ORIGINAL ARTICLE

IMMUNOHISTOCHEMICAL PROFILE OF ENDOMETRIOSIS-ASSOCIATED OVARIAN CARCINOMA

Ioana Păvăleanu[1], Cornelia Amalinei[2], Simona Eliza Giuşcă[3], Ludmila Lozneanu[2], Roxana Avădănei[2], Irina-Draga Căruntu[2], Raluca Balan[2]

[1] Mother and Child Health Department - Obstetrics and Gynecology, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania
[2] Morphofunctional Sciences Department I - Histology, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania
[3] Morphofunctional Sciences Department I - Morphopathology, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania

ABSTRACT

Objective: Endometriosis association with cancers is strongly supported by epidemiological criteria and shared protective factors, being identified as endometriosis-associated ovarian carcinoma (EOC). In this context, our study objective has been the evaluation of selected immunohistochemical markers expression (ER, PR, p53, and Ki-67) in both endometriosis and EOC as an attempt to identify common pattern of expression, in support of similar molecular pathway involved in their pathogenesis. **Material and Methods:** Our study comprised 19 cases of EOCs. The routine and immunohistochemical staining have been performed, followed by results statistical processing.
Results: The following data have been assessed in EOCs: tumor size, histological type, ovarian capsule invasion, TNM and FIGO staging. The histological types have been: endometrioid (8 cases) and non-endometrioid (11 cases of clear cell, high-grade serous, and mixed types). FIGO stages have been: stage I (4 cases), stage II (6 cases), stage III (8 cases), and stage IV (1 case). Histological grades have been: G1 (1 case), G2 (6 cases), and G3 (12 cases). **Conclusions:** The comparison between immunohistochemical staining and different clinicopathological variables supported that the altered expression of steroid receptors in ovarian endometriotic tissue and EOC are involved in malignant transformation and progression. p53 is contributing to endometriosis pathogenesis and EOC progression. EOCs are associated with low Ki-67 index compared to more aggressive types of tumors. In conclusion, the immunohistochemical expression of ER, PR, and p53 corroborated with clinicopathological features support the mechanism of endometriosis transition to EOC, provide tools for prognosis evaluation, and open new perspectives of therapy.

Keywords: endometriosis, EOC, endometrioid EOC, non-endometrioid EOC, ER, PR, p53, Ki-67

INTRODUCTION

Endometriosis has a relatively high prevalence in women, being characterized by the presence of ectopic endometrium in a multitude of possible locations and by association with infertility.

The correlation between endometriosis and cancers is strongly supported by epidemiological factors, such as common risk factors (early menarche, short menstrual cycles, nulliparity, and late menopause) and shared protective factors (oral contraceptives, multiparity, tubal ligation, and hysterectomy) [1]. Two possible mechanisms have been considered: either direct transformation of the endometriotic implants or either shared precursor mechanisms and/or risk factors, followed by divergent molecular pathways [2, 3].

The association of these two conditions has been early identified as endometriosis-associated ovarian carcinoma [4] and later on has been named endometriosis-related ovarian neoplasm (ERON or EON) [5, 6] or endometriosis-associated ovarian carcinoma (EOC or EAOC) [7, 8], mostly manifested as endometrioid carcinoma, clear cell carcinoma, sero-mucinous borderline tumor, müllerian adenosarcoma, and endometrioid stromal sarcoma.

Most of these tumors (70%) are developing during the first 10 years of endometriosis diagnosis, being associated in a majority of cases (60%) with an intermediary stage of atypical endometriosis [8].

Moreover, considering the intrinsic invasive and metastatic ability of endometriosis, its behavior has a high similitude with that of malignancies [9].

This strong association between the two diseases led to the investigation of their possible shared molecular patterns/pathways. Furthermore, by identification of some key molecules involved in their pathogenesis, the validity of endometriosis etiopathogenical theories might be evaluated.

Estrogen is recognized as a stimulator of ovarian cell proliferation, of malignant cells mobility, and of inhibition of intercellular adhesion [10, 11]. Although it has been already shown that estrogen receptor (ER) and progesterone receptor (PR) are mediating the action of steroid hormones on both endometriosis and endometrioid EOC, the correlation between ER or PR expression and clinical outcome has been recently reported in ovarian cancer [11, 12].

p53 alteration represents one of the molecular events of the transformation of endometriosis into carcinomas [13].

Ki-67 expression is strictly correlated to cell proliferation, being used in different neoplastic lesions to assess their growth [14], including endometriosis and EOC.

In this context, our study objective has been the evaluation of selected immunohistochemical markers expression (ER, PR, p53, and Ki-67) in both endometriosis and EOC as an attempt to identify common pattern of expression, in support of similar molecular pathway involved in their pathogenesis.

