

PECULIARITIES OF EPITHELIAL-MESENCHYMAL TRANSITION IN ENDOMETRIAL CARCINOMAS

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Epithelial-mesenchymal transition is an important component of tumor progression, due to which the cells of malignant neoplasms acquire invasive and migratory properties. Analysis of the literature and our own data show that the activation of proteins involved in epithelial-mesenchymal transition crucially affects the progression of endometrioid carcinoma of the endometrium and the significant variability of their expression could determine the clinical and morphological heterogeneity of this cancer. The most aggressive endometrioid carcinomas of the endometrium are characterized by a hybrid epithelial-mesenchymal phenotype, which is often associated with a collective type of invasion of endometrial tumor cells into the myometrium.

Key Words: endometrioid endometrial carcinoma, epithelial–mesenchymal transition, transcription factors, Snail, Twist, ZEB.

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Endometrial cancer (EC) is one of the most common oncogynecological diseases in Ukraine and developed countries [1, 2]. However, despite the predominantly favorable course of this disease, which in most cases (70–80%) is diagnosed as endometrioid carcinoma of the endometrium (ECE) at stage I–II with a 5-year survival of 95%, almost 20% of patients, sometimes with early EC stages, have relapses. According to longitudinal research, the clinical EC polymorphism, even within the same morphological structure of the tumor, is due to the heterogeneity of its molecular characteristics [3]. In this regard, much attention is paid to the study of the molecular features of ECE, in particular, the signaling pathways that contribute to the invasiveness and metastasis of this form of cancer.

Presently it is established that the leading role in the progression of malignant neoplasms is played by epithelial-mesenchymal transition (EMT) — an important component of both normal physiological processes (embryonic development and organogenesis, cell migration, tissue repair) and pathological conditions (fibrosis, development of invasive and migratory properties of malignant neoplasms). EMT is a multistage morphogenetic process, in which epithelial cells lose their characteristic features and acquire the properties and characteristics of mesenchymal cells (loss of apical-basal cell polarity, intercellular adhesion and integrity of the basement membrane, reorganization of cytoskeleton, i.e. the increased release of actin between the fibrous structures and the replacement of cytokeratin intermediate filaments by vimentin) [4]. That is, EMT is characterized by gradual remodeling

of the phenotype of the cells, their loss of epithelial features and progressive enhancement of mesenchymal features. According to Qin *et al.* [5], EMT is a “strategy” of adaptation and survival of malignantly transformed cells through mimicry; by partially losing the expression of epithelial markers and acquiring mesenchymal properties, they increase their chances of survival, separating from the primary tumor, migrating to other organs and forming secondary tumors.

An important aspect of molecular transformations in EMT is the reversibility of this process. It has been shown that tumor cells with mesenchymal properties undergo mesenchymal-epithelial transition (MET) during the formation of secondary lesions in distant organs [6]. It has been proven that malignantly transformed cells are characterized by epithelial-mesenchymal plasticity, due to which cells with epithelial, mesenchymal and even hybrid phenotype (in cells co-expressing both epithelial and mesenchymal markers) are present in neoplasms, which indicates tumor potency to metastasis [7–10]. There are reports that the formation of metastases may occur while maintaining the expression of epithelial markers in tumor cells. *Ex vivo* experimental studies on 3D cultures have shown that breast cancer cells can undergo a so-called partial EMT program, while retaining epithelial properties and metastasizing in clusters. However, these metastatic clusters of malignant cells express epithelial proteins, including cytokeratin-14, E-cadherin and P-cadherin, and do not express EMT markers such as Twist, Snail1/2 and vimentin [6].

It is important to note that the activation of EMT in the tumor may depend on the type of tissue and molecular characteristics of the tumor. It has been shown that breast cancer cells of the basal-like subtype respond to transforming growth factor- β (TGF- β) by an activation of the ZEB1 transcription factor and EMT induction. In contrary, in the epithelial breast cancer cells of luminal subtype, the promoter of the ZEB1 gene is blocked, which allows these cells not to respond to the action of TGF- β and remain in the

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Abbreviations used: DUBs – deubiquitinases; E – epithelial; EC – endometrial cancer; ECE – endometrioid carcinoma of the endometrium; EMT – epithelial-mesenchymal transition; EMT-TF – EMT transcription factors; HGF – hepatocyte growth factor; M – mesenchymal; MET – mesenchymal-epithelial transition; MMP – matrix metalloproteinases; ncRNA – non-coding RNA; TGF- β – transforming growth factor.

epithelial state [9]. That is, EMT implementation depends on the molecular phenotype of tumors of similar histogenesis. Nevertheless, one should be aware that the extrapolation of the conclusions about mechanisms of EMT regulation in tumor progression inferred from *in vitro* models to *in vivo* setting could be challenging [4].

It should be noted that the EMT/MET in the endometrium are of particular importance in normal physiological processes of female reproductive system, since cyclic restoration of the functional epithelial layer involves both migration of epithelial cells from the proliferating glands and MET from the mesenchymal cells of proliferating stroma with EMT/MET balance being of importance for the full repair of the endometrium in the desquamation phase [11]. The disruption of normal processes in the endometrium leads to a number of pathologies, including endometrial hyperplasia, endometriosis and adenomyosis. Malignant transformation of the endometrium is a complex multi-stage process, which in most cases is characterized by slow progression from atypical hyperplasia. The disease most often occurs in menopausal women, against the background of hyperestrogenism, obesity and diabetes, which in some way affects the biological characteristics of the tumor. Therefore, the course and progression of the disease, in particular the features of metastasis of this form of cancer may differ from tumors of other genesis [12, 13]. It is shown that the variability of EC clinical course is caused by the molecular polymorphism of tumors, which significantly affects the low effectiveness of cancer therapy [14, 15].

It is known that molecular and morphological changes of epithelial cells during EMT are controlled by several extracellular and intracellular signaling pathways. EMT is induced via the main extracellular

signaling with the involvement of Wnt, TGF- β , Notch, EGF, FGF, hepatocyte growth factor (HGF) etc., which through a cascade of intracellular kinases induce EMT transcription factors (EMT-TF) — genes of the SNAIL, ZEB i TWIST families [16–18] (Fig. 1).

