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Comparative characteristics of the transcriptional activity of *CDH1*, *CTNNB1*, *VEGFA* genes and expression of proteins E-cadherin, β-catenin and VEGFA, coded by these genes in metastatic and non-metastatic endometrioid endometrial carcinoma

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Key words: *Endometrial Neoplasms, Cadherins, beta Catenin, VEGFA protein, Human, Polymerase Chain Reaction, RNA, Messenger.*

The violation of Wnt-signaling pathway and cyclic neoangiogenesis is early events in endometrial carcinogenesis. In this process an important role is played by epigenetic modifications and mutations of *CDH1, CTNNB1, VEGFA* genes, followed by expression violations of E-cadherin, β-catenin, VEGFA encoded by them.

The aim. To study the expression levels of mRNA of *CDH1, CTNNB1, VEGFA* genes and the expression levels of E-cadherin, β-catenin, VEGFA in non-metastatic endometrioid endometrial carcinoma (EEC) and in EEC with metastases to regional lymph nodes.

Materials and methods. Immunohistochemical study of invasive $pT_{1-4}N_0M_x$ endometrioid endometrial carcinoma (EEC) (n=56; age – 42–83 years), invasive $pT_{1-4}N_{1-2}M_x$ EEC (n=30; age – 48–79 years), samples of normal proliferative endometrium (PE) (n=30; age – 41–62 years) was performed. Molecular-genetic study of invasive $pT_{1-4}N_0M_x$ EEC (n=10; age – 45–67 years), invasive $pT_{1-4}N_{1-2}M_x$ EEC (n=10; age – 44–63 years), samples of PE (n=10; age – 46–59 years) was performed.

Results. EEC is characterized by the lower mRNA expression level of *CDH1* gene and by the higher mRNA expression levels of *CTNNB1, VEGFA* genes in comparison with the PE. E-cadherin expression level is 32.6 % lower in non-metastatic EEC and also is 58.10 % lower in metastatic EEC in comparison with the PE. β-catenin expression level is 32.56 % lower in non-metastatic EEC and also is 58.11 % lower in metastatic EEC in comparison with the PE. VEGFA expression level is 27.72 % higher in non-metastatic EEC and also is 17.87 % higher in metastatic EEC in comparison with the PE. There is lower β-catenin expression level in metastatic EEC in comparison with non-metastatic EEC. Non-metastatic and metastatic EEC is characterized by the direct medium connections between the mRNA expression levels of *CTNNB1* and *VEGFA* genes (γ=0.44), between the mRNA expression level of *CTNNB1* gene and VEGFA expression level in the tumor (γ=0.37).

Conclusions. These changes contribute to tumor growth, invasion and metastasis.

Порівняльна характеристика транскрипційної активності генів *CDH1, CTNNB1, VEGFA* та експресії кодованих ними білків Е-кадгерину, β-катеніну та VEGFA в метастатичній ендометріоїдній аденокарциномі тіла матки і в аденокарциномі без метастазів

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У канцерогенезі рака ендометрію ранніми подіями є порушення Wnt-сигнального шляху та циклічного ангіогенезу. Важливу роль при цьому відіграють епігенетичні модифікації генів і мутації генів *CDH1, CTNNB1, VEGFA* з дальшим порушенням експресії кодованих ними протеїнів – Е-кадгерину, β-катеніну, VEGFA.

Мета роботи – вивчити молекулярно-генетичними методами рівень експресії мРНК генів *CDH1, CTNNB1, VEGFA*, імуногістохімічним методом – рівні експресії Е-кадгерину, β-катеніну, VEGFA в ендометріоїдній аденокарциномі ендометрію без метастазів і з метастазами в регіонарні лімфовузли.