MATERIAL AND METHODS

Our study has included a retrospective component which involved the information provided by clinical files, along with histopathological investigations of the surgical specimens (ovariectomy, adnexectomy, and hysterectomy with adnexectomy) obtained from patients diagnosed with endometriosis and endometriosis-associated ovarian carcinomas. The routine paraffin-embedded hematoxylin-eosine and immunohistochemical stainings, using a panel of antibodies, have been performed.

The material comprised 19 cases of EOC suspected by clinical and ultrasound findings, and histopathologically confirmed on surgical specimens, during 2013-2016. The patients' informed consent has been obtained in all cases.

The following data have been assessed: tumor size, histological type, ovarian capsule invasion, TNM and FIGO staging.

The antibodies used in immunohistochemical analysis and their dilutions are illustrated in **Table 1.**

The qualitative interpretation, followed by semiquantitative evaluation of the immunohistochemical reactions have been achieved, using the appropriate scores available in literature.

The processing of statistical data has been performed by using the SPSS v. 19.0 program (IBM SPSS Statistics). Continuous variables were expressed as: mean and standard deviation and categorical variables as number (%). The association level between the expression of studied markers and clinicopathological characteristics has been done using specific non-parametric tests (Chi-square test or Fisher's exact test). Student's t-test or Wilcoxon rank-sum tests have been used for continuous variables. Significant correlation has been considered at $p < 0.05$.

Antibody	Clone	Dilution
ER	6711/Novocastra	RTU
PR	312/Novocastra	RTU
p53	DO-7/Novocastra	RTU
Ki-67	MM1/Novocastra	1/50

Table 1.
Antibodies used in immunohistochemical assay

RESULTS

The patients' average age has been 59.10±8.66 years, with a median age of 57 years. All patients were menopausal at the time of the diagnosis and only 2 patients were nulliparous. The histological types have been: endometrioid (8 cases) and non-endometrioid (clear cell, high-grade serous, and mixed types – 11 cases). The distribution of FIGO stages was the following: early- localized (stage I – 4 cases), regional (stage II – 6 cases), and advanced (stage III – 8 cases and stage IV – 1 case). The tumors have been classified, according to TNM system, into: T1 – 5 cases, T2 – 6 cases, and T3 – 8 cases. Lymphadenectomy has been performed in 8 cases, revealing N0 – 5 cases and N1 – 3 cases. Histological grades have been: G1 – 1 case, G2 – 6 cases, and G3 – 12 cases.

The immunohistochemical staining in the study group has been analyzed according to the specific criteria for each marker, as following:

ER and PR analysis used a score based on positivity index (PI) added to staining intensity (SI) [15], considering PI (% positive cells): 0 – none; 1<1/100; 2=1/100-1/10; 3>1/10-1/3; 4>1/3-2/3; 5>2/3 and SI: 0-absent; 1=weak; 2=moderate; 3=strong. The total score (TS) ranged in 0-8 interval and has been considered as positive if >2.

ER and PR expressions **(Figs. 1-3)** have been quantified in ovarian endometriosis and independently in epithelial and stroma of EOC and compared with clinicopathological characteristics **(tables 2-5)**. Stromal ER expression in EOC was considered as positive when at least one cell presented a nuclear brown staining, and ranged between 0 and 60 positive cells, in the assessed areas.

ER cutoff score value has been considered as 4 (≤4 negative and low 0-4, >4 high 5-8).

Stromal PR expression in EOC was considered positive when at least one cell presented a nuclear brown staining, and ranged between 0 and 70 positive cells in the assessed areas.

PR cutoff score value was considered as 4 (≤4 negative and low 0-4, >4 high 5-8).

p53 immunohistochemical staining quantification used a score based on positivity index (PI) added to staining intensity (SI) [16], where PI (% positive cells) was: 1=1-25%; 2=26-50%; 3=51-75%; 4=76-100%, and SI was: 0-absent; 1-weak; 2-strong.

p53 cutoff score value was considered as 6 (<6 negative and low, ≥ 6 high) and its expression **(Fig. 4)** has been compared with different clinicopathological variables **(table 6).**

Ki-67 immunohistochemical staining quantification used a score based on positivity index (PI) [17], where PI (% positive cells) was: negative-absent; weak≤10%; moderate=10-40%; strong>40%.

Ki-67 expression was very low (1-2%) in 5 cases and absent in 14 cases, in endometriotic areas. Ki-67 values ranged between 3 and 75 cells, in EOC (**Figs. 5 and 6**). If Ki-67 index <10, cases were considered as having a low mitotic activity, if Ki-67 index values ranged between 10-30, cases were considered as having a moderate mitotic activity, and if Ki-67 index >30, cases were considered as having a high proliferative activity. Ki-67 index has been also compared with different clinicopathological

variables (**table 7**).