One of the most studied EMT regulators is the Wnt/Frizzled signaling pathway. It has three branches — canonical (β -catenin-dependent) and two non-canonical — Wnt/ Ca^{2+} -pathway and the pathway of cellular polarization. With the progression of the tumor process, the highly conserved canonical Wnt/Frizzled- β -catenin-dependent pathway is most often activated [19]. This leads to destruction of the E-cadherin/ β -catenin complex and intercellular connections, translocation of E-cadherin, release of β -catenin, and accumulation of these proteins in the cytoplasm. Normally, the expression of β -catenin in the cytoplasm is kept at low level due to its binding to the corresponding proteins and subsequent 26S-proteasomal degradation. However, in pathological conditions, as a result of changes in the functioning of genes that ensure the degradation of β -catenin (e.g. mutations in the *APC* gene), excessive β -catenin is translocated to the nucleus. In the nucleus β -catenin forms a transcription complex with T-cell- and lymphoid factors, which activates the expression of a number of target genes, including oncogene *C-MYC*, *cyclin D1*, *VEGF*, *Axin-2*, *Snail*, *ZEB1*, *VIM* etc. [5, 20]. That is, the activation of the canonical Wnt/ β -catenin signaling pathway increases the proliferative, invasive and angiogenic potential and triggers the EMT process in malignant neoplasms of various genesis, including ovarian, cervical and EC [5, 21, 22] (Fig. 2).

In recent years, a number of studies have been analyzing the functioning of the Wnt/ β -catenin pathway in endometrial carcinomas. It has been

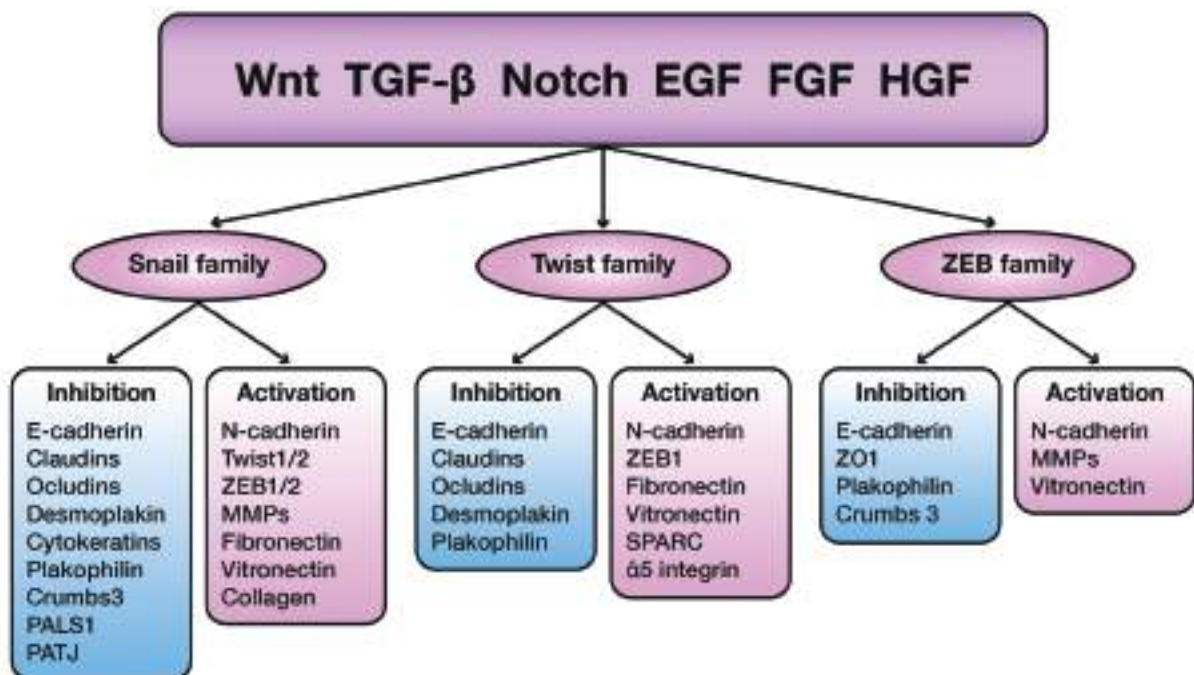


Fig. 1. The main factors that regulate EMT by activating genes of the Snail, Twist and ZEB families

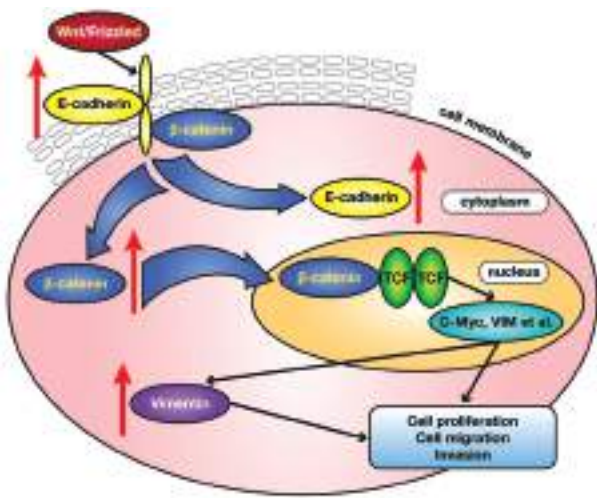


Fig. 2. Induction of EMT in tumor cells via canonical Wnt signaling

noted that the activation of this pathway in conditions of hyperestrogenism may be one of the causes of EC, because of a synergistic effect on the effectors of estrogen signaling and Wnt/β-catenin pathway [22]. Also, the aberrant functioning of β-catenin upon Wnt pathway activation is associated with decreased estrogen receptor expression, which is often observed in the course of EC progression [23]. In contrast, progesterone can inhibit Wnt/β-catenin signaling [24].

Another important EMT activator is cytokine TGF-β, which phosphorylates to bind to its TGFβRI/II receptors on the surface of a malignantly transformed cell and via the SMAD2/SMAD3/SMAD4 signaling pathway promotes the activation of Snail, Twist and ZEB. In addition, TGF-β can induce EMT via PI3K/AKT or Rho-like GTFase (Rho, Rac and Cdc42) as well as MARK signaling reducing cell-to-cell contacts and activating the reorganization of the cytoskeleton [25] (Fig. 3).

