 ± 12.2; p <0,036) and 1.68 times to the second phase of the menstrual cycle of healthy women (respectively 74.6 ± 3.9 and 125.4 ± 5.7; p <0.05) and 2.4 times lower than that of HE (respectively 74.6 ± 3.9 and 180 ± 8.3; p <0.04). The reduction of ER expression was even more significant in stroma cells, where H-index value was 5.5 times lower in comparison with the analogous index in the normal endometrium of the first phase of the menstrual cycle (respectively 30.3 ± 2.8 and 166 ± 9.7); p <0.002) and 4 times fewer than in the normal endometrium of the II phase of the cycle (respectively 30.3 ± 2.8 and 122.4 ± 4.8; p <0.05), and 5.6 times fewer than in the HE (respectively 30.3 ± 2.8 and 170.5 ± 4.1; p <0.002).

PGR H-index was also reduced both in the glandular epithelium and in stromal cells. The H-index of PGR in the glandular epithelium was 2.7 and 2.5 times lower than in the samples of healthy women in the first phase (respectively, 71.1 ± 2.3 and 193.2 ± 12.2); p <0.04) and the second phase (respectively 71.1 ± 2.3 and 178.7 ± 6.3; p <0.045) of the menstrual cycle. In the stromal cells H-index PGR was 1.7 and 1.4 times lower than this index in the normal endometrium in the first phase (respectively 80.6 ± 1.8 and 140.2 ± 4.4; p <0.05) and the second phase (80.6 ± 1.8 and 116.6 ± 3.1; p <0.05) of the cycle. However, even greater differences were found between the PGR H-index in AHE and HE compared to normal endometrium (Fig. 3).

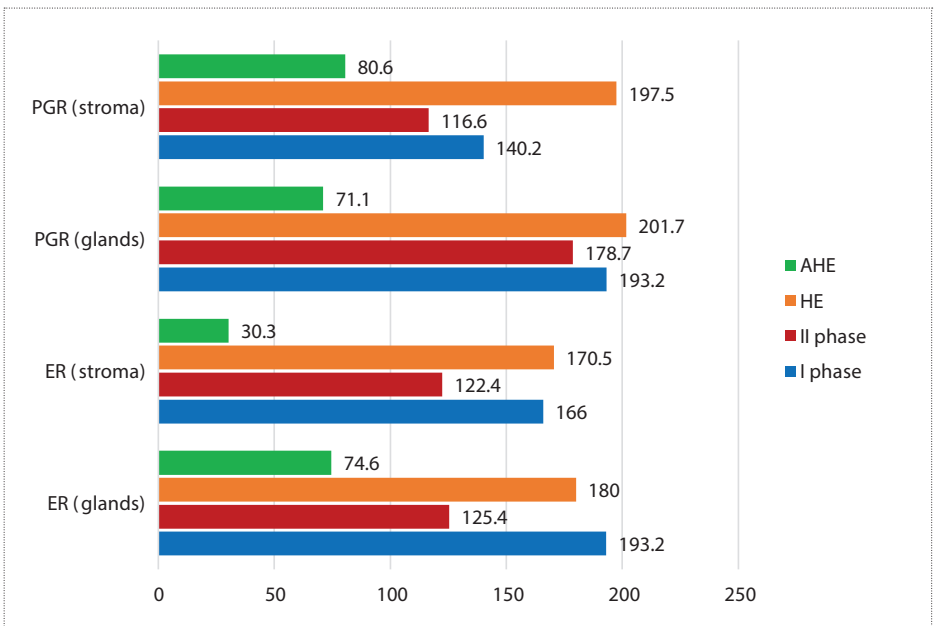

Figure 1. ER and PGR expression in the cells of normal endometrium (I and II phases of the menstrual cycle), non-atypical and atypical endometrium hyperplasia, H-index