Матеріали та методи. Імуногістохімічним методом вивчена інвазивна $pT_{1-4}N_0M_xG_{1-3}$ ендометріоїдна аденокарцинома ендометрію (ЕАЕ) (n=56; вік – 42–83 роки), інвазивна $pT_{1-4}N_{1-2}M_xG_{1-3}$ ЕАЕ (n=30; вік – 48–79 років) і зразки ендометрію фази проліферації (ПЕ) (n=56; вік – 41–62 роки). Молекулярно-генетичним методом вивчена інвазивна $pT_{1-4}N_0M_xG_{1-3}$ ЕАЕ (n=10; вік – 45–67 років), інвазивна $pT_{1-4}N_{1-2}M_xG_{1-3}$ ЕАЕ (n=10; вік – 44–63 роки) та зразки ПЕ (n=10; вік – 46–59 років).

Результати. В ЕАЕ порівняно з нормальним ПЕ знижений рівень експресії мРНК гена *CDH1*, а також підвищені рівні експресії мРНК генів *CTNNB1* і *VEGFA*. Рівень експресії Е-кадгерину в неметастатичній ЕАЕ знижений на 32,36 %, а в метастатичній ЕАЕ – на 58,10 % порівняно з нормальним ПЕ. Рівень експресії β-катеніну в неметастатичній ЕАЕ знижений на 32,56 %, в метастатичній ЕАЕ – на 58,11 % порівняно з нормальним ПЕ. Рівень експресії VEGFA в неметастатичній ЕАЕ підвищений на 27,72 %, в метастатичній ЕАЕ – на 17,87 % порівняно з нормальним ПЕ. В ЕАЕ з метастазами визначається нижчий рівень експресії β-катеніну в пухлинних клітинах, порівнюючи з інвазивною неметастатичною аденокарциномою. У неметастатичній і метастатичній ЕАЕ спостерігаються прямі помірні зв'язки між рівнями експресії мРНК генів *CTNNB1* і *VEGFA* (γ=0,44), між рівнем експресії мРНК гена *CTNNB1* і рівнем експресії судинного ендотеліального фактора росту VEGFA у тканині пухлини (γ=0,37).

Висновки. Вказані зміни сприяють пухлинному росту, інвазії й метастазуванню.

Ключові слова: *карцинома ендометрію, Е-кадгерин, β-катенін, CTNNB1, VEGFA, ПЛР, мРНК.*

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Сравнительная характеристика транскрипционной активности генов *CDH1, CTNNB1, VEGFA* и экспрессии кодируемых ими белков Е-кадгерина, β-катенина и VEGFA в метастатической эндометриоидной аденокарциноме тела матки и в аденокарциноме без метастазов

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В канцерогенезе рака эндометрия ранними событиями являются нарушение Wnt-сигнального пути и циклического неоангиогенеза. Важную роль при этом играют эпигенетические модификации и мутации генов *CDH1*, *CTNNB1*, *VEGFA* с последующим нарушением экспрессии кодируемых ими протеинов – Е-кадгерина, β-катенина, VEGFA.

Цель работы – изучить молекулярно-генетическими методами уровни экспрессии мРНК генов *CDH1, CTNNB1, VEGFA,* иммуногистохимическим методом – уровни экспрессии Е-кадгерина, β-катенина, VEGFA в эндометриоидной аденокарциноме эндометрия (ЭАЭ) без метастазов и с метастазами в регионарные лимфоузлы.

Материалы и методы. Иммуногистохимическим методом изучены инвазивная $pT_{1-4}N_0M_xG_{1-3}$ эндометриоидная аденокарцинома эндометрия (ЭАЭ) (n=56; возраст – 42–83 года), инвазивная $pT_{1-4}N_{1-2}M_xG_{1-3}$ ЭАЭ (n=30; возраст – 48–79 лет) и образцы эндометрия фазы пролиферации (ПЭ) (n=30; возраст – 41–62 года). Молекулярно-генетическим методом изучены инвазивная $pT_{1-4}N_0M_x$ ЭАЭ (n=10; возраст – 45–67 лет), инвазивная $pT_{1-4}N_{1-2}M_x$ ЭАЭ (n=10; возраст – 44–63 года) и образцы ПЭ (n=10; возраст – 46–59 лет).