To date, it has been shown that during cancer progression a cross-talk between TGF-β and WNT pathways and between TGF-β and Notch pathways could develop, which could enhance EMT and metastasis [26].

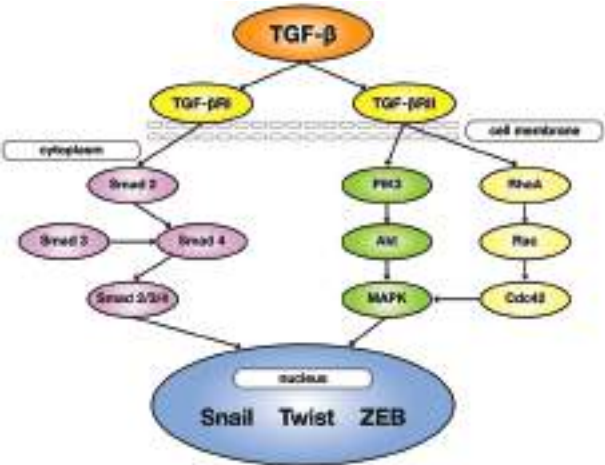


Fig. 3. TGF-β-dependent pathways of EMT-TF activation of Snail, Twist and ZEB families

At the same time, inhibition of the TGF-β pathway may occur due to the action of progesterone. In endometrial carcinoma cell lines (HEC-1B, RL-95 and Ishikawa) progesterone has been shown to inhibit the expression of TGF-β and SMAD receptors, increase the expression of E-cadherin and decrease the expression of vimentin, therefore lowering cancer cell migratory ability. Thus, the authors suggest the feasibility of progesterone therapy for EC treatment [27]. Similar findings have been made by other researchers, who have shown that progesterone alone or in combination with calcitriol can be used for treatment of EC and ovarian cancer and that TGF-β and CYP24A1 signaling proteins can be effective surrogate markers of treatment response [28]. In recent years, a series of studies have shown that components of the TGF-β- and Wnt/β-catenin pathways may be considered as potential therapeutic targets in EC [29–30].

It should be noted that a significant contribution to the activation of EMT in malignant neoplasms is made by the tumor microenvironment. In particular, it has been shown that tumor-associated fibroblasts are among the main sources of TGF-β. In experimental studies on endometrial carcinomas *in vitro* and *in vivo*, it was found that TGF-β is involved in the differentiation of stromal fibroblasts into tumor-associated myofibroblasts [31–32]. The latter, together with tumor-associated macrophages (type II macrophages), were found to actively secrete HGF and the chemokine CXCL12, which interact with their c-MET and CXCR4 receptors on EC cells. In this case, HGF/c-MET phosphorylates the Akt protein, which promotes EMT activation and proliferation of epithelial cells by modulating the transcription of cyclin D1, and CXCL12/CXCR4 induces the expression of RAS, PI3K, NF-κB, ERK1/2 which are the components of many signaling pathways, including those associated with EMT (RAS-MAPK, PI3K-AKT-mTOR, NF-κB, MAPK/ERK), thus leading to increased migration and invasion of neoplastic cells [31, 33, 34].

According to the results of our studies, endometrial carcinomas in patients with stage III EC are characterized by an increased content of CXCL12⁺-fibroblasts in the tumor microenvironment with a simultaneous decrease of CXCL12 expression and increased CXCR4 expression in tumor cells correlating with a low differentiation grade, deep invasion of the tumor into the myometrium and the presence of lymph node metastases [35].

The study of tumor-stroma interaction in endometrial carcinoma, in particular the relationship between TGF-β-signaling and estrogen receptor α (ERα) expression, is the subject of a study by Yoriki *et al.* [36]. The authors found an increase in ERα expression in EC cells, which is associated with an increase in the expression of TGF-β and ERα in stroma cells. In addition, it was found that the interaction between tumor-associated myofibroblasts and tumor cells can enhance the metastatic potential of the latter by paracrine induction of TGF-β and EMT activation.

Importantly, not only the tumor microenvironment activates EMT in tumor cells, in turn, the expression of EMT factors in tumor cells can contribute to changes in the functioning of immune cells, enhancing immunosuppression [37, 38]. Thus, it was found that the simultaneous activation of Twist transcription factor and c-Myc oncoprotein in malignant cells causes gene expression and production of CCL2 and IL13 cytokines. The latter polarize type I macrophages into M2 macrophages and recruit them to the tumor, i.e. reprogram the tumor microenvironment [39]. In our study, we found a significantly increased content of M2 macrophages in the stromal component of Twist-positive ECE compared with their number in Twist-negative endometrial carcinomas [40]. According to the modern literature, M2-macrophages are one of the leading components of the tumor microenvironment, which secrete various factors that stimulate the proliferation of tumor cells, increasing their migratory capacity [31, 33].

It should be noted that an important factor in the regulation of EMT is the influence of non-coding RNA (ncRNA) — miRNA 19–25 nucleotides in length, and lncRNA (longRNA), more than 200 nucleotides in length. ncRNAs control many biological processes in the cell and EMT is no exception [41]. Thus, it was shown that the expression of miR-200 family miRNAs (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) and miR-205 could block TGF β -induced EMT by inhibition of ZEB1 and ZEB2 activity [42]. Targets of ncRNA can be various components of EMT signaling cascades such as E- and N-cadherins, and EMT-TF or cellular receptors, small GTPases, proteins associated with cellular organelles, cytoskeletal proteins, intercellular contact components and cell cycle regulators, etc. Deng *et al.* [43] showed that miR-195 can reduce the migration and invasion of endometrial tumor cells (cancer cell lines AN 3-CA and Hec1A) through the G-protein-bound estrogen receptor 1 and the PI3K/AKT signaling pathway. There was observed a decrease in the level of mRNA and protein of the tissue inhibitor of metalloproteinase 2 and inhibition of the expression of matrix metalloproteinases (MMP) MMP-2 and -9 [43]. It has been established that lncRNA and miRNA could be divided into two categories — pro-EMT and anti-EMT, although some of them perform opposite functions depending on the histogenesis of tumors [44–45].