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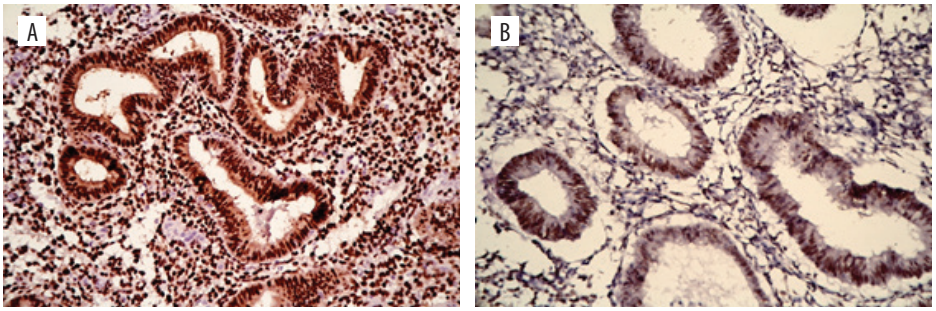

Figure 2 A, B. ER-expression in the nuclears of glandular and stromal epithelium:
A – non-atypical endometrial hyperplasia (ER+++);
B – atypical endometrial hyperplasia (ER+).
System of visualization DAKO EnVision. Magnification x400.

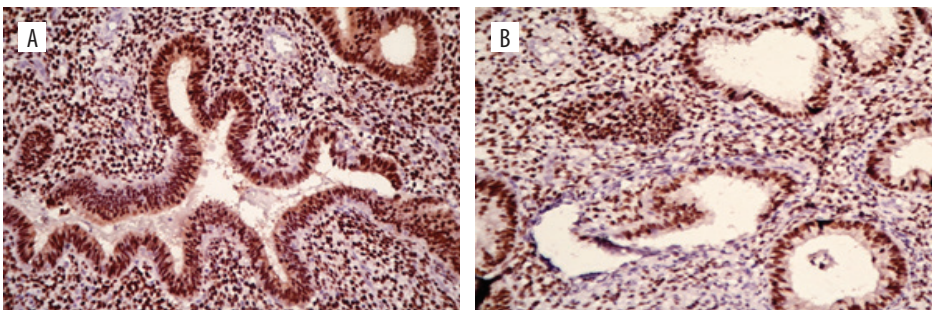

Figure 3 A, B. PGR-expression in the nuclears of glandular and stromal epithelium:
A – non-atypical endometrial hyperplasia (PGR+++);
B – atypical endometrial hyperplasia (PGR++).
System of visualization DAKO EnVision. Magnification x400.

Thus, the H-index of PGR in AHE glands was 2.8 times lower than in HE (71.1 ± 2.3 and 201.7 ± 11.5; p <0.05), and 2.4 times lower in stroma cells (80.6 ± 1.8 and 197.5 ± 9.3; p <0.05). Thus, according to the obtained results of ER and PGR expression in epithelium of the glands and stroma cells in AHE (which is considered to be a pre-cancerous state), there is a significant decrease in receptivity of the cells. As a consequence of this we can expect the reduction of both promoter and antiproliferative influence of endogenous and exogenous steroid hormones on cell cycles in this morphotype of hyperplasia [13, 28, 32].

Recently, it has become known that the interference of the natural estrogen-ER complex with cell proliferation is caused by the initiation of the cyclin D1 gene expression [17, 25, 26], which induces the cell to leave the stationary G0 phase of the cell cycle and switch to the synthetic S phase, i.e. the phase of preparation for mitosis. The most widely recognizable marker of proliferation is the Ki-67 protein, which is present in all active stages of the cell cycle (G1, S, G2, M) and is absent in the G0 phase. According to our data, in HE the distribution of cells with a positive IHC reaction to cyclin D1 was more or less the same as in the normal endometrium of healthy women. In the stromal cells of HE morphological specimens cyclin D1 expression was found only in single cells, while in the glandular epithelium a positive IHC reaction with cyclin D1 was found in 30 (49.2%) among the 61 samples studied, including 25 (40.9%) which showed that 10% to 15% of the cells were positive for cyclin D1, and 5 (8.2%) samples showed up to 25% of the cells positive. At the AHE we have seen a different situation. In stromal cells of the endometrium a positive IHC reaction to cyclin D1 antigen was also detected in the majority of cases only in a single cell (83.3%). The glandular epithelium showed positive IHC-reaction in 31 of 36 (86.1%) morphological specimens of the endometrium, including positive from 40 to 60% of the cells in 19 (52.7%) preparations, and positive from 20 to 25% of the cells in the remaining 12 (33.3%) specimens.

