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Iryna Nesina[1], Natalia Iurchenko[1], Sergey Nespriadko[2], Lubov Buchynska[1]

[1]R.E. Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology, National Academy of Sciences of Ukraine, Kyiv, Ukraine
[2]National Cancer Institute, Ministry of Health of Ukraine, Kyiv, Ukraine

Twist expression and content of tumour-associated macrophages in endometrial carcinoma

Address for correspondence:
Iryna Nesina, Ph.D. in Biology,
R. E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of National Academy of Sciences of Ukraine,
45 Vasylkivska Str., 03022 Kyiv, Ukraine
e-mail: laboncogen@gmail.com

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ABSTRACT

Introduction. This study aimed to relations between the expression of the Twist transcription factor, the content of tumour-associated macrophages (TAMs), and clinicopathological indicators of tumour progression in patients with stages I–II and III endometrial cancer (EC).

Material and methods. Surgical specimen from 45 patients with endometrioid carcinoma of the endometrium (ECE) (average age — 60.1 ± 2.3 y.o.) were investigated using morphological, immunohistochemical, flow cytofluorometry and statistical methods.

Results. Nuclear expression of Twist was determined in 47.1% of ECE samples with individual fluctuations in the range of 6.3–43.0%, which was 16.6 ± 2.9% on average. Twist expression in G3 endometrial tumours and those with deep invasion into the myometrium tended to increase (21.4 ± 4.3 and 18.0 ± 3.5%, respectively) as compared with the expression of this marker in G2-tumors and the ones, invading < 1/2 of the myometrium (13.2 ± 3.3 and 16.7 ± 3.9%, respectively). Positive expression of Twist in ECE was associated with reduced expression of E-cadherin (44.3 ± 3.8%) and increased expression of vimentin (33.9 ± 3.4%), the content of TAMs in the stromal component of the tumour (30.2 ± 3.7 cells/f.v.), and microvessels density (MVD) (46.5 ± 5.4 vessels/mm^2) as compared with the same indices for ECE with negative expression of Twist (61.4 ± 4.7%, $p < 0.05$; 14.6 ± 3.1%, $p < 0.05$; 18.0 ± 2.4 cells/f.v., $p < 0.05$ and 34.3 ± 4.7 vessels/mm^2, respectively).

Conclusions. Higher content of stromal TAMs and higher MVD are observed in Twist-positive endometrial carcinomas as compared with the same indices in Twist-negative neoplasms which are associated with different morphological specificities of invasive processes in the endometrium.

Key words: endometrioid carcinoma of endometrium, Twist, tumour-associated macrophages (CD163), microvessels density (CD31)

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Introduction

It is known that the progression of a malignant neoplasm results from the loss of genetic control over the processes of differentiation, proliferation, and apoptosis in tumour cells and molecular changes in the tumour microenvironment, which is characterized by higher growth of the tumour, neoangiogenesis, the invasion of the tumour into adjacent tissues, and metastases [1]. It was demonstrated that one of the reasons for tumour invasion and metastases is the epithelial-mesenchymal transition (EMT) due to which epithelial cells may get transformed into the cells with mesenchymal-like phenotype [2]. During carcinogenesis, EMT may be present when several signalling pathways are activated, including such transcription factors as Twist, Snail, Slug, and Zeb1 [3, 4].

It was determined that the Twist transcription factor promotes the distribution of epithelial cells not only by binding to *CDH1* gene promoter and inhibiting the

expression of E-cadherin [5]. Twist may also trigger neoplastic progression by inhibiting p53 ("wild type"). Shown that Twist1 binds p53 C terminus through the Twist box. This interaction hinders key posttranslational modifications of p53 and facilitates its MDM2-mediated degradation [6]. It has recently been demonstrated that the Twist transcription factor interacts with oncoprotein c-Myc in a tumour, thus promoting reprogramming of the tumour microenvironment [7]. It was found that Twist and c-Myc secrete cytokines CCL2 and IL13 which conduct the polarization of type I macrophages into M2-macrophages and recruit them to the tumour. It means that Twist and c-Myc may create conditions for metastasis in the neoplasm, as, according to current data, M2-macrophages are among the leading components of the tumour microenvironment to secrete different factors, stimulating the proliferation of tumour cells, enhancing their migration ability, and activating angiogenic processes [8–12]. M2-macrophages produce chemokine CXCL12 and hepatocyte growth factor (HGF), which bind to their receptors (CXCR4 and c-MET) on tumour cells thus causing the motility of the latter [10].