Many studies have also been devoted to the role of ncRNA in the regulation of EMT in EC. Thus, it has been shown that miR-326 inhibits EMT through the transcription factor Twist1 [46]. The authors believe that miR-326 can be used as a biomarker of the disease or a therapeutic target for patients with ECE. According to the results of our studies, it was found that increased expression of oncosuppressive miR-34a and miR-101 in ECE cells is associated with increased levels of E-cadherin and negative expression of vimentin, i.e. with absence of EMT signs [47]. Based on a meta-analysis, Donkers *et al.* [48] and Piergen-

tili *et al.* [49] reported the data on the peculiarities of ncRNA functioning in endometrial carcinomas, and identified their primary and secondary targets, including EMT markers. The authors selected several of the most significant ncRNAs, which are proposed to be used as prognostic EC markers.

As mentioned above, EMT-TF are the triggers of mitogenic signals in EMT. They are represented by the families of Snail, Twist and ZEB proteins, the main function of which is to inhibit the expression of epithelial phenotype genes and to activate mesenchymal phenotype gene expression [16]. Each of these proteins possesses unique properties, due to differences in the structure. Thus, EMT-TF of the Snail family (Snail, Slug and Smuc) usually contain the SNAG domain at the N-terminus and the zinc finger domains at the C-terminus. Snail additionally contains a serine-rich domain that controls its stability and nuclear localization. The Twist family (Twist1 and Twist2) are transcription factors that have similar structure with the basic helix-loop-helix domain, while the C-terminal Twist boxes determine their transcriptional activity, and interact with the DNA region of the target gene of E-cadherin in the form of a homo- or heterodimer. The ZEB family contains various regulatory domains, which include zinc finger clusters at the N- and C-terminus, homeodomain, SMAD-binding domain, and CtBP-binding sites [18, 50].

It is important to note that EMT-TFs of the Snail family are the first link in EMT, because in addition to inhibiting epithelial markers, they activate the synthesis of mesenchymal proteins, including EMT-TF of the Twist and ZEB families [16]. In addition, the existence of certain interdependence between EMT-TF Snail1, Twist and ZEB1 is shown. It has been found that reduced Twist expression is associated with low Snail1 activity; in turn, Snail1 can induce Twist1 mRNA, and thus increase its protein production. Maximum expression of both Snail1 and Twist is required for maximum ZEB1 mRNA expression [51].

Despite the fact that the transcription of EMT-TF is due to the influence of components of many signaling pathways, the regulation of the stability of their protein products, nuclear or cytoplasmic localization is provided by post-translational modifications that occur after the synthesis to form a mature protein products. By phosphorylation, ubiquitination, sumoilation, acetylation, methylation or glycosylation, post-translational modification alters the expression of EMT-TFs, affecting their stability, localization and transcriptional activity [18, 50, 52].

In addition, another mechanism of EMT-TF stabilization has been identified — via increased levels of deubiquitinases (DUBs), which remove ubiquitin and play an important role in cancer development through regulation of transcription, DNA repair, and cell cycle progression [53]. In particular, it has been shown that overexpression of deubiquitinase DUB3 increases the levels of Slug and Twist proteins in a dose-dependent manner, and DUB3 knockdown — reduces them.

Importantly, DUB3, by interacting with Slug and Twist, prevents their degradation, thereby helping to maintain stem, migratory and invasive properties of tumor cells. DUB3 has been identified as an early response gene that is activated after exposure to inflammatory cytokines such as interleukin-6 and plays a critical role in the metastasis of breast cancer cells.

It should be noted that a prerequisite for the implementation of regulatory functions of EMT-TF is their location in the nucleus. Stabilization and accumulation of EMT-TF in the cytoplasm, as well as ubiquitination and proteasomal degradation can lead to their penetration into the nucleus [18, 50]. As shown by Sreekumar *et al.* [54], the cytoplasmic localization of EMT-TF (in particular the expression of ZEB2 detected by immunohistochemical analysis) may be due to the lack of effective antibodies for this research. Although in most studies by other authors, ZEB2 expression is visualized in the cytoplasm, the authors created a new antibody SIP1/ZEB2, which allowed to detect the expression of ZEB2 in the nucleus [54].

A number of studies have been devoted to analysis of the expression of EMT markers and their prognostic value in endometrial carcinoma, but the results of these studies are far from unambiguous. Thus, Tanaka *et al.* [55] showed that positive expression of Snail and Slug in cell nucleus of endometrial carcinoma cells was observed in 16.9% and 3.7% of cases, respectively. Snail expression increased and E-cadherin decreased in low differentiated endometrial tumors with deep invasion in the myometrium and metastasis, compared with G1 or G2 tumors, with myometrial invasion $< 1/2$, and without metastatic lesions. The authors believe that low expression of E-cadherin and high nuclear expression of Snail may be independent prognostic markers in EC, and high expression of Slug — a crucial factor in EMT. A similar association between high Snail expression in the nucleus, high HIF-1 α , and low E-cadherin in ECE was also found by other researchers [56].

Xie *et al.* [57] and Feng *et al.* [58] in the study of Twist expression in ECE considered tumor cells with cytoplasmic-nuclear expression of the marker as positive. The authors found a negative correlation between Twist expression and E-cadherin and concluded that high Twist expression and low E-cadherin expression could be used as an important indicator for predicting metastatic potential and detecting aggressive ECE phenotypes.

Senol *et al.* [59] investigated nuclear expression of Twist, ZEB1, ER and PR in epithelial and stromal cells of unaltered and hyperplastic endometrial tissue and in ECE. The authors found that the number of cases with positive Twist expression in epithelial cells of atypical hyperplasia and EC increased compared to hyperplasia and unaltered endometrium and the number of cases with ZEB1 expression in EC increased significantly compared to hyperplasia. In contrast, positive expression of Snail and Slug was found in both unaltered and hyperplastic endometrium and in EC cells.