ER expression in tumor cells			
Clinicopathological characteristics	**negative and low**	**high**	**p-value (95%CI)**
Age: years ‡			
<55	1(12.5%)	7(87.5%)	0.435
≥55	3(27.3%)	8(72.7%)	
Histological subtype ‡			
Endometrioid	1(12.5%)	7(87.5%)	0.435
Non-endometrioid	3(27.3%)	8(72.7%)	
pT ‡			
I+II	3(27.3%)	8(72.7%)	0.435
III	1(12.5%)	7(87.5%)	
Grade ‡			
1-2	2(28.6%)	5(71.4%)	0.539
3	2(16.7%)	10(83.3%)	
FIGO stage ‡			
Early stage (I-II)	2(20%)	8(80%)	0.906
Late stage (III-IV)	2(22.2%)	7(77.8%)	
ER expression in endometriotic areas † 33.31 ± 32.19	47.21 ± 30.81		0.135
† Student's t-test or Wilcoxon rank-sum tests for continuous variables; ‡ Chi-square test or Fisher's exact test; (*) p <0.05.			

Table 2.
ER epithelial tumor cells expression according to different clinicopathological variables

PR expression in tumor cells			
Clinicopathological characteristics	**negative and low**	**high**	**p-value (95%CI)**
Age: years ‡			
<55	3(37.5%)	5(62.5%)	0.463
≥55	6(54.5%)	5(45.5%)	
Histological subtype ‡			
Endometrioid	2(25%)	6(75%)	0.096
Non-endometrioid	7(63.6%)	4(36.4%)	
pT ‡			
I+II	5(45.5%)	6(54.5%)	0.845
III	4(50%)	4(50%)	
Grade ‡			
1-2	1(14.3%)	6(85.7%)	0.027*
3	8(66.7%)	4(33.3%)	
FIGO stage ‡			
Early stage (I-II)	4(40%)	6(60%)	0.498
Late stage (III-IV)	5(55.6%)	4(44.4%)	
PR expression in endometriotic areas † 30.31 ± 31.44	27.94 ± 30.86		0.807
† Student's t-test or Wilcoxon rank-sum tests for continuous variables; ‡ Chi-square test or Fisher's exact test; (*) p <0.05.			

Table 4.
PR epithelial tumor cells expression according to different clinicopathological variables

ER expression in tumor stroma			
Clinicopathological characteristics	**negative**	**positive**	**p-value (95%CI)**
Age: years ‡			
<55	5(62.5%)	3(37.5%)	0.125
≥55	3(27.3%)	8(72.7%)	
Histological subtype ‡			
Endometrioid	2(25%)	6(75%)	0.198
Non-endometrioid	6(54.5%)	5(45.5%)	
pT ‡			
I+II	5(45.5%)	6(54.5%)	0.729
III	3(37.5%)	5(62.5%)	
Grade ‡			
1-2	3(42.9%)	4(57.1%)	0.960
3	5(41.7%)	7(58.3%)	
FIGO stage ‡			
Early stage (I-II)	4(40%)	6(60%)	0.845
Late stage (III-IV)	4(44.4%)	5(55.6%)	
ER expression in stroma of endometriotic areas † 30.21 ± 25.30	6 ± 13.58		0.001*
† Student's t-test or Wilcoxon rank-sum tests for continuous variables; ‡ Chi-square test or Fisher's exact test; (*) p <0.05.			

Table 3.
ER stromal tumor cells expression according to different clinicopathological variables

PR expression in tumor stroma			
Clinicopathological characteristics	**negative**	**positive**	**p-value (95%CI)**
Age: years ‡			
<55	3(37.5%)	5(62.5%)	0.729
≥55	5(45.4%)	6(54.5%)	
Histological subtype ‡			
Endometrioid	2(25%)	6(75%)	0.198
Non-endometrioid	6(54.5%)	5(45.5%)	
pT ‡			
I+II	4(36.4%)	7(63.6%)	0.552
III	4(50%)	4(50%)	
Grade ‡			
1-2	0(0%)	7(100%)	0.005*
3	8(66.7%)	4(33.3%)	
FIGO stage ‡			
Early stage (I-II)	3(30%)	7(70%)	0.260
Late stage (III-IV)	5(55.6%)	4(44.4%)	
PR expression in stroma of endometriotic areas † 42.63 ± 25.78	9.15 ± 17.09		0.000*
† Student's t-test or Wilcoxon rank-sum tests for continuous variables; ‡ Chi-square test or Fisher's exact test; (*) p <0.05.			