It is believed that the availability of a high number of tumour-associated macrophages (TAMs) in patients with solid tumours is an unfavourable prognostic marker, associated with the aggravated clinical course [11–13]. For instance, Jackute et al. [12] demonstrated that high content of $CD163^+$-macrophages in a stromal component of the tumour was related to the decline in the survival rate of patients with non-small-cell lung cancer.

The same is true regarding endometrial cancer (EC), one of the most common gynaecological malignant neoplasms among women both in Ukraine and globally [14]. Many authors note that the clinical course of EC is associated with specific morphological and molecular traits of neoplasms and the specificities of the tumour microenvironment [3, 4, 15]. However, the issue of the integral impact of molecular changes in tumour cells and components of tumour microenvironment with immunosuppressive properties in the formation of some invasive potential of malignant endometrial tumours is studied insufficiently.

Taking the abovementioned into consideration, the work aimed to study the relations between the expression of EMT marker — Twist transcription factor, the content of TAMs, and clinicopathological indicators of tumour progression in patients with EC stages I–II and III.

Material and methods

The samples of surgical material of 45 patients with EC, stages I–III, aged 32 to 78 y.o. (average age — 60.1 ± 2.3 y.o.). All patients were treated at the Oncogynaecology department of the National Cancer Institute, Ministry of Health of Ukraine in 2014–2018 (the head of the research and experimental unit of the Oncogynaecology department — Professor V.S. Svintsitsky, Doctor of Science in Medicine). They did not have preoperative therapy and gave their informed consent to the use of their biological material for scientific studies. During the study, all the required ethical standards were complied with according to the universally accepted international requirements of the Declaration of Helsinki 2008.

The final morphological diagnosis was verified by examination of histological preparations, stained with haematoxylin and eosin (H & E).

The immunohistochemical (IHC) determination of biomolecular markers was done using the deparaffinized sections of endometrial tumours. Twist, the marker of epithelial-mesenchymal transition, was determined using the polyclonal antibody Twist1/Twist2 (Thermo Fisher Scientific, USA), catalogue No. PA5-78211. The expression of other markers was determined with monoclonal antibodies (McAbs): M2-macrophages were detected using McAb to CD163 (the one, detecting M2-macrophages [13]), the clone of Mob460-05 and *de novo* microvessels were detected by the expression of a vascular endothelium marker — antigen CD31, McAb to CD31, clone EP78 (Diagnostic BioSystems, USA).

The mentioned proteins were detected with the visualizing PolyVue HRP/DAB Detection System (Diagnostic BioSystems, USA). Cell nuclei were additionally stained with Mayer's haematoxylin.

The results of the IHC reaction were assessed by the semi-quantitative method. About 700–1,000 cells were analysed in each preparation, separately in glandular and solid structures, to determine Twist protein product. The results of IHC reaction were assessed by the semi-quantitative method, by counting the number of stained cells — the labelling index (LI, %). Usually, both cytoplasmic and nuclear localization of this protein were observed, but, since Twist is a transcription factor, only tumour cells with nuclear localization of the marker were considered.

The data obtained were compared with the results of the previous studies, in which the authors determined the expression of EMT markers in these very cases of ECE [16–18].

In addition, the authors counted the number of positively stained $CD163^+$-macrophages (TAMs) — the number of cells per one field of vision (cells/f.v.) of the microscope, analysing them in 10 fields of vision with ×400 magnification. Both the total number of TAMs and their separate amounts in intratumoural and stromal components were determined.

