According to available literature, endometrial cancer is predominantly diagnosed at early stages (80% at stage I) and, as a rule, has a relatively favorable course with a five-year survival of over 95%. However, the five-year survival rates of such patients are much lower in the presence of metastases in regional lymph nodes or relapse of the disease (respectively, 68% and 17%) [1]. In a number of cases, this is due to the low effectiveness of therapeutic measures that do not take into account the molecular mechanisms of cancer progression.

It is known that the biological features of endometrial carcinoma (EC) are formed under the influence of a number of different factors, such as the hormonal background in which tumors have developed, changes in the functioning of oncogenes and suppressor genes, and the composition of the tumor microenvironment. All this contributes to the emergence of genome instability and the accumulation of genetic alterations in cancer cells [2–5].

To some extent, the progression of malignant neoplasms (increased proliferative activity, invasive, angiogenic and metastatic potential) depends on the changes in the expression of molecules that provide intercellular contacts. The loss of adhesive properties by tumor cells contributes to their increased mobility and, as a consequence, penetration into surrounding tissues, blood and lymphatic vessels [6]. Recent studies have shown that the migration of neoplastic cells during tumor progression can be the result of consistent molecular changes and a complex of morphological alterations that are accompanied by the destruction of the basement membrane of cancer cells and the loss of the polar orientation of their nuclei, while epithelial cells may acquire mesenchymal-like morphology. This phenomenon was called epithelial-mesenchymal transition (EMT) and, according to Qin et al. [7] is a strategy of immune rescue, adaptation of cancer cells to increase their chances of survival in mesenchymal tissues by mimicry. In this case, abnormal epithelial cells not only lose the expression of epithelial markers, but they may acquire expression of an unusual protein for epithelial cells, a marker of mesenchymal cells — vimentin.

The role of vimentin in determining the malignancy of solid tumors of various genesis has not been clarified yet [8], however, the appearance of this protein in EC cells is associated with tumor invasiveness and metastasis [9]. In contrast, in endometrial serous carcinomas, which are more aggressive tumors with an unfavorable course of the disease, vimentin expression is usually absent or is significantly lower than in endometrioid type EC [10, 11].

The main proteins that provide stable intercellular contacts between epithelial cells and inhibit their mobility are cadherins and catenins, in particular E-cadherin and β-catenin. E-cadherin belongs to the family of transmembrane glycoproteins being one of the key cadherins that provides Ca²⁺-dependent homophilic adhesion in epithelial tissues by the formation of transdimers in two adjacent cells via the interaction of extracellular domains [12]. Its intracellular domain is bound to a number of proteins and, in the first place, with β-catenin, which, apart from E-cadherin, binds with α-catenin and actin microfilaments, providing dense intercellular contacts [12, 13]. The interaction of E-cadherin with β-catenin isregulated
by the level of serine and threonine phosphorylation in β-catenin [14–16].

In most epithelial neoplasias, including EC, there is a decrease in the expression of E-cadherin, including hypermethylation of the promoter of its gene — CDH1, sometimes with gene mutations or deletions on the 3rd chromosome [17, 18]. In addition, the functional loss of E-cadherin may be due to the activation of transcription factors Snail1 and Snail2 or ZEB1 and ZEB2, which suppress the expression of the CDH1 gene [14, 19]. As a result, the destruction of E-cadherin/β-catenin complex and intercellular bonds occurs, E-cadherin is translocated into the cytoplasm, and β-catenin is released and accumulated in the cytoplasm followed by its translocation into the nucleus [13, 14].

The aim of the study was to analyze the features of the expression of adhesion markers E-cadherin, β-catenin and vimentin and their role in progression of ECs.

MATERIALS AND METHODS

The study was conducted on the samples of surgical material of 55 EC patients (47 with stage I and II EC and 8 with stage III EC according to TNM and FIGO classifications). The patients did not receive preoperative therapy, their age ranged from 32 to 73 years (average 58.9 ± 6.5 years). All patients were treated at the Department of Oncogynecology of the National Cancer Institute of the Ministry of Health of Ukraine from 2014 to 2017 and provided informed consent on the use of their biological material for scientific research. Morphological diagnosis was verified on histological preparations stained with hematoxylin and eosin. The differentiation grade of tumors was determined according to WHO criteria [20].

