

**I.O.S.U.D. UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA  
DOCTORAL SCHOOL U.M.F CRAIOVA**

**PHD THESIS**

*The role of epithelio-mesenchymal transition in the progression of  
endometrial carcinomas*

Scientific coordinator:

**Prof. Univ. Dr. Cristiana Eugenia Simionescu**

PhD

**Ciucă (Florescu) Mirela-Marinela**

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### III.2. THE ROLE OF EPIHELIO-MEZENCHIMAL TRANSITION IN ENDOMETRIAL CARCINOMAS

The TEM process has been extensively described in many cancers but is insufficiently studied in endometrial carcinomas. Recent studies suggest that endometrial tumors, regardless of their classification in Type I and Type II carcinomas, are capable of undergoing the TEM process, which facilitates myometrial invasion and subsequent metastasis.

- The essential molecular characteristic of the TEM process is the decrease in E-cadherin expression, a protein-controlled process such as Snail, the expression of both markers, E-cadherin and Snail, with predictive value in endometrial endometrial carcinomas [4].

Oncogene BMI-1 can induce TEM in cancer cells, and recent studies have suggested that uncodified microRNA (miRNAs) act as a crucial modulator for TEM [69].

Activation of the PI3K / AKT pathway is a common mechanism in all endometrial cancer (endometrial and non-endometrial) subtypes and plays an important role in TEM [72]. MiRNAs are non-coding RNA molecules that simultaneously affect several target genes and regulate a wide range of genes involved in TEM modulation. Several recent studies have revealed the impact of miRNAs on TEM and cancer cell phenotype in endometrial cancer by regulating the PI3K / AKT pathway [72]. In endometrial carcinoma cells, miRNAs can activate or attenuate TEM and cancer stem cells through PTEN and other TEM-associated genes such as Twist1, ZEB1 and BMI-1 [72]. Direction of the PI3K / AKT pathway signaling key to restore or inhibit miRNAs may be a potential therapeutic approach to suppress TEM and cancer stem cells in endometrial cancer [72].

- Kruppel 17 (KLF17) is a member of the KLF transcription factor family, which can inhibit TEM and tumor growth. However, expression, cell function and KLF17 mechanism in endometrioid endometrial cancer remain elusive. In a study by Dong et al., The authors found that among members of the KLF family, KLF17 was consistently overexpressed in the cell lines of endometrioid endometrial carcinomas [70]. Overexpression of KLF17 in the cell lines of endometrial carcinomas induced

TEM and promoted cell invasion and drug resistance, resulting in increased Twist1 expression [70]. Instead, KLF17 suppression reverses the TEM process, diminishes cell invasion, restores susceptibility to drugs, and suppresses Twist1 [70]. The authors reported correlation of KLF17 expression to tumor grade, KLF17 having an oncogenic role in progression of endometrial endometrial carcinomas by initiation of TEM [70]

Growth factor D derived from platelets (PDGF-D) can promote growth and invasion of malignant tumors. PDGF-D is part of the PDGF family involved in various cellular mechanisms, such as proliferation, migration, invasion, transformation and survival [83,307]. PDGF-D interacts primarily with PDGFR- $\beta$  (PDGF- $\beta$  receptor) to activate its downstream signaling pathways containing Wnt /  $\beta$ -catenin, PI3K / Akt / mTOR and NF- $\kappa$ B, which ultimately accelerates tumorigenesis and tumor progression [286, 288].

Epidermal growth factor receptors (EGFR), EGFR1 (HER1), Erb2 (Her2 / neu), Erb3, and erbB4, are heavily expressed in endometrial carcinomas and were at the top of specific therapies [23].

These data could contribute to a better understanding of the pathogenesis of endometrial carcinomas and can provide important information for better management of endometrial cancer [169].

## **CHAPTER IV. MATERIAL AND METHODS**

### **IV.A. STUDIED MATERIAL**

The study was of analytical, retrospective and prospective type in which we analyzed a series of clinical-epidemiological, histopathological and biomolecular characteristics of endometrial carcinomas.