It should be noted that on the background of the above-mentioned activation of cyclin D1 expression, which corresponds to the initiative of proliferation, the number of glandular epithelium cells with Ki-67 expression, according to our data, decreased significantly in HE and AHE compared to normal endometrium and was the smallest in AHE (Fig. 4).

Figure 4 shows that in the examined AHE samples the index of Ki-67 in the epithelium of the glands was 2.9 times lower than the average value of the analogous index in the normal endometrium in the first phase of the cycle (respectively, 19.8 ± 1.2% and 57.8 ± 3.1%; p <0.05) and in the second phase of the cycle – 2.1 times (respectively, 19.8 ± 1.2% and 41.6 ± 1.7%; p <0.05). The average Ki-67 values HE was also statistically lower than those of the normal endometrium in 2.6 times (respectively 22.6 ± 1.2% and 57.8 ± 3.1%; p <0.05) in the first phase of the cycle, and 1.8 times (respectively, 22.6 ± 1.2% and 41.6 ± 1.7%; p <0.05) in the second phase of the menstrual cycle. At the same time, this tendency was less noticeable in stromal cells of the endometrium and the frequency of detection of positive affection of cells for Ki-67 antigen did not differ significantly not only between AHEs (8.0 ± 0.7%) and HE (8.9 ± 0.5%), but also between these two morphotypes and normal endometrium (Fig. 4).

E-cadherin plays a major role in maintaining normal adhesion in epithelial cells restricts their excessive proliferation and

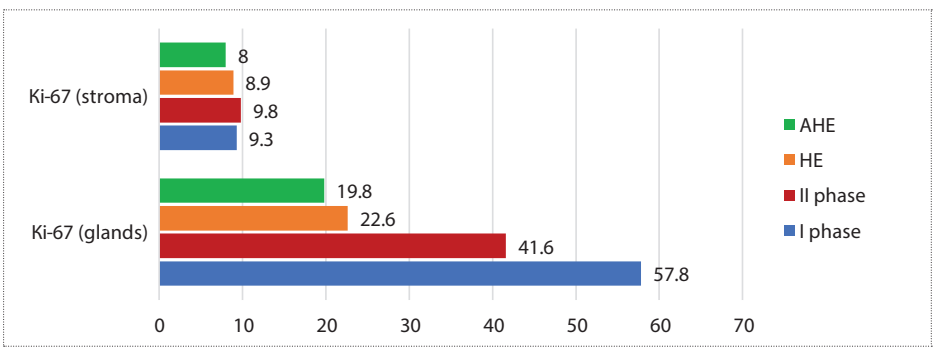

Figure 4. Indicators of Ki-67 protein expression in cells of normal endometrium (I and II phases of the menstrual cycle), nonatypical and atypical endometrium hyperplasia, % of positive cells

inhibits the processes of tumor of the endometrium by contact inhibition, due to the fact that the mechanisms of proliferation are excluded at the brush contact of the cells. It has also been shown that E-cadherin is a signal glycoprotein for the normal differentiation of the cells [7, 16]. An analysis of the results of IHC reaction with E-cadherin in the glandular epithelium of the endometrium in women with HE showed that 10 (16.4%) morphological patterns were negative for this antigen. In other 51 (83.6%) cases we obtained positive IHC-staining of the cells, while in 30 (49.2%) cases it was weak and in 21 (34.4%) was moderate. In the group of women with AHE, the absence of E-cadherin expression was observed in 24 (66.7%) of 36 tested samples of the endometrium, i.e., 2.4 times more frequently than in HE. In the other 12 (33.3%) morphological preparations, the IHC response to this antigen was weak (Fig. 5).

Figure 5 shows that epithelial cells in all samples of normal endometrium are positive for glycoprotein E-cadherin expression in two phases of the menstrual cycle. If we consider that E-cadherin expression is mainly assigned to mature epithelial cells of the endometrium, which have completed the stage of differentiation and have lost their ability to proliferate, the results obtained for the negative status of a significant number of E-cadherin signatures in AHE are the most likely to indicate the accumulation of cells with incomplete morphogenesis that pose a risk of carcinogenic transformation of endometrial cells. Therefore, the IHC marker of negative E-cadherin expression can be an additional indicator of atypical potential of the endometrium not only in AHE but also in doubtful or insufficiently identified endometrial morphotypes. E-cadherin expression in the normal endometrium and different morphotype of its hyperplasia shows at the Fig. 6.