Immunohistochemical (IHC) detection of E-cadherin, β-catenin and vimentin was performed on deparaffinized sections, using MoAbs to E-cadherin (clone NCH-38), β-catenin (clone β-catenin-1) (DakoCytomation, Denmark), vimentin (clone V9) (Diagnostic BioSystems, the Netherlands). In each slide 700–1000 cells were analyzed. The results of the IHC reaction were evaluated by the semi-quantitative method counting the number of stained cells and the intensity of the reaction (H-score, points). The analysis took into account the number of cells (%) with the membrane-cyttoplasmic localization of E-cadherin and cytoplasmic-nuclear — of β-catenin [9, 21].

The proliferation index (PI, %, i.e. the number of cells in the S + G2/M phases of the mitotic cycle) was determined using the flow cytometry method after staining cells with fluorochrome — propidium iodide [22]. The studies were conducted on a flow cytometric analyzer EPICS-XL (Beckman Coulter, USA).

To objectivize the evaluation of the expression of the biomolecular markers examined, we determined the median (Me) of the number and intensity of stained cells (H-score) or the proliferative potential — MePI. The values of all indices less than Me were considered low, and above Me — high.

Statistical data were processed using Statistica 8.0 software package (StatSoft, Inc.) by non-parametric Mann — Whitney U Test. Differences at p < 0.05 were considered significant.

RESULTS

According to morphological analysis of histological preparations, all patients had endometrioid form of EC of high (7.2%), moderate (41.8%) and low (50.9%) differentiation grade. 40% of tumors had minor invasion in myometrium (< ½) and 60% invaded by > ½ of myometrium.

According to the results of flow cytometry, it was found that while S + G2/M ratio varied from 8.5% to 48.7%, PI was 27.6 ± 4.3%, with Me = 25.4%.

The results of IHC study showed that positive expression of E-cadherin was observed in 78.2% of EC, β-catenin — 100% and vimentin in 74.5%. The protein product was detected diffusely, or in separate sections of the tumor (Fig. 1).

At that, a significant variation in the expression of the investigated proteins was observed: individual variations in the expression of E-cadherin were 14.6–190.6 points (66.3 ± 5.3 points on the average), β-catenine — 55.0–237.4 points (155.0 ± 6.4 points on the average), vimentin — 15.4–242.1 points (53.4 ± 4.8 points on the average). Me values of the expression of these markers were 51.5, 152.5 and 41.6 points, respectively.

Comparison of expression of EMT-associated molecular markers in EC of different grade showed that the expression of E-cadherin was higher, and vimentin lower (significantly or at the level of tendency) in low-differentiated tumors than in tumors deeply invaded the myometrium (Table 1).

Table 1. Expression of E-cadherin, β-catenin and vimentin in EC related to clinical and pathological characteristics of tumors

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Expression of markers of EMT transition, H-score, points</th>
</tr>
</thead>
<tbody>
<tr>
<td>M ± m</td>
<td>E-cadherin</td>
</tr>
<tr>
<td>Min–Max</td>
<td>14.6–190.6</td>
</tr>
<tr>
<td>PI:</td>
<td></td>
</tr>
<tr>
<td>&lt; Me (25.4%)</td>
<td>52.9 ± 4.2</td>
</tr>
<tr>
<td>&gt; Me (25.4%)</td>
<td>93.9 ± 4.7**</td>
</tr>
<tr>
<td>Differentiation grade:</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>49.7 ± 5.4</td>
</tr>
<tr>
<td>G2</td>
<td>77.1 ± 6.0*</td>
</tr>
<tr>
<td>G3</td>
<td>75.6 ± 6.1*</td>
</tr>
</tbody>
</table>