We selected a total of 50 endometrial carcinomas within 4 years (2011-2014). The casuistry analyzed came from patients hospitalized and operated at the clinics of Gynecology and Surgery of the County Clinical Emergency Hospital of Craiova.

The study material was represented by the anatomo-pathological records from which we extracted information on the analyzed clinical-epidemiological data and the histopathological parameters of the investigated tumors. For retrospectively analyzed cases, we also used paraffin blocks as well as histological blades found in histologies of the Pathological Anatomy Laboratory of the Craiova County Emergency Clinical Hospital. For the present cases, we used hysterectomy pieces, which were subjected to the usual histological processing by the paraffin inclusion technique within the same laboratory.

Subsequently, the histopathologically investigated cases were subjected to the immunohistochemical examination within the Laboratory of Morphopathology Discipline of UMF Craiova.

## **CHAPTER V**

### **RESULTS**

#### **V.4. IMMUNOHISTOCHEMICAL STUDY OF ENDOMETRIOID CARCINOMAS**

Immunohistochemical analysis comprised a total of 40 cases of endometrioid carcinomas, the results were then statistically interpreted in relation to the clinical-pathological parameters.

Of the total of 40 patients, morphological parameters analysis indicated an average age of 60.8 years. Most of the injuries presented were well and moderately differentiated (19 cases, respectively 12 cases), with invasion in the inner half of the myometrium (23 cases), and no metastasis in the lymph nodes (38 cases), most of them framed Stage I pTNM disease (23 cases) (Table 21). In this study the number of cases in relation to tumor extensions (T category) and tumor stage was the same.

Markers used for immunohistochemical analysis of selected endometrial carcinoma cases are involved in the epithelio-mesenchymal transition, which will then be analyzed in relation to the clinical-morphological parameters investigated, in order to assess their involvement in the prognosis of these neoplasias. For this

purpose we used markers of intercellular adhesion, mesenchymal markers, transcription factors and decreasing factors

#### V.4.1. STUDY OF IMMUNOEXPRESSION OF INTERCELLULAR ADHESIVE MARKERS

The markers of cellular adhesiveness investigated for the 40 cases of endometrioid carcinomas were: P-cadherin, E-cadherin and N-cadherin.

##### *IMMUNOEXPRESSION OF E-CADHERIN*

Immunoreaction for E-cadherin was identified in 85% of the 40 cases of immunohistochemically analyzed endometrioid carcinoma with membrane localization (Table 22)

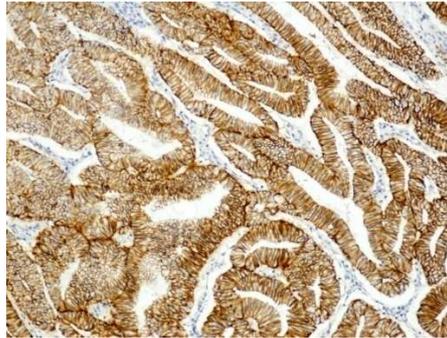
**Table 22. E-cadherin imunoexpression in relation to clinical-morphological parameters**

Parametres		No. cases	E-cadherin		p
			% celule +	SMI	
Age	<50	3	96.3±6.3	9.3	0.145
	>50	37	54.9±34.1	6.1	
Differentiation degree	G1	19	69.6±31.4	8.3	<b>0.004</b>
	G2	12	53±36.1	5.2	
	G3	9	30.5±30.2	2.8	
N category	N0	38	67.3±26.1	7.5	0.413
	N	2	50±70.7	4	
T category/ Stage	T1/ I	23	59.5±33.3	7.3	0.229
	T2/ II	12	65.3±29.6	5.8	
	T3/ III	5	34±47.7	3.2	

SMI - Medium immunocoloring score

The E-cadherin marker varied according to the degree of tumor differentiation, both in terms of the percentage of immunomarcated cells and its intensity.