To determine the microvessels density (MVD) in endometrial tumours, the number of vessels in 10 fields of vision of the microscope was counted at ×100 magnification. The area of one field of vision was limited with

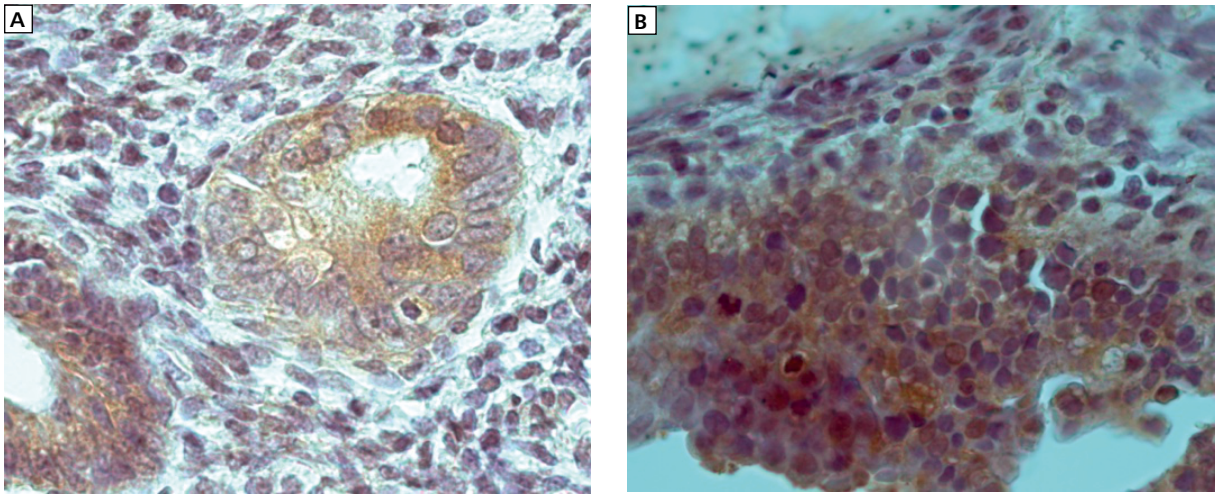

Figure 1. The expression of Twist in the glandular (**A**) and solid (**B**) areas of the moderately differentiated endometrial carcinoma (IHC method, additional staining with Mayer's haematoxylin); ×1,000 magnification, oil immersion

the measuring square grid (the side of 1.25 mm). MVD (number of vessels/mm^2) was defined by the formula: MVD = n: 1.56 vessels/mm^2, where (n) — the average number of vessels per one field of vision; 1.56.mm^2 — the area of one field of vision. The criteria for assessing the mentioned indices were as follows: the expression of Twist LI < 1.0% was considered negative; the values of M2-macrophages and MVD under the median (Me) were considered low, and the ones above or equal to Me were considered high.

The proliferative activity of the investigated endometrial carcinomas was determined by the proliferation index (PI, %) using flow cytofluorometry [19]. The studies were conducted in the flow cytofluorometer EPICS-XL (Beckman Coulter, USA).

The statistical processing of the data was conducted in Statistica 8.0 (StatSoft, Inc.) using the non-parametric Mann-Whitney test and Spearman's correlation. Here $p < 0.05$ was accepted as a reliable significance level.

Results

The morphological analysis of neoplasms demonstrated that the tumours under investigation were endometrioid carcinomas of the endometrium (ECE) of different differentiation degrees: 18 cases (40.0%) of moderate (G2) and 27 cases (60.0%) of low differentiation degree (G3). 16 (35.6%) patients had tumours, which invaded < 1/2 myometrium and 29 (64.4%) cases had tumours with deep (> 1/2) invasion of the myometrium. Most patients, 24 (53.3%), had stage I tumour progression, 13 (28.9%) — stage II, and 8 (17.8%) patients — stage III. All tumours of patients with stage III tumour progression were of low differentiation degree and invaded the myometrium deeply.

Most investigated ECE were highly proliferating tumours with the average LI value of 31.0 ± 3.1% (the range of 13.4–69.2%, Me = 29.1%).

The results of the IHC investigation demonstrated that positive expression of Twist transcription factor was mostly manifested in the cytoplasm, while in a smaller number of tumours it was found in the nucleus (Fig. 1).

The nuclear expression of this marker was determined in 47.1% of ECE samples with individual fluctuations in the range of 6.3–43.0%, which was 16.6 ± 2.9% on average. The tumours of 69.5% of patients with stage I–II EC and 50.0% tumours of patients with stage III of tumour progression were positive in terms of the expression of this protein. It was determined that positive expression of Twist was associated with the decreased expression of E-cadherin and the increased expression of vimentin as compared with these indices for ECE with negative expression of Twist [16, 18] (Tab. 1).