It is known that the protein β-catenin can function as a transcription factor, thus acting as a regulator of the cell cycle. One of the main targets of ß-catenin is the cyclin D1 gene, which results in activation of cyclin-dependent kinases. In turn, cyclin-dependent kinases are responsible for the transition of the cell cycle from the presynthetic phase (G1) to the DNA replication phase (S), i.e. to the cell proliferation phase [19]. In morphological samples of normal endometrium, we found a negative IHC reaction to β-catenin in 32.5% of cases in phase I and 35% of cases in phase II of the menstrual cycle. Accordingly, it was weak in 50% and 57.5% of the studied samples, as well as more pronounced in the proliferative endometrium (17.5% of cases) than in the secretory one (7.5% of cases) (Fig. 7).

Interestingly, all 36 morphological samples of AHEs were positive for β-catenin, and in 12 (33.3%) cases it was high and significant, and in other 24 (66.7%) cases it was weak. A strong and significant IHC reaction to β-catenin was also detected in 40 (65.6%) of the 61 HE samples, weak reaction in 11 (18%) cases, and negative reaction in 10 (16.4%). Thus, a high and significant excess of β-catenin was observed in a significant number of

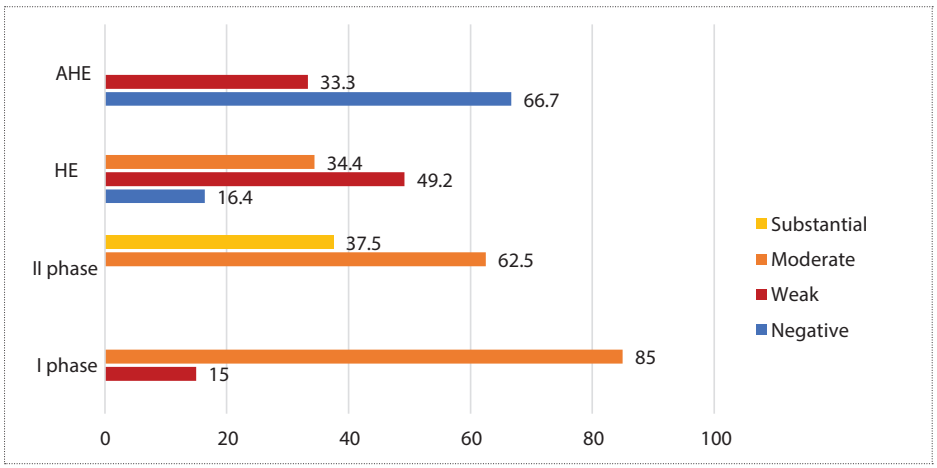

Figure 5. Indicators of E-cadherin expression in the cells of normal endometrium (I and II phases of the menstrual cycle), nonatypical and atypical endometrial hyperplasia, % of cells

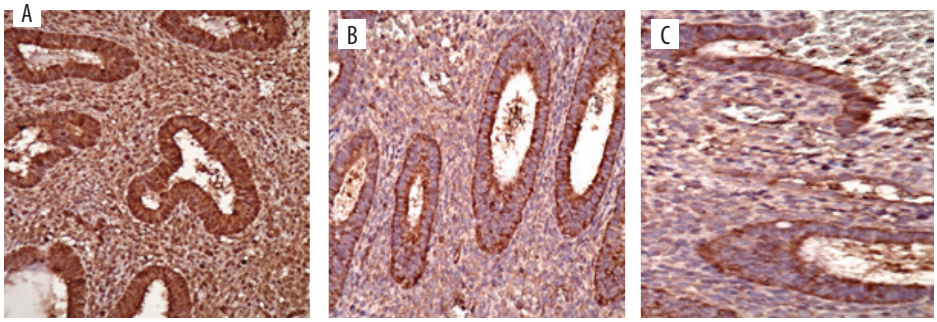

Figure 6 A–C. E-cadherin expression in the glandular epithelium:
A – normal secretory endometrium (sample of strong expression);
B – non-atypical endometrial hyperplasia (sample of moderate expression);
C – atypical endometrial hyperplasia (sample of week expression).
System of visualization DAKO EnVision. Magnification x400.