Результаты. В ЭАЭ в сравнении с нормальным ПЭ снижен уровень экспрессии мРНК гена *CDH1*, а также повышены уровни экспрессии мРНК генов *CTNNB1* и *VEGFA*. Уровень экспрессии Е-кадгерина в неметастатической ЭАЭ снижен на 32,36 %, в метастатической ЭАЭ – на 58,10 % в сравнении с нормальным ПЭ. Уровень экспрессии β-катенина в неметастатической ЭАЭ снижен на 32,56 %, в метастатической ЭАЭ – на 58,11 % в сравнении с нормальным ПЭ. Уровень экспрессии VEGFA в неметастатической ЭАЭ повышен на 27,72 %, в метастатической ЭАЭ – на 17,87 % в сравнении с нормальным ПЭ. В ЭАЭ с метастазами определяется более низкий уровень экспрессии β-катенина в опухолевых клетках по сравнению с инвазивной неметастатической аденокарциномой. В неметастатической и метастатической ЭАЭ прослеживаются прямые умеренные связи между уровнями экспрессии мРНК генов *CTNNB1* и *VEGFA* (γ=0,44), между уровнем экспрессии мРНК гена *CTNNB1* и уровнем экспрессии сосудистого эндотелиального фактора роста VEGFA в ткани опухоли (γ=0,37).

Выводы. Данные изменения способствуют опухолевому росту, инвазии и метастазированию ЭАЭ.

Ключевые слова: *карцинома эндометрия, Е-кадгерин, β-катенин, VEGFA, ПЦР, мРНК.*

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The features of endometrial carcinogenesis, in which the violation of Wnt-signaling pathway and the violation of cyclic neoangiogenesis are ones of the earliest events, have become the subject of many researches in recent years [1,2]. It is known that epigenetic modifications and also mutations of β-catenin and E-cadherin genes (*CTNNB1* and *CDH1* accordingly), that encode molecules which regulate cell polarity and provide reorganization of the cytoskeleton, and also encode intercellular adhesion molecules which ensure the maintenance of normal tissues histoarchitectonics, play an important role in the carcinogenesis of endometrioid endometrial carcinoma (EEC) [3–6]. *CTNNB1* gene encodes β-catenin, which is an intercellular adhesion molecule and a transcription factor of the canonical Wnt-signaling pathway [7]. Mutations of *CTNNB1, APC, AXIN1* genes lead to the abnormal stabilization of β-catenin, resulting in nuclear accumulation of this protein and subsequent uncontrolled proliferation of the tumor cells [8,9]. It is considered that endometrioid adenocarcinoma with *CTNNB1*-mutation has a relatively better prognosis [10]. *CDH1*-supressor gene encodes E-cadherin, which is also an intercellular adhesion molecule. But the loss of E-cadherin expression due to mutations of *CDH1* gene contribute to the tumor invasion and metastasis, hence it correlates with the tumor aggressiveness [10].The aberrant expression of E-cadherin and β-catenin was revealed in 51.1 and 62.2 % cases (respectively) of endometrioid adenocarcinoma with using of immunohistochemical study. The aberrant expression of these proteins has been rated as a factor of the tumor progression [11]. The process of angiogenesis, in which the interaction of vascular endothelial growth factor (VEGF) and its receptors (VEGFR) plays a key role, is equally important in the progression of the endometrial cancer. These tumors with severe VEGF expression have a worse prognosis than VEGF-negative carcinomas, accordingly, VEGF regarded as an important, clinically relevant inducer of angiogenesis in endometrial adenocarcinoma [12].

Comparative studies of mRNA expression of *CDH1, CTNNB1, VEGFA* genes and immunohistochemical (IHC) expression of the proteins encoded by them in non-invasive and invasive EEA, and also in non-metastatic EEA and EEA with regional metastases are not numerous. Moreover, the results of these studies are contradictory.

Aim – to study the expression levels of mRNA of *CDH1, CTNNB1, VEGFA* genes and the expression levels of E-cadherin, β-catenin, VEGFA, which are encoded by the genes, in non-metastatic endometrioid adenocarcinoma of uterus on the one hand and in endometrioid adenocarcinoma with metastases to regional lymph nodes on the other hand.