Table 5.
PR stromal tumor cells expression according to different clinicopathological variables

P53 expression in tumor cells			
Clinicopathological characteristics	**negative and low**	**high**	**p-value (95%CI)**
Age: years ‡			
<55	4(50%)	4(50%)	0.845
≥55	5(45.5%)	6(54.5%)	
Histological subtype ‡			
Endometrioid	6(75%)	2(25%)	0.040*
Non-endometrioid	3(27.3%)	8(72.7%)	
pT ‡			
I+II	7(63.6%)	4(36.4%)	0.096
III	2(25%)	6(75%)	
Grade ‡			
1-2	4(57.1%)	3(42.9%)	0.515
3	5(41.7%)	7(58.3%)	
FIGO stage ‡			
Early stage (I-II)	7(70%)	3(30%)	0.037*
Late stage (III-IV)	2(22.2%)	7(77.8%)	
P53 expression in endometriotic areas †			
14.32 ± 10.75	45.74 ± 40.43		0.003*
† Student's t-test or Wilcoxon rank-sum tests for continuous variables; ‡ Chi-square test or Fisher's exact test; (*) p <0.05.			

Table 6.
p53 tumor cells expression according to different clinicopathological variables

Ki-67 index in tumor cells			
Clinicopathological characteristics	**negative and low**	**high**	**p-value (95%CI)**
Age: years ‡			
<55	5(62.5%)	3(37.5%)	0.729
≥55	6(54.5%)	5(45.5%)	
Histological subtype ‡			
Endometrioid	3(37.5%)	5(62.5%)	0.125
Non-endometrioid	8(72.7%)	3(27.3%)	
pT ‡			
I+II	5(45.5%)	6(54.5%)	0.198
III	6(75%)	2(25%)	
Grade ‡			
1-2	4(57.1%)	3(42.9%)	0.960
3	7(50%)	5(50%)	
FIGO stage ‡			
Early stage (I-II)	5(40%)	5(60%)	0.463
Late stage (III-IV)	6(66.7%)	3(33.3%)	
‡ Chi-square test or Fisher's exact test; (*) p <0.05.			

Table 7.
Ki-67 tumor cells expression according to different clinicopathological variables

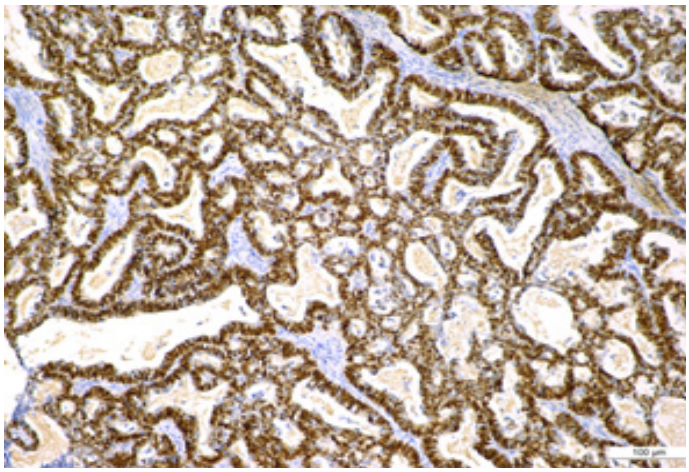

Fig.1
ER nuclear expression in endometrioid EOC (x10)

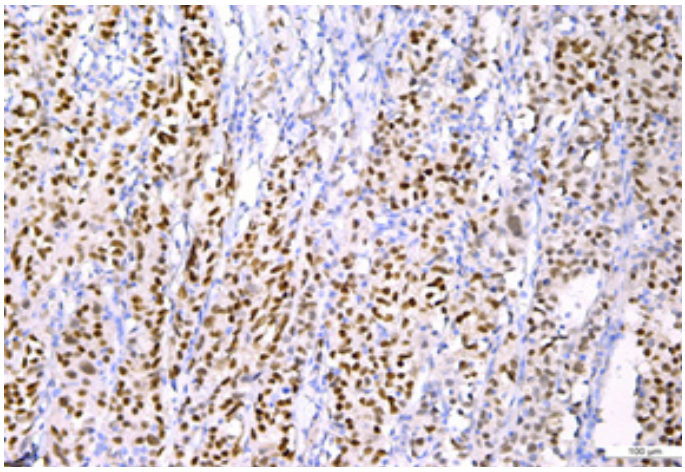

Fig.2
ER nuclear expression in non-endometrioid EOC (x20)