At the same time, neither complete absence of E-cadherin expression was found in Twist-positive endometrial carcinomas nor the complete absence of vimentin expression — in Twist-negative ECE. It allows for the assumption that most tumour cells of the endometrium are characterized by hybrid phenotype (with the expression of both epithelial and mesenchymal markers) [20]. Positive expression of vimentin in some Twist-negative ECE may probably result from the activation of other transcription factors (Snail, Slug, and Zeb) or reduced functioning of other adhesive proteins which promotes the occurrence of EMT features in tumour cells of the endometrium.

While determining the connection between Twist expression and the indices of endometrial carcinoma progression, it was found that in low differentiated endometrial carcinomas and the ones with a deep invasion of the tumour into myometrium the expression of Twist

Table 1. The comparison of the expression of epithelial-mesenchymal transition markers and Twist transcription factor in tumour cells of the endometrium

Molecular markers of EMT	Expression of EMT markers, M ± m, %	
	Twist-positive ECE	Twist-negative ECE
E-cadherin	44.3 ± 3.8	61.4 ± 4.7*
β-catenin	78.6 ± 4.2	86.3 ± 5.4
Vimentin	33.9 ± 3.4	14.6 ± 3.1*

*p < 0.05 as compared with the expression of the corresponding marker in tumours with positive expression of Twist; EMT — epithelial-mesenchymal transition; ECE — endometrioid carcinoma of the endometrium

Table 2. The expression of Twist in endometrioid carcinoma of the endometrium of different differentiation degree, depth of tumour invasion into the myometrium and the stage of tumour progression

Investigated parameters of ECE	Twist expression, LI%		
	Glands	Solid areas	Total
Degree of tumour differentiation			
G2	3.8 ± 2.2	9.4 ± 2.4	13.2 ± 3.3
G3	8.2 ± 2.9	13.2 ± 3.6	21.4 ± 4.3
Depth of tumour invasion into the myometrium			
< 1/2	8.4 ± 2.9	8.3 ± 2.9	16.7 ± 3.9
> 1/2	7.0 ± 2.3	11.0 ± 3.0	18.0 ± 3.5
Stage of tumour progression			
Ia + Ib	6.1 ± 1.9	8.7 ± 2.9	14.8 ± 3.3
Ic	2.2 ± 0.8	9.5 ± 3.0	11.7 ± 3.2
II	2.4 ± 0.9	10.0 ± 3.1	12.4 ± 3.3
III	2.6 ± 1.1	7.1 ± 2.2	9.7 ± 2.9

ECE — endometrioid carcinoma of the endometrium; LI — labelling index

tended to increase as compared with the expression of this marker in G2-tumors and the ones, invading < 1/2 of the myometrium. It was lower in the tumours of patients with stage III disease (9.7 ± 2.8%) as compared with the tumours of patients with stage I–II (13.7 ± 3.5%). It should be noted that the increase in Twist expression occurred mainly in solid areas of tumours, while in glandular structures the changes were ambiguous (Tab. 2).

Some authors believe that it may be conditioned by the fact that solid structures are located in the areas with more evident hypoxia which promotes the occurrence of EMT traits in tumour cells.

As demonstrated using breast cancer tumours, the expression of the Twist transcription factor in tubular and trabecular structures, which did not lose their contact with the surrounding stroma, was observed only in 5.0–8.0% of tumours, while in the alveolar and solid areas, characterized by the accumulation of tumour cells and limited contact with stroma, the number of tumours with Twist expression increased up to 18.0–19.0% respectively [21].

Taking into consideration the scientific data about the role of Twist in the polarization of M1-macrophages into M2-macrophages, which promotes the occurrence of immunosuppressive, proangiogenic, and invasive properties in tumours [3, 7, 9], the following stage of this study was to determine the relationship between Twist expression, the content of TAMs, MVD, and other indices of ECE progression.