However, the authors studied the expression of these markers in the peritumoral stroma of the endometrium and found that the loss of expression of β -catenin, Twist, Snail, Slug, ER and PR can be an important marker of the transition from atypical hyperplasia to malignant endometrial neoplasms.

Sadłacki *et al.* [60] demonstrated that Snail, Twist1, and ZEB1 expression in the nucleus and Twist2 in the cytoplasm of endometrial carcinoma cells is associated with such clinical and pathological characteristics of the tumor process as the differentiation grade, invasion into lymphatic vascular and cervical canal, and lymph node metastasis. In contrast, ZEB2 expression was significantly higher in tumors of patients with ovarian metastases than in patients without metastases, and Slug expression was significantly higher in tumors of patients with stage III–IV by International Federation of Gynaecology and Obstetrics (FIGO) compared to that with less advanced EC. In addition, Slug expression was significantly increased in endometrial carcinomas in patients with deep tumor invasion into the myometrium and the spread of the tumor to the ovary and with distant metastases compared with patients without these adverse events. Analyzing the results, the researchers concluded that only high values of Slug and ZEB2 expression in primary endometrial tumor could serve as prognostic markers of adverse clinical course of this form of cancer [60].

In our study, we found a tendency to increased nuclear expression of Twist1 in low-differentiated and deeply invasive ECE, with a simultaneous increase of E-cadherin expression and a decrease of vimentin expression compared with similar values in G2 tumors and those invading less than 50% of the myometrium [40]. In addition, in some Twist-positive carcinomas, positive expression of E-cadherin was observed, and in Twist-negative ECE expression of vimentin was detected. In addition, we found (unpublished data) that in 66.7% of cases of moderately differentiated ECE with metastasis, high (above median values) expression of vimentin was observed, and in 33.3% of such cases — positive Twist expression was detected along with complete absence of E-cadherin expression. In contrast, metastatic carcinomas of low differentiation grade were characterized by positive expression of vimentin only in 14.3% of cases, by high expression of E-cadherin in 37.5% of cases and positive expression of Twist in 25.0% of cases. The obtained data suggest that the invasion and metastasis of ECE of moderate differentiation grade are associated with higher expression of mesenchymal markers than in ECE of low differentiation grade. It is possible that the positive expression of vimentin in a part of Twist-negative ECE may be due to activation of other transcription factors (Slug, ZEB1 and ZEB2) or reduced functioning of other adhesive molecules (β -catenin, CD44, CD24, etc.), which contribute to development of EMT features. Our results suggest that a large part of endometrial tumor cells are characterized by a hybrid epithelial-mesenchymal phenotype.

Therefore, the study of Krögera *et al.* [61], which showed that the expression of EMT-TF is associated with the mesenchymal or hybrid phenotype of malignant tumors, is of considerable interest. The authors identified populations of breast cancer cell lines with epithelial (E) (CD104^{high}/CD44⁻), mesenchymal (M) (CD104⁻/CD44^{high}) and hybrid phenotype (CD104⁺/CD44^{high}), in which single cell is co-expressing both epithelial and mesenchymal markers. In experiments both *in vitro* and *in vivo* (in mice), the authors showed that the transition of cells from state E to the state of the hybrid E/M phenotype requires stimulation of the expression of Snail or Twist, Slug. A necessary condition for further transition to the M-phenotype is the active functioning of the ZEB1 protein. In addition, it was found that in triple-negative breast cancer, cells with hybrid E/M-phenotype possess more oncogenicity, stem properties, potential for invasiveness and metastasis while cells with M-phenotype exert lower oncogenic ability and stemness. It should be noted that the mechanical mixture of tumor cells with exclusively E-phenotype and M-phenotype was less oncogenic and aggressive than cells with a hybrid E/M-phenotype.

Another group of researchers [62], who studied the dependence of E- and N-cadherin expression on their topology in endometrial carcinomas, showed that high E-cadherin expression was observed mainly in the center of the tumor and was lower in the invasive front, while N-cadherin expression changed oppositely. In addition, a decreased E-cadherin expression was also observed in endometrial carcinoma cells invading the lymphatic space.

We have found the association of morphological features of ECE of low differentiation grade with the features of expression of adhesion markers and EMT, i.e. E-cadherin, β -catenin, CD44, CD24 and vimentin; also we have revealed the relation between the level of expression of these markers with features of EC cell invasion [63]. In addition, morphological analysis of the studied ECE revealed that tumors with positive Twist expression, in most cases, were characterized by significant variability in the number of cells invading the myometrium: from single cells to small clusters of tumor cells. Twist expression in such tumors was found mainly in solid areas, which were often located close to or directly in the myometrium. Often in such tumors the phenomenon of “gland in the gland” or cystic-diffuse growth of glands in the myometrium was observed [40]. In contrast, in Twist-negative endometrial neoplasms we found both high expression of epithelial markers such as E-cadherin, β -catenin, CD24 and mesenchymal marker — CD44, along with low expression of mesenchymal marker — vimentin, i.e. hybrid E/M phenotype, which in some cases was associated with the migration of tumor cells into the myometrium in the form of collective invasion.

The presence and causes of this type of invasion in endometrial carcinomas are discussed by Wilson *et al.* [64] showing that the collective migration of ECE

cells can occur with simultaneous mutations in the *ARID1A* and *PI3K* genes. The authors note that the *ARID1A* gene, which encodes the BAF250a protein and maintains the epithelial cell phenotype, is mutated in approximately 30–40% of ECE cases. Mutations in *PI3K* that promote EMT are observed in 84% ECE cases and are considered one of the initial changes in the occurrence of hyperplasia and EC. In experimental studies on a model of mouse endometrial carcinoma, it was found that in most cases *PI3K* mutations in ECE were associated with abnormalities in the *ARID1A* gene and the loss of the latter inhibits the effect of *PI3K* on EMT-associated proteins (in particular, in cells transfected with both *mtARID1A* and *mtPI3K* there is a decrease in the induction of Twist1), which leads to partial inhibition of the EMT phenotype and the collective invasion of endometrial tumor cells into the myometrium.