Depth of tumor invasion into myometrium:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Expression of markers of EMT transition, H-score, points</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>&lt; ½</td>
</tr>
<tr>
<td></td>
<td>69.1 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>&gt; ½</td>
</tr>
<tr>
<td></td>
<td>83.3 ± 5.0</td>
</tr>
<tr>
<td>II</td>
<td>73.6 ± 4.1</td>
</tr>
<tr>
<td>III</td>
<td>72.3 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>92.0 ± 5.7</td>
</tr>
</tbody>
</table>

Notes: E-cadherin: *p < 0.05 compared with G1 tumors; **p < 0.01 compared with PI < Me; β-catenin: *p < 0.05 compared to G1 tumors; vimentin: *p < 0.05 compared with G1 tumors *p < 0.01 compared to G1 tumors, with invasions in myometrium < ½, with stage I.

Expression of the studied markers was significantly reduced in stage III EC compared with these at stages I and II of the disease.

The direction of changes in β-catenin expression in the investigated tumors of the endometrium was different, namely, lower than Me in tumors of moderate and low differentiation grades and neoplasms with deep
invasion into myometrium, and in patients with stage I of the tumor process.

In high-proliferating tumors, the expression of E-cadherin was significantly increased, and β-catenin tended to decrease compared to those in tumors with low PI. Instead, the expression of vimentin practically did not depend on PI index.

Taking into account the available literature, according to which the detection of vimentin in epithelial tumors is one of the characteristics of EMT and the absence or low expression of this protein is more common in serous carcinoma of the endometrium [9, 10], we have compared clinical morphological and molecular-biological indices in tumors of patients with high expression of vimentin and with vimentin-negative EC (Table 2).

The analysis of the data showed that in the group of vimentin-negative EC, tumors of low differentiation grade and deep invasion in myometrium prevailed compared with the cases with high expression of vimentin. In addition, in the tumors with high expression of vimentin, a significant decrease in the expression of E-cadherin was observed as well as the number of cells expressing E-cadherin in the cytoplasm (78.9 ± 3.6%) and β-catenin — with cytoplasmic-nuclear localization (73.7 ± 3.2%) compared to these indices in vimentin-negative tumors (respectively, 45.4 ± 4.2%, p < 0.001 and 54.5 ± 2.6%, p < 0.005). It was found that in EC without E-cadherin expression, high expression of vimentin (76.9 ± 6.8 points) was observed, which was higher than in tumors with high expression of E-cadherin (56.1 ± 4.6 points).

In tumors without vimentin expression, a high correlation was found between the expression of E-cadherin and β-catenin (r = 0.7, p < 0.05), which was absent in the vimentin-positive tumors suggesting the shift in E-cadherin/β-catenin ratio.

It should be noted that the proliferative potential in vimentin-negative tumors and tumors with high expression of vimentin was higher than Me. That is, most EC are highly proliferating tumors.

**DISCUSSION**

The study revealed a significant EC heterogeneity by clinical and morphological characteristics, and such molecular characteristics as the expression of markers providing intercellular adhesion and associated with EMT. It is known that tumor progression is associated with the acquisition of invasive and metastatic properties by tumor cells. The latter arise as a result of protein-protein and protein-DNA interactions in a number of signaling cascades, which leads to a decrease in intercellular adhesion, which determines the EMT and accelerates the growth rate of the tumor [19].

**Table 2. Comparative study of clinical-morphological and molecular-biological indices in EC related to the level of vimentin expression**

<table>
<thead>
<tr>
<th>Expression of vimentin, points</th>
<th>Number of cases, %</th>
<th>Expression of markers, M ± m, points</th>
<th>PI, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1</td>
<td>G2</td>
<td>G3</td>
</tr>
<tr>
<td></td>
<td>&lt; ½</td>
<td>&gt; ½</td>
<td></td>
</tr>
<tr>
<td>&gt; Me (41.6)</td>
<td>(n = 25)</td>
<td>12.0</td>
<td>64.0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 14)</td>
<td>0</td>
<td>35.7</td>
</tr>
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<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