The results of IHC studies demonstrated that ECE were notable for a considerable variability by the number of such components of microenvironment as the content ($CD163^+$-macrophages) of TAMs and the number of *de novo* microvessels. It was determined that individual fluctuations in TAMs content in ECE were in the range of 7.8–81.5 cells/f.v., which on average was 32.9 ± 2.9 cells/f.v. (Fig. 2).

Individual fluctuations of MVD in the ECE under investigation were in the range of 9.2–88.5 vessels/mm^2, which on average was 35.5 ± 4.3 vessels/mm^2 (Fig. 3).

It was demonstrated that the number of TAMs in the malignant endometrial tumours was related to their localization. The number of intratumoural TAMs was almost twice smaller (12.7 ± 1.4 cells/f.v.) than their number in the stromal component of neoplasms (20.3 ± 2.2 cells/f.v., p < 0.05).

We found the relationship between the content of TAMs and MVD and the expression of the Twist transcription factor in ECE. A reliable increase in TAMs content in the stromal component of endometrial carcinomas and the increase in MVD (at the tendency level)

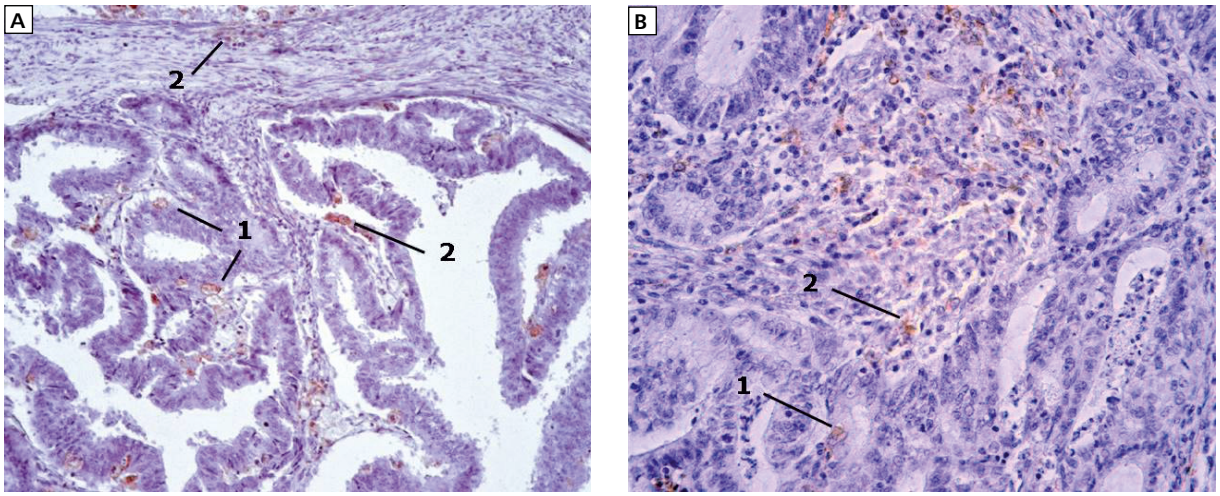

Figure 2. Tumour-associated macrophages (TAMs) in moderately differentiated endometrial carcinoma: 1 — intratumoural TAMs; 2 — stromal TAMs (IHC method, additional staining with Mayer's haematoxylin); Magnification: **A**. ×200; **B**. ×400