Thus, the analysis of literature sources and the data of own research shows that the activation of proteins leading to EMT definitely affects the progression of endometrial carcinoma and the significant variability of their expression can determine the clinical and morphological heterogeneity of this form of cancer. The most aggressive ECEs are characterized by a hybrid epithelial-mesenchymal phenotype, which is manifested by the expression of epithelial and mesenchymal markers, often associated with a collective type of invasion of endometrial tumor cells into the myometrium.

REFERENCES

1. Fedorenko ZP, Mikhailovich YU, Gulak LO, *et al.* Cancer in Ukraine, 2019–2020. Morbidity, mortality, indicators of oncology service activity. Bul Nat Registry of Ukraine 2021; 22: 82 p. <http://www.ncru.inf.ua>
2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics 2021. CA. Cancer J Clin 2021; 71: 7–33. doi: 10.3322/caac.21654
3. Nyen TV, Moiola CP, Colas E, *et al.* Modeling endometrial cancer: past, present, and future. Int J Mol Sci 2018; 19: 2348. doi: 10.3390/ijms19082348
4. Francou A, Anderson KV. The epithelial-to-mesenchymal transition in development and cancer. Annu Rev Cancer Biol 2020; 4: 197–220. doi: 10.1146/annurev-cancerbio-030518-055425
5. Qin J-H, Wang L, Li Q-L, *et al.* Epithelial-mesenchymal transition as strategic microenvironment mimicry for cancer cell survival and immune escape? Genes Dis 2017; 4: 16–8. doi: 10.1016/j.gendis.2016.10.001
6. Jolly MK, Ware KE, Gilja S, *et al.* EMT and MET: Necessary or permissive for metastasis? Mol Oncol 2017; 11: 755–69. doi:10.1002/1878-0261.12083
7. Williams ED, Gao D, Redfern A, Thompson EW. Controversies around epithelial-mesenchymal plasticity in cancer metastasis. Nat Rev Cancer 2019; 19: 716–32. doi:10.1038/s41568-019-0213-x
8. Bhatia S, Wang P, Toh A, Thompson EW. New insights into the role of phenotypic plasticity and EMT in driving cancer progression. Front Mol Biosci 2020; 7: 71. doi.org/10.3389/fmolb.2020.00071
9. Chaffer CL, Juan BPS, Lim E, Weinberg RA. EMT, cell plasticity and metastasis. Cancer Metastasis Rev 2016; 35: 645–54. doi: 10.1007/s10555-016-9648-7