Materials and methods

For this purpose, pathohistological, immunohistochemical and molecular-genetic methods were used. Pathohistological and immunohistochemical studies of invasive non-metastatic ($pT_{1-4}N_0M_x$) EEA (56 patients, the age of the patients ranged from 42 to 83 years) and invasive metastatic ($pT_{1-4}N_{1-2}M_x$) EEA (30 patients, the age ranged from 48 to 79 years) were performed. The comparison group consisted of normal endometrium (proliferative phase) of 30 patients (the age ranged from 41 to 62 years), in all cases the diagnosis was estimated based on clinical and morphological data.

The pieces of the tissue, which were cut from the main

body of the EEA and also from the EEA invasion zone, were fixed in 10 % neutral buffered formalin and then were embedded in paraffin blocks. The microstructure of the cancer, the histological type and the degree of tumor differentiation were evaluated in the paraffin sections, which were stained with hematoxylin and eosin. IHC study was performed according to the standard procedures [13] using the primary monoclonal mouse antibodies against the β-Catenin (DAKO, USA) – undiluted, *Cloneβ-Catenin-1*, E-Cadherin – undiluted, *Clone NCH-38* (DAKO, USA), VEGFA (Vascular endothelial growth factor A), *Clone VG1* (DAKO, USA) – dilution 1:50, and also detection system EnVisionFLEX with diaminobenzidine (DAKO, USA). After that, sections were stained with Meyer's hematoxylin and embedded in balsam.

An immunohistochemical expression of β-catenin, E-cadherin, VEGF in the tumor cells of EEA was assessed by the method of digital morphometry [14] using the program Image J [15].

Molecular-genetic study of invasive non-metastatic ($pT_{1-4}N_0M_x$) EEC (10 patients) and invasive metastatic ($pT_{1-4}N_{1-2}M_x$) EEC (10 patients) was performed. The comparison group consisted of normal endometrium (proliferative phase) of 10 patients. Molecular-genetic study was conducted on the material, which was fixed in formalin and embedded in paraffin blocks. In order to obtain a total RNA preliminary dewaxing in xylene and rehydration in descending concentrations of ethanol (100 %, 96 %, 70 %) were performed. Prepared samples were homogenized using a mortar and pestle, then they were placed in a test tube "Axygen" (USA) and then samples were further dewaxed and rehydrated. An isolation of a total RNA was performed using a set "Trizol RNA Prep 100" (Izogen Lab., LTD, Russian Federation), which contains the *Trizol reagent* (lysing reagent, that includes denaturant guanidintiotsionat and phenol with pH=4.0) and *ExtraGene E* (ion exchangers slurry mixture). An RNA was isolated according to the kit protocol.

For reverse transcription and obtaining cDNA a set OT-1 ("Syntol", Russian Federation) was used. The reaction mixture had a total volume of 25 μl, contained μl of Random-6 primer, 2 μl of the total RNA, 8.5 μl of deionized H_2O, which was cleared from nucleases, 12.5 μl 2.5x of the reaction mixture and 1 μl of reverse transcriptase MMLV-RT. Reverse transcription was performed at 45 °C during 45 minutes, followed by heating to 92 °C during 5 minutes in order to inactivate the MMLV-RT.

To determine the expression level of mRNA of genes, which were investigated, a thermal cycler CFX96 ™ Real-Time PCR Detection Systems ("Bio-Rad Laboratories, Inc.", USA) and a reagent kit for RT-PCR in the presence of SYBR Green R-402 ("Synthol", Russian Federation) were used. The final reaction mixture consisted of SYBR Green colorant, a DNA-polymerase SynTaq with antibodies that suppress the enzyme activity, 0.2 μl of forward and 0.2 μl of reverse specific primers, dNTP-deoxynucleoside triphosphates and 1 μl of the matrix (cDNA). The reaction mixture was brought to the total volume of 25 μl by addition of deionized H_2O. Specific primer pairs (5'–3') for analysis of the test genes and the reference genes were chosen using the Primer-Blast software (www.ncbi.nlm.nih.gov/tools/primer-blast) and were manufactured by Thermo Scientific, USA (*Table 1*).