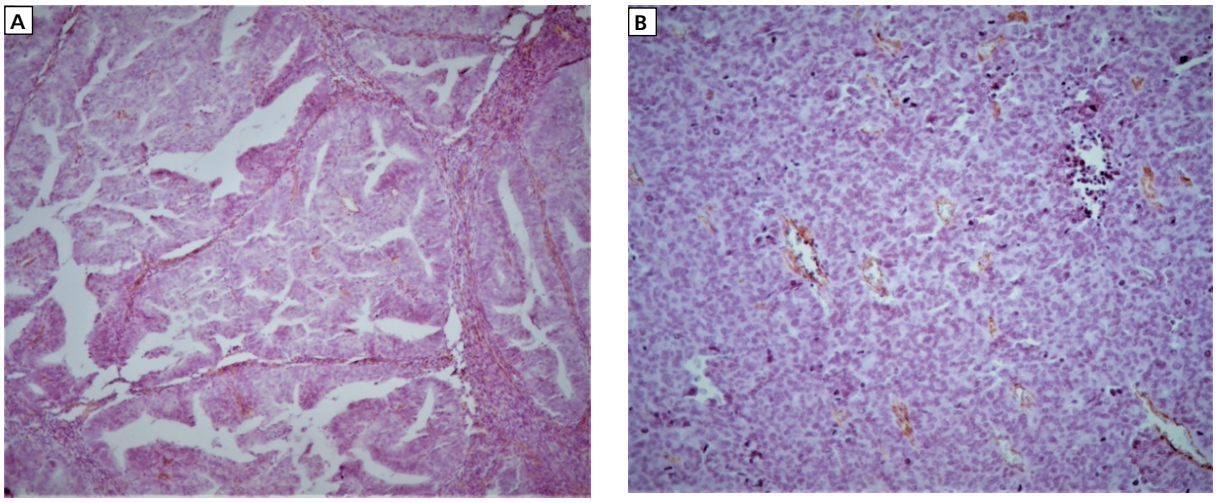

Figure 3. The microvessels (**A**) in moderately and (**B**) low differentiated endometrial carcinoma (IHC method, additional staining with Mayer's haematoxylin); Magnification: **A**. ×100; **B**. ×200

in Twist-positive ECE was demonstrated as compared with the number of these indices in Twist-negative endometrial carcinomas (Tab. 3).

At the same time, it was determined that the number of TAMs and MVD in ECE fluctuated depending on such indices of tumour progression as high proliferative potential, low degree of differentiation, deep invasion of a tumour into the myometrium, and the stage of tumour progression.

For instance, highly proliferating endometrial carcinomas were characterized by a higher content of intratumoural TAMs (15.7 ± 2.1 cells/f.v.) as compared with their number in ECE with IP < Me (10.0 ± 1.3 cells/f.v., $p < 0.05$). The number of intratumoural TAMs was also increasing in G3-tumors (14.3 ± 1.9 cells/f.v.) and in the tumours which deeply invaded the myometrium (14.3 ± 1.8 cells/f.v.) as compared with their content in G2-tumors and the tumours with the invasion of <1/2 myometrium (11.7 ± 2.1 and 9.1 ± 1.2 cells/f.v., $p < 0.05$ respectively). The content of TAMs in stroma also tended to increase in ECE with IP>Me and with a low degree of differentiation and increased reliably in the tumours which deeply invaded the myometrium as compared with ECE which had IP<Me, a moderate differentiation degree and invaded less than 1/2 of the myometrium (Tab. 4).

MVD had similar changes: it was reliably higher in highly proliferating, low differentiated, and deeply invading endometrial carcinomas as compared with the tumours of IP < Me, in G2-tumors and the ones with the invasion of < 1/2 of the myometrium. A correlative

Table 3. The content of tumour-associated macrophages and microvessels density in Twist-positive and Twist-negative endometrial carcinomas

Investigated parameters	Twist-positive ECE	Twist-negative ECE
Number of TAMs		
Intratumoural	15.3 ± 1.9 cells/f.v.	15.3 ± 2.1 cells/f.v.
In stroma	30.2 ± 3.7 cells/f.v.*	18.0 ± 2.4 cells/f.v.**
MVD	46.5 ± 5.4 vessels/mm^2	34.3 ± 4.7 vessels/mm^2

*p < 0.05 as compared with the content of intratumoural TAMs; **p < 0.05 as compared with the content of stromal TAMs in Twist-positive endometrial tumours; TAMs — tumour-associated macrophages; ECE — endometrioid carcinoma of the endometrium; MVD — microvessels density

Table 4. The content of tumour-associated macrophages and microvessels density in endometrial carcinomas with different proliferative potential, differentiation degree, depth of tumour invasion into the myometrium, and the stage of tumour progression