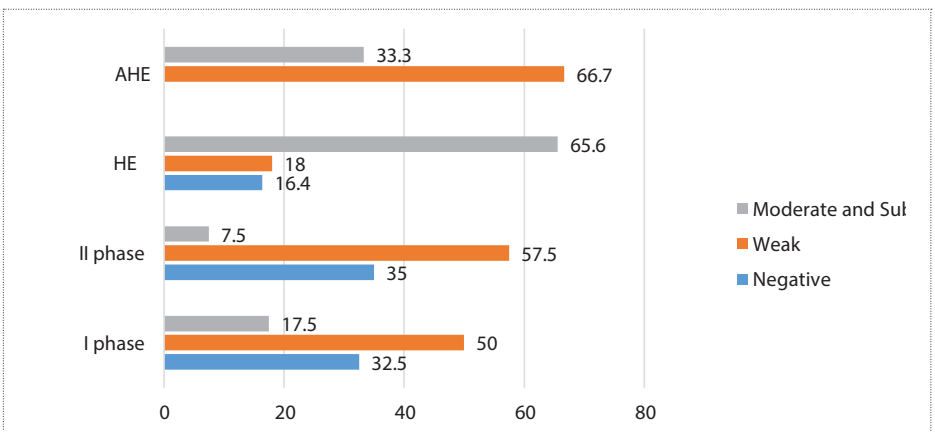

Figure 7. Indicators of β-catenin expression in eutopic endometrial cells (I and II phases of the menstrual cycle), non-atypical and atypical endometrial hyperplasia, % of cells

women with AHE and HE, with 1.96 times more frequent in HE than in AHE. However, the negative IHC reaction for β-catenin was mostly observed in the normal endometrium.

DISCUSSION

The process of proliferation and differentiation of endometrial cells is regulated by sex-steroid hormones in a complex with the corresponding nuclear receptors in the regulation zone of the estrogen-dependent genes, as well as by low proteins that form the central system of control over the passage of the cell cycle. Cell transition to different stages of the cycle is regulated by a family of cyclin-dependent kinases in a complex with corresponding cyclins, one of which cyclin D1 is responsible for the initiation of mitosis and procreation of the cell cycle from phase G0 (the phase of cellular calmness) and phase G1 (the presynthetic phase) to S-phase (the phase of DNA replication and segregation). Subsequent steps of the cell cycle are closely related to additional factors of cellular function, which include the most studied groups of proteins known as Ki-67, E-cadherin, β-catenin, etc. [2, 19, 26]. Therefore, according to the results of Ki-67 expression obtained in this study, the increase in the number of endometrial cells in HE does not occur due to the increased activity of mitosis, but due to the accumulation of cellular material in time, even if the proliferation of cells is significantly decreased. This is probably the scenario for irregular menstrual cycles with a 2–3-month delay in menstruation, which can often be observed in clinical practice in premenopausal women. At the same time, a significant increase in cyclin D1 expression that is not accompanied by adequate expression of Ki-67, E-cadherin and β-catenin, and, accordingly, by complete repositioning and segregation of DNA, can indicate the appearance of a significant number of cells with an unstable genome, which in our opinion can result in the appearance of endometrial cells with an atypical phenotype, aberrant growth or malignant transformation. This way of cells aberration could lead endometrium to malignancy [10, 14, 23, 24]. Moreover, the differences in the values of the key IHC markers of proliferation of endometrial cells in women with HE and AHE indicate that these morphotypes are different diseases with different epigenetic profile that should be taken into account when developing an individualized strategy of their treatment. Specifically, IHC markers of proliferation in endometrial cells did not vary significantly in women who had isolated morphotypes of endometrial hyperplasia or in those with uterine leiomyoma. Women's using combined oral contraceptives for contraception, which had been discontinued 2–3 years before the study, also had no effect on the results.

CONCLUSION

Information about epigenetic profiles of hyperplastic processes in endometrium can be used not only to assess the risks of progression of carcinogenesis in endometrial cells at early stages of its development, detection of individuals with increased risk of malignant process development, as well as for the selection of optimal influence on the individual pathological process in endometrium, evaluation of the therapy success, and in some cases, for the review of the therapeutic strategy.