After an initial denaturation at 95 °C during 10 min, amplification consisted of 45–50 cycles and was carried out under the following conditions: denaturation – 95 °C, 15 seconds; annealing – 59–61 °C, 30–60 seconds; elongation – 72 °C, 30 seconds. As a referent gene for determining the values of changes in the expression levels of the genes, which were studied, glyceraldehyde 3-phosphate dehydrogenase gene (*GAPDH*) was used. The relative normalized amount of cDNA targeted genes was determined by the $_{\Delta\Delta}$Ct method. Statistical analysis of PCR results was performed using CFX Manager™ software (Bio-Rad, USA). The negative controls were included in the experiment: without the addition of the matrix of cDNA in the PCR reaction; without the addition of the matrix of mRNA template into the cDNA synthesis; without the addition of the enzyme into the cDNA synthesis. All the reactions were performed on the individual samples in three replicates.

Statistical processing of the results was performed on a personal computer using program "STATISTICA® for Windows 6.0" (StatSoft Inc., License № AXXR712D-833214FAN5). Data was presented as Me (Q_1, Q_3) – the median of expression levels, and the lower and the upper quartiles. The analysis of the differences between the mRNA expression levels (for *CDH1, CTNNB1, VEGFA* genes) and the expression levels of the proteins encoded by the genes, in non-metastatic EEA, metastatic EAE and in normal endometrium was performed using a nonparametric Kruskal-Wallis test. In the absence of the statistically significant difference in the 3 groups of study, the pairwise analysis of the groups using the nonparametric Mann-Whitney test was carried

Table 1

Gene	Primer	T, °C	Product length (bp)	Exon junction
cateninbeta 1 (CTNNB1)	F = CCTGTTCCCCTGAGGGTATT R = CCATTGTCCACGCTGGATTT	58.4 58.82	66	220/ 221
E-cadherin, (CDH1)	F = CAGTACAACGACCCAACCCA R = ACCCACCTCTAAGGCCATCT	59.89 59.96	63	2060/ 2061
VEGFA	F = TCACCATGCAGATTATGCGGA R = TCTCTCCTATGTGCTGGCCT	59.86 60.03	54	1353/ 1354
GAPDH	F = CTCTGCTCCTCCTGTTCGAC R = CGATGTGGCTCGGCTGG	59.83 60.58	63	165/ 166

out. The links between the mRNA expression levels and the corresponding proteins expression levels were determined by the calculating of the γ-coefficient. The results were considered as statistically significant at the level of 95 % ($p<0.05$).

Results and discussion

It was found that the levels of relative normalized mRNA expression of E-cadherin gene (*CDH1*) in non-metastatic and metastatic EEA reduced by 12.5 times in comparison with the normal endometrium (for non-metastatic EEC the median of expression level is 0.08 (0.05; 0.54) and for metastatic EEC the median of expression level is 0.08 (0.03; 0.29) (*Table 2*). The difference between the mRNA expression levels of E-cadherin gene (*CDH1*) in metastatic EEA and non-metastatic EEA aren't statistically significant ($p>0.05$).

The results of the molecular-genetic studies correspond to the results of our immunohistochemical studies, which were carried out in parallel: the expression level of E-cadherin in normal endometrium is higher, the figure is 112.82 (99.72, 139.34) CUOD; the expression level of the protein in non-metastatic EEC is 46.49 % lower, the figure is 60.37 (30.15; 74.33) CUOD; the expression level of E-cadherin in EEC with metastasis is 56.19 % lower, the figure is 49.43 (30.34; 73.23) CUOD (*Table 3*). In our previous study the 2-times lower expression level of E-cadherin in invasive non-metastatic EEC compared to the normal endometrium has been demonstrated [16].

It is established that the loss of E-cadherin expression is more typical for type II endometrial carcinomas, but the loss of E-cadherin expression is also observed in 6–57 % cases of type I endometrial carcinoma. [17]. The loss of E-cadherin expression in EEC is associated not only with the *CDH1* gene mutations [17], but also with *CDH1* promoter hypermethylation [18] and dysregulation of the proteins, which normally inhibit the E-cadherin expression. The loss of E-cadherin expression is a poor prognostic factor for the patients with EEC [10], because this loss is associated with the phenomenon of epithelial-mesenchymal transformation, which includes the loss of apical-basal polarity of the cancer cells, the increasing of the mesenchymal integrins synthesis by these cells, the migration of the tumor cells into the adjacent tissues and also the tumor metastasis [19].