10. **Liao T-T, Yang M-H.** Hybrid epithelial/mesenchymal state in cancer metastasis: clinical significance and regulatory mechanisms. *Cells* 2020; **9**: 623. doi: 10.3390/cell9030623
11. **Tolibova GK, Tral TG, Ailamazyan EK, et al.** Molecular mechanisms of cyclic transformation of the endometrium. *J Obstetrics Women's Dis* 2019; **68**: 5–12. doi.org/10.17816/JOWD6815-12 (in Russian)
12. **Sanderson PA, Critchley HO, Williams AR, et al.** New concepts for an old problem: The diagnosis of endometrial hyperplasia. *Hum Reprod Update* 2017; **23**: 232–54. doi: 10.1093/humupd/dmw042
13. **Chiu H-C, Li C-J, Yang G-T, et al.** Epithelial to mesenchymal transition and cell biology of molecular regulation in endometrial carcinogenesis. *J Clin Med* 2019; **8**: 439. doi:10.3390/jcm8040439
14. **Buchynska LG, Borykun TV, Iurchenko NP, et al.** Expression of microRNA in tumor cells of endometrioid carcinoma of endometrium. *Exp Oncol* 2020; **42**: 289–94. doi: 10.32471/exp-oncology.2312-8852
15. **Škovierová H, Okajčková T, Strnádel J, et al.** Molecular regulation of epithelial-to-mesenchymal transition in tumorigenesis (Review). *Int J Mol Med* 2018; **41**: 1187–200. doi:10.3892/ijmm.2017.3320
16. **Lamouille S, Xu J, Derynck R.** Molecular mechanisms of epithelial–mesenchymal transition. *Nat Rev Mol Cell Biol* 2014; **15**: 178–96. doi: 10.1038/nrm3758
17. **Ribatti D, Tamma R, Annese T.** Epithelial–mesenchymal transition in cancer: a historical overview. *Transl Oncol* 2020; **13**: 100773. doi: 10.1016/j.tranon.2020.100773
18. **Kang E, Seo J, Yoon H, Cho S.** The post-translational regulation of epithelial–mesenchymal transition-inducing transcription factors in cancer metastasis. *Int J Mol Sci* 2021; **22**: 3591. doi.org/10.3390/ijms22073591
19. **Huang L, Jin Y, Feng S, et al.** Role of Wnt/ β -catenin, Wnt/c-Jun N-terminal kinase and Wnt/ Ca^{2+} pathways in cisplatin-induced chemoresistance in ovarian cancer. *Exp Ther Med* 2016; **12**: 3851–8. doi: 10.3892/etm.2016.3885
20. **Makker A, Goel MM.** Tumor progression, metastasis, and modulators of epithelial–mesenchymal transition endometrioid endometrial carcinoma: an update. *Endocr Relat Cancer* 2016; **23**: 85–111. doi: 10.1530/ERC-15-0218
21. **McMellen A, Woodruff ER, Corr BR, et al.** Wnt signaling in gynecologic malignancies. *J Mol Sci* 2020; **21**: 4272. doi:10.3390/ijms21124272
22. **Goad J, Ko YA, Kumar M, et al.** Oestrogen fuels the growth of endometrial hyperplastic lesions initiated by overactive Wnt/ β -catenin signalling. *Carcinogenesis* 2018; **39**: 1105–16. doi:10.1093/carcin/bgy079
23. **Kasoha M, Dernektsi C, Seibold A, et al.** Crosstalk of estrogen receptors and Wnt/ β -catenin signaling in endometrial cancer. *Cancer Res Clin Oncol* 2020; **146**: 315–27. doi: 10.1007/s00432-019-03114-8
24. **Wang Y, Hanifi-Moghaddam P, Hanekamp EE, et al.** Progesterone inhibition of Wnt/ β -catenin signaling in normal endometrium and endometrial cancer. *Clin Cancer Res* 2009; **15**: 5784–93. doi: 10.1158/1078-0432.CCR-09-0814
25. **Ahmadi A, Najafi M, Farhood B, Mortezaee K.** Transforming growth factor β signalling: Tumorigenesis and targeting for cancer therapy. *J Cell Physiol* 2019; **234**: 12173–87. doi:10.1002/jcp.27955
26. **Derynck R, Muthusamy BP, Saetern KY.** Signaling pathway cooperation in TGF- β -induced epithelial–mesenchymal transition. *Curr Opin Cell Biol* 2014; **31**: 56–66. doi: 10.1016/j.ceb.2014.09.001
27. **Bokhari AA, Lee LR, Raboteau D, et al.** Progesterone inhibits endometrial cancer invasiveness by inhibiting the TGF- β pathway. *Cancer Prev Res* 2014; **7**: 1045–55. doi:10.1158/1940-6207.CAPR-14-0054
28. **Paucarmayta A, Taitz H, Casablanca Y, et al.** TGF- β signaling proteins and CYP24A1 may serve as surrogate markers for progesterone calcitriol treatment in ovarian and endometrial cancers of different histological types. *Transl Cancer Res* 2019; **8**: 1423–37. doi: org/10.21037/tcr.2019.07.36
29. **Fatima I, Barman S, Rai R, et al.** Targeting Wnt signaling in endometrial cancer. *Cancers (Basel)* 2021; **13**: 2351. doi.org/10.3390/cancers13102351
30. **Kurnit KC, Draisey A, Kazen RC, et al.** Loss of CD73 shifts transforming growth factor- β 1 (TGF- β 1) from tumor suppressor to promoter in endometrial cancer. *Cancer Let* 2021; **505**: 75–86. doi: 10.1016/j.canlet.2021.01.030
31. **Sahoo SS, Zhang XD, Hondermarck H, Tanwar PS.** The emerging role of the microenvironment in endometrial cancer. *Cancers (Basel)* 2018; **10**: 408. doi:10.3390/cancers10110408
32. **Subramaniam KS, Omar IS, Kwong SC, et al.** Cancer associated fibroblasts promote endometrial cancer growth via activation of interleukin-6/STAT-3/c-Myc pathway. *Am J Cancer Res* 2016; **6**: 200–13. www.ajcr.us/ISSN:2156-6976/ajcr0021359
33. **Chen Y, Song Y, Du W, et al.** Tumor-associated macrophages: an accomplice in solid tumor progression. *J Biomed Sci* 2019; **26**: 78. doi: 10.1186/s12929-019-0568-z
34. **Li M, Xin X, Wu T, et al.** Stromal cells of endometrial carcinoma promotes proliferation of epithelial cells through the HGF/c-Met/Akt signaling pathway. *Tumour Biol* 2015; **36**: 6239–48. doi: 10.1007/s13277-015-3309-2
35. **Buchynska LG, Movchan OM, Iurchenko NP.** Expression of chemokine receptor CXCR4 in tumor cells and content of CXCL12⁺-fibroblasts in endometrioid carcinoma of endometrium. *Exp Oncol* 2021; **43**: 135–41. doi: 10.32471/exp-oncology.2312-8852.vol-43-no-2.16240
36. **Yoriki K, Mori T, Kokabu T, et al.** Estrogen-related receptor alpha induces epithelial–mesenchymal transition through cancer–stromal interactions in endometrial cancer. *Sci Rep* 2019; **9**: 6697. doi.org/10.1038/s41598-019-43261-z
37. **Chen H-Y, Chiang Y-F, Huang J-S, et al.** Isoliquiritigenin reverses epithelial–mesenchymal transition through modulation of the TGF- β /Smad signaling pathway in endometrial cancer. *Cancers (Basel)* 2021; **13**: 1236. doi.org/10.3390/cancers13061236
38. **Romeo E, Caserta CA, Rumio C, Marcucci F.** The vicious cross-talk between tumor cells with an EMT phenotype and cells of the immune system. *Cells* 2019; **8**: 460. doi:10.3390/cells8050460
39. **Dhanasekaran R, Baylot V, Kim M, et al.** MYC and Twist1 cooperate to drive metastasis by eliciting crosstalk between cancer and innate immunity. *Cancer Biol* 2020; **14**: 202. doi: 10.7554/eLife.50731
40. **Nesina I, Iurchenko N, Nespriado S, Buchynska L.** Twist expression and content of tumour-associated macrophages in endometrial carcinoma. *Oncol Clin Pract* 2021; **17**: 139–47. doi: 10.5603/OCP.2021.0026
41. **Abba ML, Patil N, Leupold JH, Allgayer H.** MicroRNA regulation of epithelial to mesenchymal transition. *J Clin Med* 2016; **5**: 8. doi:10.3390/jcm5010008
42. **Zaravinos A.** The regulatory role of microRNAs in EMT and cancer. *J Oncol* 2015; **2015**: 865816. doi:10.1155/2015/865816
43. **Deng J, Wang W, Yu G, Ma X.** MicroRNA-195 inhibits epithelial–mesenchymal transition by targeting G protein-coupled estrogen receptor 1 in endometrial carcinoma. *Mol Med Reports* 2019; **20**: 4023–32. doi: 10.3892/mmr.2019.10652