Investigated parameters	Number of intratumoural TAMs, cells/f.v.	Number of stromal TAMs, cells/f.v.	MVD, number of vessels/mm^2
IP < Me	10.0 ± 1.3	19.1 ± 3.7	29.5 ± 5.0
IP > Me	15.7 ± 2.1	23.1 ± 3.0	40.0 ± 6.4*
ECE differentiation degree			
G2	11.7 ± 2.1	21.7 ± 3.4	26.6 ± 4.8
G3	14.3 ± 1.9	23.9 ± 2.9	40.8 ± 5.8**
Tumour invasion into the myometrium			
< 1/2	9.1 ± 1.2	14.8 ± 2.4	24.0 ± 5.7
>1/2	14.3 ± 1.8	24.9 ± 3.0***	41.4 ± 5.3***
Stage of tumour progression			
Ia + Ib	11.4 ± 2.1	18.2 ± 2.8	27.2 ± 4.7
Ic	13.3 ± 1.8	23.9 ± 3.1	42.1 ± 5.4
II	16.0 ± 2.3	22.8 ± 2.9	50.6 ± 5.9****
III	20.7 ± 2.2****	25.8 ± 2.9	48.9 ± 5.6****

*p < 0.05 as compared with the index at LI < Me; **p < 0.05 as compared with the index in G2-tumors; ***p < 0.05 as compared with the index during the tumour invasion into the myometrium < 1/2; ****p < 0.05 as compared with the index at Ia + Ib stage of tumour progression; TAMs — tumour-associated macrophages; MVD — microvessels density; ECE — endometrioid carcinoma of the endometrium

relationship of moderate density (R = 0.4, p < 0.05) was found between MVD and IP in the investigated endometrial carcinomas.

While determining the content of TAMs and MVD in ECE depending on the stage of tumour progression, it was found that the number of both intratumuoral and stromal TAMs was gradually increasing starting with Ia+Ib towards Ic, stages II and III of the disease, and MVD was twice higher in tumours of stage II and III as compared with the tumours on Ia + Ib stages of tumour progression.

Taking into consideration the scientific data about the dependence of angiogenic processes in tumours on the content of TAMs [11, 22], MVD in ECE were determined depending on the content of TAMs. A simultaneous increase in MVD and the number of intratumoural and stromal TAMs (15.9 ± 2.3 and 22.7 ± 2.5 cells/f.v., respectively) was demonstrated as compared with their content in tumours with low MVD (MVD<Me), 10.7 ± 2.2 and 15.3 ± 2.4 cells/f.v., respectively, p < 0.05 (Fig. 4).

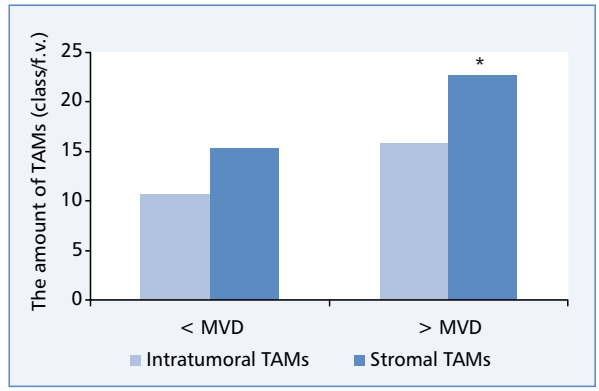

Figure 4. The determination of the relationship between the content of intratumoural and stromal tumour-associated macrophages and microvessels density in endometrial carcinomas; *p < 0.05 as compared with the index in endometrioid carcinoma of the endometrium with microvessels density < Me; TAMs — tumour-associated macrophages; MDV — microvessels density

In the group of tumours, invading less than 1/2 of the myometrium, evident correlative relationships were observed between MVD and the content of intratumoural and stromal TAMs ($R = 0.52$ and $R = 0.68$, $p < 0.05$, respectively), which confirms the dependence of angiogenic and invasive processes in endometrial carcinomas on the content of TAMs. However, the correlative relationships between MVD and the content of TAMs were absent in endometrial carcinomas, which deeply invaded the myometrium.

Discussion

The latter may be related to the fact that even in the initial stages of the invasive process, tumour cells induce the expression of the vascular endothelial growth factor (VEGF, promoting the activation of angiogenesis and remodelling of vessels) in macrophages and matrix metal proteinases (which ensure the destruction of the basal membrane). With further progression of the neoplasm, the activation of endothelial cells is most likely to result from the impact of many factors, including circulating inflammatory cytokines, such as tumour necrosis factor (TNF) and interleukins (IL), reactive oxygen intermediates (ROI), etc. [8, 9–11, 23].