It was established, that the content of mRNA of *CTNNB1* gene in non-metastatic EEC is increased by 6.88 (2.78; 13.31) times ($p<0.05$) and in EEC with metastases it's increased by 28.20 (3.85; 217.65) times ($p<0.05$) in compar-

Table 2

The levels of relative normalized mRNA expression of *CDH1, CTNNB1, VEGFA* genes in non-metastatic and metastatic endometrioid adenocarcinoma of uterus

Gene	The levels of relative normalized mRNA expression of the genes			p
	Normal proliferative endometrium, n=10*	EEA without metastases, n=10	EEA with metastases, n=10	
		Me (Q_1; Q_3)	Me (Q_1; Q_3)	
E-cadherin (CDH1)	1	0.08 (0.05; 0.54)	0.08 (0.03; 0.29)	>0.05
Cateninbeta 1 (CTNNB1)	1	6.88 (2.78; 13.31)	28.20 (3.85; 217.65)	>0.05
VEGFA	1	2.78 (1.31; 4.71)	5.27 (2.37; 19.57)	>0.05

Notes: Me – the median of relative normalized expression level; Q_1 – the lower quartile of relative normalized expression level; Q_3 – the upper quartile of relative normalized expression level. The reliabilities of the differences of the normalized expression levels in non-metastatic and metastatic EEC were evaluated using the non-parametric Mann-Whitney U test; * – the statutory rate – the level of mRNA gene expression in normal proliferative endometrium is taken as 1, and the relative normalized levels of mRNA expression in adenocarcinomas is defined in relation to this indicator.

Table 3

The expression levels of E-cadherin, β-catenin and VEGFA in normal endometrium, non-metastatic and metastatic endometrioid adenocarcinoma of uterus

Protein	The expression levels of the proteins			p
	Normal endometrium, n=56	Non-metastatic EEA, n=30	EEA with metastasis, n=30	
	Me (Q1; Q3)	Me (Q1; Q3)	Me (Q1; Q3)	
E-cadherin	112.82 (99.72; 139.34) CUOD	60.37 (30.15; 74.33) CUOD	49.43 (30.34; 73.23) CUOD	>0.05
β-catenin	161.47 (151.46; 168.47) CUOD	108.90 (89.11; 124.35) CUOD	67.64 (55.40; 96.21) CUOD	<0.05*
VEGFA	111.43 (106.36; 116.02) CUOD	142.32(128.90; 152.39) CUOD	131.34 (107.19; 163.17) CUOD	>0.05

Notes: Me – the median of relative normalized expression level; Q_1 – the lower quartile of relative normalized expression level; Q_3 – the upper quartile of relative normalized expression level. The reliabilities of the differences of the expression levels of the studied proteins in normal proliferative endometrium, in non-metastatic and metastatic EEA were evaluated using non-parametric Kruskal-Wallis test; * – statistically significant results.

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ison with normal endometrium (*Table 2*). At the same time, the content of mRNA of *CTNNB1* gene in metastatic EEA wasn't significantly different from the mRNA of the gene in EEC without metastases (p>0.05). According to the literature mutations of *CTNNB1* gene occur in the early stages of EEC development, they are found in 20–40 % cases of EEC [4]. Mutations of *CTNNB1* gene are accompanied by activation of the canonical signaling Wnt/β-catenin-pathway and abnormal nuclear accumulation of β-catenin, that is observed in 16–47 % cases of EEC [17], and also by Cyclin-D activation with subsequent uncontrolled division of the tumor cells [8,20].