Well-differentiated endometrioid carcinomas showed a mean percentage of  $69.63 \pm 31.44$  cells, the intensity of the reactions being strongly moodyed, with a mean immunocolouration score of 8.3 (Fig. 43, Table 22).



**Figure 43 Endocrine G1 Carcinoma, E-cadherin Immunodeficiency, Ob. X100**

For moderate and poorly differentiated carcinomas, mean percentage of positive cell counts were  $53 \pm 36.19$  and  $30.55 \pm 30.25$ , respectively, and the intensity of the reactions varied from mild to intense, with median immunocolorization scores of 5.25 and 2, respectively, 8 (Fig. 44, Fig. 45, Table 22).

In relation to the extent of tumor extension, the mean percentage of immunomarcated tumor cells was higher in the pT1 and pT2 categories ( $59.5 \pm 33.3$ ;  $65.3 \pm 29.6$ ), the intensity of the reactions being variable and the mean score of 7.3 and 5.8, respectively. The pT3 category average percentage of the immunostained tumor cells was  $34 \pm 47.7$ , the intensity of the reactions also variable, and the mean score was 3.2 (Table 22).

The sublots analyzed for the extent of tumor extension and tumor status coincided, so that the highest values of E-cadherin markers were present in tumorous stage I and II carcinomas.

#### *IMMUNOEXPRESSION OF N-CADHERIN*

Immunoreaction was identified in 45% of cases in tumor cells with cytoplasmic localization (Table 24).

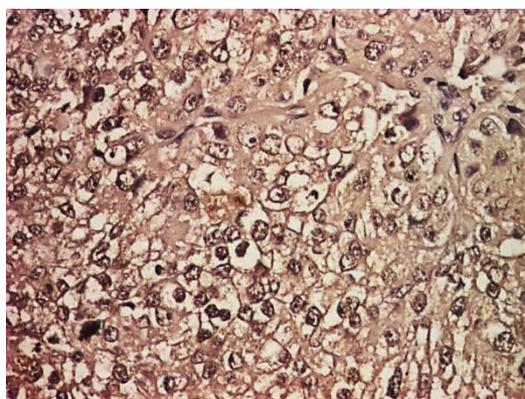
**Tabel 24. Imunoexpresia N-caderinei în raport cu parametrii clinico-morfologici**

Parametres		No. cases	N-cadherin		p
			% celule +	SMI	
Age	<50	3	13.3±23	1	0.648
	>50	37	41±21.6	5.8	
Differentiation degree	G1	19	33.2±13.7	4.7	<b>0.028</b>
	G2	12	40±21.9	6.3	
	G3	9	50.7±25.8	6.1	
N category	N0	38	43.2±20.5	5.9	0.465
	N	2	60 ±28.2	7	
T category/ Stage	T1/ I	23	38.5±19.2	5.4	0.138
	T2/ II	12	46.1±22.7	6.2	
	T3/ III	5	56.2±22.1	7	

SMI - Medium immunocoloring score

Well-differentiated carcinoma analysis revealed a mean percentage of marked tumor cells of  $33.2 \pm 13.7$ , the intensity of the reactions being mild / moderate with an average score of 4.7 (Figure 61, Table 24).

Moderately differentiated and poorly differentiated carcinomas showed mean percentages of immunoprecipitated cells of  $40 \pm 21.9$  and  $50.7 \pm 25.8$ , respectively, the intensity of the reactions being variable and the mean scores of 6.3 and 6.1 respectively (Fig. 62, Fig. 63, Table 24).



**Figure 63. Endometroid carcinoma G3, N-cadherin immunomarker, ob. X200**

The degree of tumor extension and tumor stage respectively showed average percentages of higher immunomarcated tumor cells in the pT2 / II and pT3 / III stage ( $46.1 \pm 22.7$  and  $56.2 \pm 22.1$  respectively), the intensity of the reactions being variable in these cases and the score The mean being 6.2 and 7. The average percentage of tumor cells marked in pT1 / I was  $38.5 \pm 19.2$ , the intensity of the reactions being variable, with a mean score of 5.1 (Table 24).