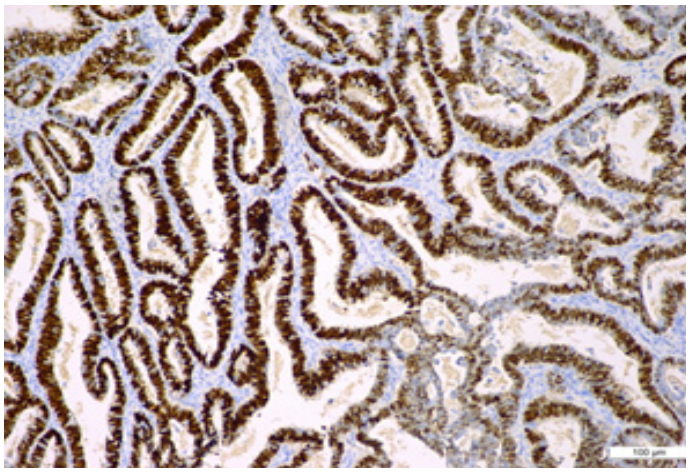

Fig.3
PR nuclear expression in endometrioid EOC (x 10)

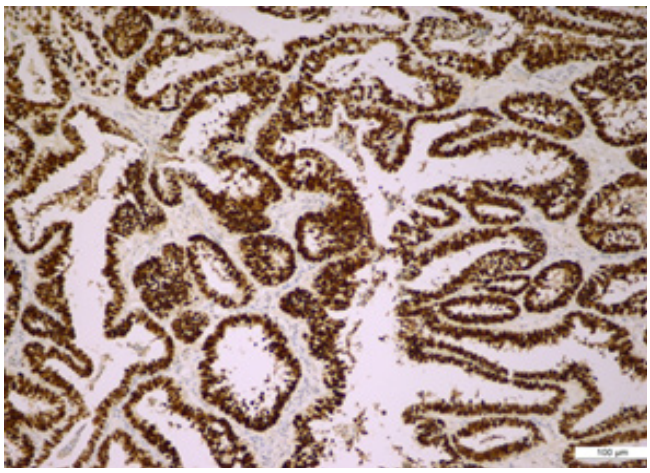

Fig.4
Strong p53 nuclear expression in endometrioid EOC (x 10)

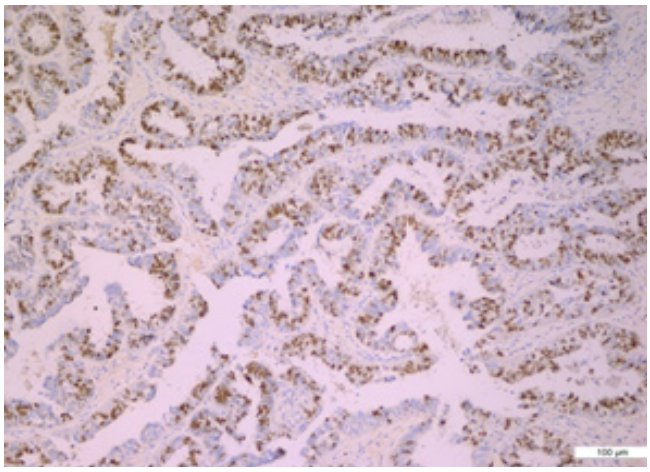

Fig.5
Ki-67 nuclear expression in endometrioid EOC (x 10)

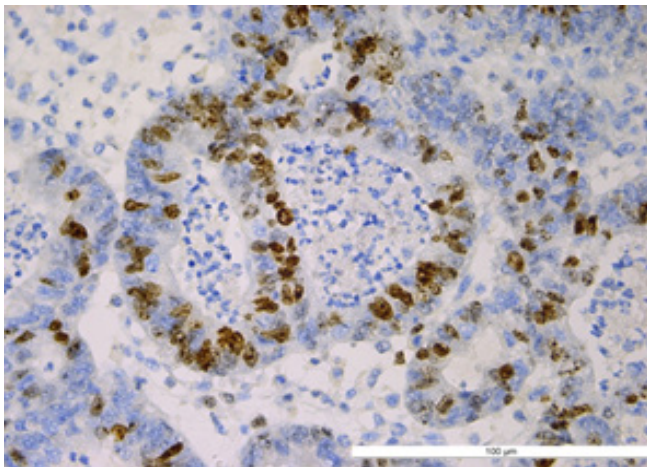

Fig.6
Ki-67 nuclear expression in endometrioid EOC (x 40)

DISCUSSIONS

Since its identification, the hypothesis of endometriosis' intrinsic capacity of malignant transformation has been launched [18], with an estimated low frequency of 0.7-1.6% [19]. However, endometriosis is a disease characterized by many intrinsic neoplastic attributes and, considering its invasive and metastatic abilities, endometriosis behavior resembles that of malignancies [9].

This has been attributed to two possible mechanisms: either the endometriotic implants are suffering a direct transformation, or either both processes have a common precursor mechanisms and/or predisposing factors, subsequently registering divergent evolutive pathways [2, 3].