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ЕПІГЕНЕТИЧНИЙ ПРОФІЛЬ ПРОЛІФЕРАЦІЇ ЕНДОМЕТРІЯ ПРИ РІЗНИХ МОРФОТИПАХ ГІПЕРПЛАЗІЇ

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Мета дослідження: визначення проліферативної активності нормального та гіперплазованого ендометрія на підставі ключових молекулярних маркерів клітинного циклу.
Матеріали та методи. Обстежено 137 жінок: 40 – з нормальним станом ендометрія (НЕ), 61 – з неатиповою гіперплазією ендометрія (НГЕ), 36 – з атиповою (АГЕ). Досліджували експресію гена циклін D1, антигена Ki-67, глікопротеїнів E-кадгерину та β-катеніну, рецепторів до естрадіолу (ER) та прогестерону (PGR).
Результати. Клітини залоз і строми НЕ мали високу експресію ER у проліферативну фазу циклу, яка значно знижувалась у секреторній фазі. Експресія PGR була високою в обидві фази циклу. При НГЕ експресія ER як в епітелії залоз ($180 \pm 8,3$), так і в стромі ($170,5 \pm 4,1$) перевищувала показники секреторної фази. Експресія PGR в клітинах строми НГЕ ($197,5 \pm 9,3$ балів) достовірно перевищувала показники НЕ. Навпаки, при АГЕ експресія ER і PGR значно зменшувалася. В залозах Н-індекс ER ($74,6 \pm 3,9$) був в 2,4–2,6 разу нижчим, ніж у проліферативному ендометрії та при НГЕ ($p < 0,05$); в стромі АГЕ знизився до $30,3 \pm 2,8$, що в 5,5–5,6 разів нижче, ніж в НЕ проліферативної фази та при НГЕ ($p < 0,002$). В залозах АГЕ експресія PGR була в 2,5–2,7 разу меншою ($71,1 \pm 2,3$), ніж в НЕ, та в 2,8 разу меншою, ніж при НГЕ ($p < 0,05$). Експресія гена циклін D1 достовірно збільшувалась при АГЕ порівняно з НЕ та НГЕ. Експресія білка Ki-67 в залозах при НГЕ була нижчою в 2,6 разу, при АГЕ – у 2,9 разу порівняно з НЕ фази проліферації ($p < 0,05$). Експресія E-кадгерину була найнижчою при АГЕ. Найбільший відсоток позитивних за β-катеніном клітин був при АГЕ (100%), негативних – при НЕ (32,5–35%).
Висновок. Дані про епігенетичний профіль ендометрія в нормі та при гіперплазії можуть бути застосовані для розробки оцінки ризиків малігнізації, виявлення осіб із підвищеним ризиком канцерогенезу, вибору оптимального впливу на патологічний процес в ендометрії.

Ключові слова: епігенетичний профіль, неатипова гіперплазія ендометрія, атипова гіперплазія ендометрія, експресія рецепторів до естрадіолу та прогестерону, циклін D1, ядерний антиген Ki-67, E-кадгерин, β-катенін.

EPIGENETIC PROFILE OF ENDOMETRIAL PROLIFERATION IN THE DIFFERENT MORPHOTYPES OF ENDOMETRIAL HYPERPLASIA

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Research aim: to investigate the proliferative status of endometrium in the different morphotypes of endometrial hyperplasia based upon the identification of key molecular markers of the cell cycle.
Materials and methods. Endometrial samples taken from 137 women were investigated: 40 – normal endometrium (NE), 61 – non-atypical endometrial hyperplasia (EH), 36 – atypical hyperplasia (AHE). Expression of gene cyclin D1, nuclear antigen Ki-67, glycoproteins E-cadherin and β-catenin, estradiol receptors (ER) and progesterone receptors (PGR) were investigated.
Results. ER expression of NE was high in the proliferative phase and decreased significantly in the secretory phase. PGR expression was high in both phases. ER expression of EH in glandular (180 ± 8.3) and in stromal cells (170.5 ± 4.1) exceed the indicators of the secretory phase. PGR expression in the stromal cells of EH (197.5 ± 9.3) exceed significantly indicators of NE. ER and PGR expression significantly and reliably decreased if there was AHE. ER expression of glandular cells was 2.6 times lower (74.6 ± 3.9) compere to proliferative NE ($p < 0.05$) and 2.4 times lower to EH ($p < 0.05$). ER of stromal AHE cells dropped to 30.3 ± 2.8, which was 5.5–5.6 times lower than in the proliferative NE and EH ($p < 0.002$). PGR expression was 2.5–2.7 times lower (71.1 ± 2.3) in AHE glands than in NE and 2.8 times lower than in EH ($p < 0.05$). Gene cyclin D1 expression was reliably increased in AHE cells compere to NE and EH. Protein Ki-67 expression in the glandular cells of EH was 2.6 times lower ($p < 0.05$) and in AHE 2.9 times lower ($p < 0.05$) than NE proliferative phase. We discovered strong direction to decreasing E-cadherin expression in EH and it was lowest for AHE. Opposite direction was expression of β-catenin. The highest numbers of positive samples were observed in AHE and it was 100%. The highest numbers of negative β-catenin samples were in the NE cells (32,5–35%).
Conclusion. The epigenetic profile investigation of endometrial hyperplasia will be useful for future development of carcinogenesis risk stratification, identifying patients with high risk of endometrial cancer and also for choosing the optimal way to influence the pathological process in the endometrium.