Therefore, the study demonstrated the increase in Twist expression and the content of TAMs in ECE, which was associated with such tumour progression indices as low differentiation degree, deep tumour invasion into the myometrium, and the increase in MVD. At the same time, a correlative relationship was determined between such components of the tumour microenvironment as TAMs and MVD and the increase in the content of TAMs in stroma and MVD in Twist-positive ECE. The reasons for this interaction lie in the functional properties of the mentioned markers. It is well-known that Twist promotes the polarization of M1-macrophages into M2-macrophages, and the latter, in their turn, produce several cytokines, chemokines, and growth factors, including VEGF, which, in addition to activating neoangiogenesis, fulfils a function of chemoattractant, getting TAMs and tumour-associated fibroblasts (one of the main sources of VEGF) involved in hypoxic regions of the tumour, which increases MVD [10, 22–26]. It was determined that TAMs may induce EMT via the activation of EGFR which, in its turn, promotes the expression of ERK1/2, Slug, and vimentin [27].

It should be noted that the formation of new vessels leads to further progression of the neoplasm, as tumour angiogenesis is functionally inadequate — the endothelium of such vessels is not homogeneous in its structure — it is faulty and intermittent which promotes increased intravasation of tutor cells [28]. The uneven location of microvessels in the tumour complicates the efficient supply of oxygen, which reduces the response of the tumour to radiation therapy. In addition to the abovementioned, tumour blood vessels promote avoiding the immune response due to the absence of reaction to the activation of inflammation, thus creating an immune-tolerant tumour microenvironment [23].

The observed phenotypic characteristics of tumour cells and tumour microenvironment was associated with certain morphological specificities of endometrial carcinomas. For instance, in some tumours with positive expression of Twist, were found structures, described in scientific literature as the ones observed in ECE with EMT traits. These are areas with the accumulation of histiocyte-like cells with hyperchromatic nuclei, small groups of glands, diffusely located in the myometrium or microcystic, elongated, and fragmented glands (MELF) [29–31]. Many authors demonstrated that the mentioned morphological structures in endometrial tumours are often associated with decreased expression of E-cadherin, nuclear expression of β-catenin, and inhibited expression of ER and PR along with the deep invasion of the myometrium and unfavourable prognosis of the disease. It was shown that ECE with the MELF pattern of invasion is notable for the increase in MVD in the tumour stroma, which, in the authors' opinion, may be a predictive marker of the unfavourable clinical course [31].

On the contrary, Twist-negative endometrial tumours had a different pattern of invasive growth. Such ECEs often had large, convoluted glands, tightly surrounding the myometrium, and "invasive front areas". As it was shown in the authors' previous study while investigating the morphological traits of such neoplasms, they invaded the myometrium by large groups of tumour cells in the form of solid bands which is a morphological manifestation of collective migration [17]. These results agree with the data of other researchers who demonstrated that such morphological traits of ECE are associated with the decreased expression of EMT markers [29, 30]. As noted above, in the present study, half of the tumours of patients with metastases were Twist-negative. This is consistent with other authors providing evidence that EMT is not required for metastasis in vivo. [32]. Some authors believe that the motility of cells with preserved adhesive properties may be a more efficient way of spreading for transformed cells compared to single cells [5].

At the same time, many authors proved that tumour cells are remarkable for epithelial-mesenchymal plasticity, due to which a malignant neoplasm has cells with epithelial, mesenchymal, and even hybrid phenotype (co-expression of both epithelial and mesenchymal markers) which enhances its ability to form metastases [4, 20, 22, 33, 34].

Conclusions

Thus, the presented study demonstrated that invasion and metastasis of ECE may occur in the setting of various molecular changes in tumour cells and tumour microenvironment, particular, Twist-positive endometrial carcinomas have a higher content of stromal TAMs and MVD as compared with the same indices in Twist-negative neoplasms. The identified differences are associated with various morphological features of invasive processes in the endometrium and can be used as markers of possible ways of invasion and metastasis of endometrial cancer and the aggressiveness of the tumour process in patients.

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Conflict of interest

The authors declare to have no conflict of interest.

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