As a highlight of the correlation between these two diseases, different terms have been introduced in literature, exhibiting a large spectrum of pathologic entities. EOC may be classified according to the histology findings into three types: with evident transition from endometriosis to carcinoma, both entities within the same ovary but without evident transition, and ovarian carcinomas with concomitant pelvic endometriosis [1]. Our cases have been selected to comply with the first type.

Numerous studies have been trying to identify a common molecular pattern between endometriosis and ovarian carcinomas associated with endometriosis. Additionally, the understanding of the mechanisms involved in these diseases has a preventive role for ovarian malignancies, in risk populational groups. Moreover, based on genomic, transcriptomic, and proteomic profiles, by correlating the genetic mechanisms corresponding to transition from normal to malignant transformation of endometriosis, new therapeutic algorithms may be achieved.

In this context, numerous studies have been focusing on the identification of a common molecular pattern of these two diseases. Consequently, by the investigation of key molecules involved in endometriosis and EOC, one or more etiopathogenical mechanisms could be validated.

Ovarian ECs and CCCs are associated to endometriosis as a precursor lesion in 20-40% and 40-55%, respectively [20]. As prevalence, these carcinomas are associated to 5-10% of endometriosis cases, with an intermediary stage of atypical endometriosis identified in 0.7-1.6% of cases, with a predilection in those with a long pre-existent history of the disease [20].

Some authors have launched the hypothesis of micromedium influence, or of the ferrous concentration from endometriotic cysts, by persistence of oxidative stress ferrous-induced, with consecutive alteration of DNA and frequent genic mutations, both in endometriosis and EOC, such as PTEN, ARID1, PIK3CA, along with heterozygosity loss [20].

As hormonal-driven disease, endometriosis pathogenesis is characterized by increased local estrogen levels, with high ER-β/ER-α ratio that leads to a reduced PR expression [21, 22]. Moreover, a low PR-B/A ratio

and PR-B immunoreactivity have been demonstrated in endometriosis [23], suggestive of an alteration of endometrial responsivity to progesterone and the persistence of proliferative profile, with an increased ER expression in endometriosis [24].

ER expression is high in ovarian carcinomas, mainly in serous and endometrioid types ≥80%, while PR is mostly highly expressed in endometrioid carcinomas (72%), decreasing to 30-50% in serous type [25], unlike endometrial carcinomas, in which ER and PR expressions are obviously higher in endometrioid type, than in serous histological variant [26]. Clear cell carcinomas have a low expression of steroid receptors (13% for ER and 6% for PR) [25], possibly due to a shift to to sex-hormone independent receptors, similar to the mechanism identified in endometrium [27, 28].

In our study group, ER score in tumor cells was high in majority of cases (78.94%), with a strong nuclear expression, supporting the role of hormone mediation in pathogenesis of ovarian malignancies. ER epithelial expression was also high in endometriosis, but large variations of expression were registered. These could be related to one isoform expression, as high levels of ERβ, associated with low ERα expression are reported [24, 29], as a probable consequence of compliance with the physiological changes in the eutopic endometrium.

There are reports of 91% ER overexpression in endometrioid EOCs compared to 8% in clear cell EOCs [13] and also a negative association between ER positivity and advanced stages has been demonstrated in endometrioid ovarian carcinoma [11, 30, 31]. Due to the fact that clear cell ovarian carcinomas do not usually express steroid receptors positivity, the characteristic loss of ER expression in this histological type represents the carcinogenic event which separates ovarian carcinomas in estrogen-dependent and estrogen-independent tumors [32]. Furthermore, ER lost expression in ovarian clear cell carcinomas in comparison to its maintained expression in endometriosis and endometrioid EOC has suggested its late intervention in carcinogenesis [33]. Although we expected an agreement with literature findings, no significant difference in ER expression in tumor cells between endometrioid and non-endometrioid carcinomas or between more differentiated and poorly differentiated histological types or between early stage and late FIGO stage has been detected in our study group. These differences are possibly related to our limited group of study or to our choice of the cutoff value.

ER score in stromal cells was high in more than half of cases (57.89%) supporting the role of hormone mediation in pathogenesis of ovarian malignancies and the important role of stroma in carcinogenesis.

ER stromal expression in endometriosis has been significantly lower when compared to tumor stroma (p=0.001), possibly as an indicator of a less responsive component to hormonal stimulation, or by a reduced expression of one of the two ER isoforms.

There was no significant difference in ER expression in stromal cells between endometrioid and non-endometrioid carcinomas, nor between more differentiated and poorly differentiated histological types, nor between early stage and late FIGO stage in our study group, although a stromal involvement is attested by the frequent staining of its component cells.

PR score in epithelial cells was high in approximate half of the cases (52.63%) supporting a less important role of progesterone when compared to estrogen in pathogenesis of ovarian malignancies, or, according to literature over-expression of PR-B in aggressive ovarian carcinomas, similar to its endometrial counterpart expression [34].