Keywords: epigenetic profiles, endometrial hyperplasia without atypia, endometrial hyperplasia with atypia, estradiol receptor expression, progesterone receptor expression, cyclin D1, nuclear antigen Ki-67, E-cadherin, β-catenin.

ЭПИГЕНЕТИЧЕСКИЙ ПРОФИЛЬ ПРОЛИФЕРАЦИИ ЭНДОМЕТРИЯ ПРИ РАЗЛИЧНЫХ МОРФОТИПАХ ГИПЕРПЛАЗИИ

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Цель исследования: определение пролиферативной активности нормального и гиперплазированного эндометрия на основе изучения ключевых молекулярных маркеров клеточного цикла.
Материалы и методы. Обследовано 137 женщин: 40 – с нормальным состоянием эндометрия (НЭ), 61 – с неатипичной (НГЭ), 36 – с атипичной гиперплазией эндометрия (АГЭ). Исследовали экспрессию гена циклин D1, антигена Ki-67, E-кадгерина и β-катенина, рецепторов к эстрадиолу (ER) и прогестерону (PGR).
Результаты. Как железы, так и строма НЭ имели высокую экспрессию ER в пролиферативную фазу цикла, которая значительно снижалась в секреторной фазе. Экспрессия PGR была высокой в обе фазы цикла. При НГЭ экспрессия ER как в эпителии желез ($180 \pm 8,3$), так и в строме ($170,5 \pm 4,1$) превышала показатели секреторной фазы. Экспрессия PGR в клетках стромы НГЭ ($197,5 \pm 9,3$) достоверно превышала показатели НЭ. Напротив, при АГЭ экспрессия ER и PGR значительно уменьшалась. В железах Н-индекс ER был в 2,4–2,6 раза ниже ($74,6 \pm 3,9$), чем в пролиферативном эндометрии и при НГЭ ($p < 0,05$); в строме АГЕ снизился до $30,3 \pm 2,8$, что в 5,5–5,6 раза ниже, чем в НЭ пролиферативной фазы и при НГЭ ($p < 0,002$). В железах АГЭ экспрессия PGR была в 2,5–2,7 раза ниже ($71,1 \pm 2,3$), чем в НЭ, и в 2,8 раза меньше, чем при НГЭ ($p < 0,05$). Экспрессия гена циклин D1 достоверно увеличивалась в сравнении с НЭ и НГЭ. Экспрессия белка Ki-67 в железах при НГЭ была ниже в 2,6 раза, при АГЭ – в 2,9 раза по сравнению с НЭ фазы пролиферации ($p < 0,05$). Экспрессия E-кадгерина была самой низкой при АГЭ. Наибольшая часть положительных по β-катенину клеток выявлена при АГЭ (100%), отрицательных – при НЭ (32,5–35%).
Вывод. Данные об эпигенетическом профиле эндометрия в норме и при гиперплазии могут быть использованы для разработки оценки рисков малигнизации, выявления пациенток с повышенным риском канцерогенеза, выбора оптимального воздействия на патологический процесс в эндометрии.

Ключевые слова: эпигенетический профиль, неатипичная гиперплазия эндометрия, атипичная гиперплазия эндометрия, экспрессия рецепторов к эстрадиолу и прогестерону, циклин D1, ядерный антиген Ki-67, E-кадгерин, β-катенин.