*Note: * p < 0.05 compared with the vimentin-negative tumors.
According to current data, one of the causes of EMT may be a hormonal imbalance, in particular a decrease in the progesterone content. For example, van der Horst et al. [23] showed that progesterone stimulates the activity of intratumoral T-lymphocytes and inhibits the Wnt/β-catenin pathway associated with EMT. It is possible that, as was shown in our previous studies, the low level of progesterone in the peripheral blood of EC patients, along with the low expression of progesterone receptors in tumors and the increase in the number of intratumoral FOXR3 lymphocytes in low-differentiated EC, created conditions for EMT, which led to a deep invasion of myometrium [24–26]. In fact, in some EC samples under study, EMT-associated changes (low or no expression of E-cadherin, translocation of E-cadherin into the cytoplasm, and β-catenin — into the nucleus) may be supposed, which is consistent with the literature data [13–15, 27, 28].

These translocations of E-cadherin and β-catenin can occur during activation of various signaling cascades (TGFβ, FGF and Wnt), and due to disorders in the functioning of the genes of CDH1, CTNND1 or genes that provide degradation of β-catenin (e.g., APC gene) [13, 14]. In a nucleus, a transcriptional complex formed between β-catenin and T-cell- and lymphoid factors (TCF LEF) activates the expression of a number of target genes, including the C-MYC, CCND1, VEGF, Axin-2, Snail, VIM and others, which leads to an increase in proliferative and angiogenic potential of malignant neoplasms, which is accompanied by the appearance of a marker of the mesenchymal tissues vimentin [13, 14, 16, 19].

Increased expression of vimentin associated with an increase in the migration and invasive properties of cancer cells was noted by other researchers in malignant neoplasms and cell lines derived from the tumors of other genesis [11, 29]. The presence of such a link has been shown in cell lines and surgical samples of breast tumors with low estrogen receptor levels. The authors emphasize that the expression of vimentin influences the invasive phenotype of cancer cells only in cooperation with other, yet clearly unidentified proteins [30].

Separate attention should be given to the cases of EC with high-level expression of E-cadherin and β-catenin, and without expression of vimentin. In 64.3% of cases, such tumors were characterized by a low differentiation grade and in 78.6% of cases — by a deep invasion in myometrium. That is, more than 60% of these tumors were more aggressive than vimentin-positive tumors. It is possible that in vimentin-negative tumors, could be present a EGFR-mediated activation of PI3K/AKT signaling, which is accompanied by an increase in the expression of E-cadherin and the formation of tumor cell complexes — microembolies with dense intercellular contacts with so-called “collective or chain migration” [12, 14].

Summing up the results obtained, it can be hypothesized that the formation of a more aggressive phenotype in the vimentin-negative tumors of the endometrium is not due to the reduction of the expression of the markers of intercellular adhesion E-cadherin and β-catenin, but via the changes in the functioning of a number of proteins, oncosuppressors p53, p21WAF1/CIP1, p16INK4a and others [11, 31, 32].

To confirm our experimental data, a bioinformatics analysis of the VIM mRNA expression was performed [33]. The portal Oncomine, which contains data on microarrays and RNA sequencing, was used. The evaluation of the array data showed that VIM mRNA expression in EC of various differentiation grades and the stages at the level of mRNA is similar to our results obtained during IHC analysis of vimentin expression.

The level of VIM mRNA expression in moderately and low-differentiated EC is reduced in comparison with highly differentiated tumors (Fig. 2).

In addition, it has been shown that VIM expression also progressively decreased in tumors of patients with stage II and III compared to that in patients with stage I (Fig. 3).

Thus, the data of the bioinformatics analysis show that the decrease in the expression of vimentin in EC correlates with the aggressiveness of this type of cancer.

In conclusion, our data show that the progression of the endometrioid carcinoma may occur due
to various molecular changes, in particular, decreased expression of E-cadherin and β-catenin proteins, and high expression of the marker of mesenchymal tissues — vimentin, or in the absence of vimentin expression, with the involvement of other mechanisms of regulating proliferative and metastatic potential.

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REFERENCES