## **CHAPTER VI**

### **DISCUSSIONS**

#### **VI.2. DISCUSSIONS ON THE IMMUNOHISTOCHEMICAL STUDY**

The epithelium-mesenchymal transition is a biological process in which epithelial cells lose their polarity and cell-cell contact to acquire a migratory mesenchymal phenotype [173, 280]. The process is characterized by loss of expression of epithelial markers and the acquisition of mesenchymal ones, thus playing a key role in the invasion and metastasis of cancer [248]. The association of epithelio-mesenchymal transition with cancer progression has been reported in several cancers, including breast, prostate, pancreatic and hepatocarcinoma [118, 156].

##### **VI.2.1. DISCUSSIONS ON THE IMMUNOHISTOCHEMICAL STUDY OF ADHESIVE MARKERS**

###### ***E-CADHERIN***

One of the features of the epithelial-mesenchymal transition process is the loss of intercellular adhesion, which is associated with low expression of E-cadherin [45, 94].

Immunoreaction for E-cadherin has been identified in 85% of cases of immunohistochemically analyzed endometriod carcinomas. The results of the study revealed significant differences between E-cadherin expression and the degree of

tumor differentiation, reported in similar studies. E-cadherin expression was more pronounced in well-differentiated endometriotic carcinomas, tumors with superficial myometric invasion, and tumors at incipient stages. These histopathological characteristics are associated in many studies with a more favorable prognosis [45, 233].

### ***N-CADHERIN***

As a characteristic of aggressive tumors, the epithelio-mesenchimal transition is characterized by the reduction of E-cadherin expression and the increase of N-cadherin expression, contributing to the shaping of the cell adhesion profile, the increase of tumor cell motility and invasive properties, aspect recently reported in several Tumors [49, 64, 130].

In order to verify this hypothesis for endometrial cancers, we studied the cytoplasmic expression of N-cadherin in tumor cells. The percentage of marked tumor cells was higher in cases of poorly differentiated endometroid carcinoma (50.7%) compared to moderate or well differentiated tumors (40%, 33.2%). The statistical analysis allowed a statistically significant association to increase N-cadherin expression with tumor growth ( $p = 0.028$ ). In addition, we noticed an increase in N-cadherin expression depending on the tumor status of the tumor. The mediated percentage of marked cells was higher in pT3 / III than in pT2 / II, respectively pT1 / I.

## **CHAPTER VII**

### **CONCLUSIONS**

The study, which included 50 cases of endometrial carcinomas, allowed the following conclusions:

- Immunoreaction for E-cadherin was identified in 85% of the carcinomas analyzed; The marks being superior to well-differentiated tumors, as well as early stages of the disease;
- The statistical analysis revealed statistically significant associations of E-cadherin expression with the degree of tumor differentiation ( $p = 0.004$ );

Immunoreaction for N-cadherin was present in 45% of cases, with superior markers in moderate and poorly differentiated tumors, as well as advanced disease states;

- Statistical analysis revealed significant associations of increase in N-cadherin expression and tumor growth ( $p = 0.028$ );
- The cadherin switching characteristic of the epithelio-mesenchymal transition was highlighted by the presence of a negative linear correlation between E-cadherin and N-cadherin.
- Changing epithelium-mesenchimal phenotype in endometrial carcinomas by altering E-cadherin expression, along with overexpression of P-cadherin, N-cadherin and Snail in high-grade and advanced lesions, are mechanisms involved in tumor progression.
- Investigation of epithelio-mesenchymal transition in endometrial carcinomas supports the additional study of both E-cadherin and aggressive markers (N-cadherin, P-cadherin and Snail) for the stratification of high-risk patients and also potential targets therapeutic.

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