PR epithelial expression in endometriosis has not been significantly lower when compared to tumor stroma (p=0.807), possibly as an indicator of a less responsive component to progesterone stimulation, in both instances, in agreement with the concept of progesterone resistance by increased ER-β binding to PR promoter, resulting in inhibition of estradiol-dependent paracrine induction of PR expression [35].

There was no significant difference in PR expression in epithelial cells between endometrioid and non-endometrioid carcinomas, although literature data highlight the favorable PR expression in endometrioid types, without peritoneal metastases, being correlated to inhibition of tumoral cell proliferation and of metastases development [11, 30, 32].

There was no significant difference in PR expression in epithelial cells between early stage and late FIGO stage in our study group. Significant differences were registered between PR epithelial expression in more differentiated and poorly differentiated histological types (p=0.027), demonstrating a negative correlation between PR expression and advanced stages, thus suggesting a partial protective role of progesterone in carcinogenesis.

PR score in stromal cells was positive in more than half of the cases (57.89%) supporting a less important role of this progesterone when compared to estrogen in pathogenesis of ovarian malignancies, or, according to literature of unbalanced estrogen stimulation in both endometriosis and EOCs.

PR stromal expression in endometriosis has been significantly lower when compared to tumor stroma (p=0.000), possibly as an indicator of unbalanced estrogen stimulation as being involved in the pathogenesis of endometriosis but less involved in its malignant development. The weaker PR expression found in both epithelial and stromal endometriotic cells may exhibit the features sometimes encountered in endometriosis without EOC, where the early loss of PR expression has been attributed to early gene alterations preceeding morphological atypia, as an important immunohistochemical marker in monitoring the risk of malignant transformation of endometriosis [36].

There was no significant difference in PR expression in stromal cells between endometrioid and non-endometrioid carcinomas, or between early stage and late FIGO stage in our study group. Significant differences were registered between PR stromal expression in more differentiated and poorly differentiated histological types (p=0.005), demonstrating a negative correlation between PR expression and advanced stages, suggesting a partial protective role of progesterone in carcinogenesis, similar to that of the epithelial component.

p53 is a tumor suppressor gene which is mutated both in endometriosis and in ovarian carcinomas [37], mainly in endometrioid EOC [38], supporting divergence in their carcinogenesis pathways and different prognosis [39, 40].

According to WHO recommendations, mutated or aberrant p53 is characterized either by a strong nuclear expression in >60% of tumor cells, either by lack of staining (expressed in <5% of tumor cells) [25]. Our score, based on two variables, corresponded to this recommendation.

p53 expression score in tumor cells was high in more than half of the cases (52.63%) in our study group, supporting the role of this mutated protein in cell proliferation. According to literature, p53 is losing its expression in premalignant lesions and in low grade carcinomas, while high grade carcinomas, similar to endometrial location, may have common features with type II ovarian tumors [41]. A recent concept of ovarian carcinogenesis has emerged [42] in which the origin of serous ovarian carcinomas is fallopian tube epithelium and ovarian endometrioid and clear cell carcinomas arise from endometrial epithelial and stromal implants and our findings are in agreement with this concept.

p53 expression in endometriosis has been significantly lower when compared to tumor tissue (p=0.003), as an indicator of less aggressive behavior of endometriosis compared to its malignant counterpart or an early role of p53 in the malignant transformation of endometriosis. Literature data are in agreement with our findings [16, 43, 44].

There was a significant difference in p53 expression in tumor cells between endometrioid and non-endometrioid carcinomas (p=0.040), corresponding to a better prognosis associated to endometrioid ovarian carcinomas compared to more aggressive types (clear cell, high-grade serous). These findings are consistent with the data revealed by a study in which p53 alterations were found especially in high-grade ovarian clear cell carcinomas, irrespective of their association with endometriosis [45]. In a larger study group, of 79 cases, p53 was over-expressed in 24% of endometrioid EOCs, in a similar manner to serous EOCs (24%), but much lesser in clear cell EOCs [13]. These data are different from the main features of ovarian tumors without associated endometriosis, in which mutated p53 is highly suggestive of high-grade serous carcinoma (approximate 93%), compared to just 12% in clear cell carcinomas and only 11% in endometrioid carcinomas [25].

There was also a significant difference of p53 expression between early stage and late FIGO stage in our study group (p=0.037), corresponding to literature data [16].

No significant differences were registered between p53 expression in more differentiated and poorly differentiated histological types, suggesting a more important involvement of tumor type than of its degree of differentiation in tumor behavior determination.

Ki-67 is a nuclear marker of cell cycle, being absent in G0 cellular stage, and consequently studied for the evaluation of the proliferation status of different types of tumors [14]. Supplementary, its expression has been proposed as a predictor of endometriosis development [46].