44. **Gugnoni M, Ciarrocchi A.** Long noncoding RNA and epithelial mesenchymal transition in cancer. *J Mol Sci* 2019; **20**: 1924. doi: 10.3390/ijms20081924
45. **Landeros N, Santoro PM, Carrasco-Avino G, Corvalan AH.** Competing endogenous RNA networks in the epithelial to mesenchymal transition in diffuse-type of gastric cancer. *Cancers (Basel)* 2020; **12**: 2741. doi:10.3390/cancers12102741
46. **Liu W, Zhang B, Xu N, et al.** miR-326 regulates EMT and metastasis of endometrial cancer through targeting TWIST1. *Eur Rev Med Pharm Sci* 2017; **21**: 3787–93.
47. **Buchynska LG, Borykun TV, Iurchenko NP, et al.** Expression of microRNA in tumor cells of endometrioid carcinoma of endometrium. *Exp Oncol* 2020; **42**: 289–94. doi: 10.32471/exp-oncology.2312-8852
48. **Donkers H, Bekkers R, Galaal K.** Diagnostic value of microRNA panel in endometrial cancer: A systematic review. *Oncotarget* 2020; **11**: 2010–23. doi: 10.18632/oncotarget.27601
49. **Piergentili R, Zaami S, Cavaliere AF, et al.** Non-coding RNAs as prognostic markers for endometrial cancer. *Int J Mol Sci* 2021; **22**: 3151. doi.org/10.3390/ijms22063151
50. **Serrano-Gomez SJ, Maziveyi M, Alahari SK.** Regulation of epithelial-mesenchymal transition through epigenetic and post-translational modifications. *Mol Cancer* 2016; **15**: 18. DOI: 10.1186/s12943-016-0502
51. **Dave N, Guaita-Esteruelas S, Gutarra S, et al** Functional cooperation between Snail1 and twist in the regulation of ZEB1 expression during epithelial to mesenchymal transition. *J Biol Chem* 2011; **286**: 12024–32. doi: 10.1074/jbc.M110.168625
52. **Xu R, Won J-Y, Kim C-H, et al.** Roles of the phosphorylation of transcriptional factors in epithelial-mesenchymal transition. *J Oncol* 2019; ID: 5810465. doi.org/10.1155/2019/5810465
53. **Lin Y, Wang Y, Shi Q, et al.** Stabilization of the transcription factors Slug and Twist by the deubiquitinase Dub3 is a key requirement for tumor metastasis. *Oncotarget* 2017; **8**: 75127–40. doi: 10.18632/oncotarget.20561
54. **Sreekumar R, Al-Saihati H, Emaduddin M, et al.** The ZEB2-dependent EMT transcriptional programme drives therapy resistance by activating nucleotide excision repair genes ERCC1 and ERCC4 in colorectal cancer. *Mol Oncol* 2021; **15**: 2065–83. doi:10.1002/1878-0261.12965
55. **Tanaka Y, Terai Y, Kawaguchi H, et al.** Prognostic impact of EMT (epithelial-mesenchymal-transition)-related protein expression in endometrial cancer. *Cancer Biol Ther* 2013; **14**: 13–9. doi: 10.4161/cbt.22625
56. **Abouhashem NS, Ibrahim DA, Mohamed AM.** Prognostic implications of epithelial to mesenchymal transition proteins (E-cadherin, Snail) and hypoxia inducible factor endometrioid endometrial carcinoma. *Ann Diagn Pathol* 2016; **22**: 1–11. doi: 10.1016/j.anndiagpath.2016.01.004
57. **Xie X, Zheng X, Wang J, Chen L.** Clinical significance of Twist, E-cadherin, and N-cadherin protein expression in endometrioid adenocarcinoma. *J Can Res Ther* 2017; **13**: 817–22. doi.org/10.1155/2019/5810465
58. **Feng Z, Gan H, Cai Z, et al.** Aberrant expression of hypoxia-inducible factor 1 α , TWIST and E-cadherin is associated with aggressive tumor phenotypes in endometrioid endometrial carcinoma. *Jpn J Clin Oncol* 2013; **43**: 396–403. doi: 10.1093/jjco/hys237
59. **Senol S, Sayar I, Ceyran AB, et al.** Stromal clues in endometrial carcinoma: loss of expression of β -catenin, epithelial-mesenchymal transition regulators, and estrogen-progesterone receptor. *Int J Gynecol Pathol* 2016; **35**: 238–48. doi:10.1097/PGP.0000000000000233
60. **Sadłcecki P, Jóźwicki J, Antosik P, Walentowicz-Sadłcecka M.** Expression of selected epithelial-mesenchymal transition transcription factors in endometrial cancer. *Biomed Res Intern* 2020; 2020: 4584250. doi.org/10.1155/2020/4584250
61. **Krögera C, Afeyana A, Mraz J, et al.** Acquisition of a hybrid E/M state is essential for tumorigenicity of basal breast cancer cells. *PNAS* 2019; **116**: 7353–62. doi/10.1073/pnas.1907473116
62. **Roberta R-M, Emina B, Danijela V-M, et al.** The immunohistochemical pattern of epithelial-mesenchymal transition markers in endometrial carcinoma. *Appl IHC Mol Morph* 2020; **28**: 339–46. doi: 10.1097/PAI.0000000000000754
63. **Buchynska LG, Naleskina LA, Nesina IP.** Morphological characteristics and expression features of adhesion markers in cells of low differentiated endometrial carcinoma. *Exp Oncol* 2019; **41**: 335–41. doi:10.32471/exp-oncology.2312-8852.vol-41-no-4.13965
64. **Wilson MR, Reske JJ, Holladay J, et al.** ARID1A and PI3-kinase pathway mutations in the endometrium drive epithelial transdifferentiation and collective invasion. *Nat Commun* 2019; **10**: 3554. doi.org/10.1038/s41467-019-11403-6

ОСОБЛИВОСТІ ЕПІТЕЛІАЛЬНО-МЕЗЕНХІМАЛЬНОГО ПЕРЕХОДУ В КАРЦИНОМАХ ЕНДОМЕТРІА

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Епітеліально-мезенхімальний перехід є важливою складовою прогресування пухлинного процесу, за допомогою якого клітини злоякісних новоутворень набувають інвазивних і міграційних властивостей. Аналіз літературних джерел і дані власних досліджень свідчать, що активація білків, які залучені до епітеліально-мезенхімального переходу, безумовно, впливає на прогресування карциноми ендометрія, і саме значна варіабельність їх експресії може зумовлювати клінічну і морфологічну гетерогенність цієї форми раку. Найбільш агресивні пухлини характеризуються гібридним епітеліально-мезенхімальним фенотипом, який часто асоціюється з колективним типом інвазії пухлинних клітин ендометрія у міометрій.

Ключові слова: ендометріодна карцинома ендометрію, епітеліально-мезенхімальний перехід, транскрипційні фактори, Snail, Twist, ZEB.