Our immunohistochemical studies, which were carried out in parallel, confirmed previous data. It was found, that β-catenin expression level in non-metastatic and metastatic EEA is lower, than in normal endometrium. The expression level of β-catenin in normal endometrium is 161.47 (151.46, 168.47) CUOD; in the tumor cells of non-metastatic EEC the median is 32.56 % below and the figure is 108.90 (89.11; 124.35) CUOD; in the tumor cells of metastatic EEC the median is 58.11 % below and the figure is 67.64 (55.40; 96.21) CUOD (*Table 3*).

It was found that the level of mRNA expression of *VEGFA* gene in non-metastatic and metastatic EEC in comparison with the normal endometrium increased by 2.78 (1.31; 4.71) and 5.27 (2.37; 19.57) times, respectively (p<0.05). The difference between the mRNA expression of *VEGFA* gene in metastatic EEC and in non-metastatic EEC isn't statistically significant (p>0.05) (*Table 2*). Moreover, a statistically significant increase of the level of immunohistochemical expression of VEGFA in the EEC tumor cells as compared to the normal endometrium was revealed (p<0.05): the VEGFA expression level in normal endometrium is 111.43 (106.36, 116.02) CUOD; in the tumor cells of non-metastatic EEA the expression level of the marker is 27.72 % higher, the figure is 142.32 (128.90; 152.39) CUOD; in the tumor cells of metastatic EEC the expression level of the marker is 17.87 % higher, the figure is 131.34 (107.19, 163.17) CUOD (*Table 3*). At the same time, the difference between the expression levels of this marker in non-metastatic and metastatic EEC isn't statistically significant (p>0.05).

It is well known that angiogenesis plays a critical role in the progression of endometrial cancer [21]. One of the most powerful pro-angiogenic factors, which are involved in the angiogenesis during type 1 endometrial cancer, is vascular endothelial growth factor (VEGF) [12]. The angiogenesis, which progresses in the endometrial cancer, is supposed by the alteration in VEGF/VEGF-receptors signaling pathways [3], the participation of WNT-proteins and β-catenin, but the role of inhibitors and inducers of Wnt-signaling pathway in this process is still unknown [22].

The direct medium connections between the mRNA expression levels of *CTNNB1* gene and *VEGFA* gene (γ=0.44) in non-metastatic and metastatic EEC, and also between immunohistochemical expression level of VEGFA in the tumor tissue (γ=0.37) were revealed. Such connections may be a manifestation of the Wnt-signaling pathway effect on the VEGFA expression.

Conclusions

1. Endometrioid adenocarcinoma of uterus is characterized by the lower mRNA expression level of E-cadherin gene (*CDH1*) and also by the higher mRNA expression level of β-catenin gene (*CTNNB1*) and vascular endothelial growth factor A gene (*VEGFA*) in comparison with the normal endometrium.

2. The immunohistochemical expression level of E-cadherin is 32.6 % lower in the tumor cells of non-metastatic endometrioid adenocarcinoma and also is 58.10 % lower in the tumor cells of metastatic endometrioid adenocarcinoma in comparison with the normal endometrium.

3. The immunohistochemical expression level of β-catenin is 32.56 % lower in the tumor cells of non-metastatic endometrioid adenocarcinoma of uterus and also is 58.11 % lower in the tumor cells of metastatic endometrioid adenocarcinoma in comparison with the normal endometrium.

4. The immunohistochemical expression level of vascular endothelial growth factor A (VEGFA) is 27.72 % higher in non-metastatic endometrioid adenocarcinoma and also is 17.87 % higher in metastatic endometrioid adenocarcinoma of uterus in comparison with the normal endometrium.

5. Endometrioid adenocarcinoma of uterus with metastases in the regional lymph nodes differs by the lower expression level of β-cateninin comparison with the non-metastatic endometrioid adenocarcinoma.

6. Non-metastatic and metastatic endometrioid adenocarcinoma of uterus is characterized by the direct medium connections between the mRNA expression level of β-catenin gene (*CTNNB1*) and the mRNA expression level of *VEGFA* gene (γ=0.44) and also between the immunohistochemical expression level of VEGFA in the tumor tissue (γ=0.37).

The prospects of further research

Considerable interest is the further study of transcriptional activity of other genes, which are involved in the EEA carcinogenesis.

Conflicts of interest: authors have no conflict of interest to declare.

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