Ki-67 index in tumor cells was low in more than half of the cases (57.89%) but even lower or absent in endometriosis, as an indicator of less proliferation activity in the pathogenesis of endometriosis compared to its malignant counterpart [47]. According to literature, Ki-67 has a lower expression in endometriosis than in proliferative endometrium [45; 47, 48] and in ovarian endometriosis compared to non-ovarian endometriosis [49], while it is high in type II ovarian tumors.

There was no significant difference in Ki-67 expression in tumor cells between endometrioid and non-endometrioid carcinomas, or between early stage and late FIGO stage, or between grades of differentiation in our study group, corresponding to a relative low proliferation rate in comparison with type II ovarian tumors.

CONCLUSIONS

The altered expression of steroid receptors in ovarian endometriotic tissue and EOC are involved in malignant transformation and progression.

P53 is contributing to endometriosis pathogenesis and EOC progression. EOCs are associated with low Ki-67 index compared to more aggressive types of tumors, supporting the latest hypothesis of ovarian carcinomas origin.

Immunohistochemical expression of ER, PR, and p53 corroborated with clinicopathological features support the mechanism of endometriosis transition to EOC, provide tools for prognosis evaluation, and open new perspectives of therapy for these types of tumors.

REFERENCES

1. Van Gorp T, Amant F, Neven P et al. Endometriosis and the development of malignant tumours of the pelvis. A review of literature. Best Pract Res Clin Obstet Gynaecol. 2004; 18:349-71.
2. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100(1):57-70.
3. Hanahan D, Weinberg RA. The hallmarks of cancer: the next generation. Cell. 2011; 144(5):646-74.
4. Scott R. Malignant change in endometriosis. Obstet Gynecol. 1953; 2:293-9.
5. Maeda D, Shih IM. Pathogenesis and the role of ARID1A mutation in endometriosis-related ovarian neoplasms. Adv Anat Pathol. 2013; 20:45-52.
6. Mikami Y. Endometriosis-related ovarian neoplasms: pathogenesis and histopathologic features. Diagn Histopathol. 2014; 20: 357-63.
7. Matias-Guiu X, Stewart CJR. Endometriosis-associated ovarian neoplasia. Pathology. 2018; 50(2):190-204.
8. Taniguchi F. New knowledge and insights about the malignant transformation of endometriosis. J Obstet Gynaecol Res. 2017; 43(7):1093-100.
9. Zeitvogel A, Baumann R, Starzinski-Powitz A. Identification of an invasive, N-cadherin-expressing epithelial cell type in endometriosis using a new cell culture model. Am J Pathol. 2001; 159(5):1839-52.
10. Park SH, Cheung LW, Wong AS et al. Estrogen regulates Snail and Slug in the down-regulation of E-cadherin and induces metastatic potential of ovarian cancer cells through estrogen receptor alpha. Mol Endocrinol. 2008; 22:2085-98.
11. Chen S, Dai X, Gao Y et al. The positivity of estrogenreceptor and progesterone receptor may not be associated with metastasis and recurrence in epithelial ovarian cancer. Sci Rep. 2017; 7(1):16922.
12. Tkalia IG, Vorobiova LI, Svintsisky et al. Clinical significance of hormonal receptor status of malignant ovarian tumors. Exp Oncol. 2014; 36:125-133.
13. Lai CR, Hsu CY, ChenYJ et al. Ovarian cancers arising from endometriosis: A microenvironmental biomarker study including ER, HNF1ß, p53, PTEN, BAF250a, and COX-2. J Chin Med Assoc. 2013; 76:629-34.
14. Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. J Cell Physiol. 2000;182:311-22.
15. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol. 1998; 11: 155-68.
16. Esmaili H, Vahedi A, Mohajeri S et al. Evaluation of the pathogenesis of tumor development from endometriosis by estrogen receptor, p53 and bcl-2 immunohistochemical staining. Asian Pac J Cancer Prev. 2016; 17(12):5247-50.
17. Hafez NH, Tahoun NS. Diagnostic value of p53 and ki67 immunostaining for distinguishing benign from malignant serous effusions. J Egypt Natl Canc Inst. 2011; 23(4):155-62.
18. Sampson JA. Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol. 1927; 14:422-69.
19. Kobayashi H, Sumimoto K, Moniwa N et al. Risk of developing ovarian cancer among women with ovarian endometrioma: a cohort study in Shizuoka, Japan. Int J Gynecol Cancer. 2007;17:37-43.
20. Matsumoto T, Yamazaki M, Takahashi H et al. Distinct β-catenin and PIK3CA mutation profiles in endometriosis-associated ovarian endometrioid and clear cell carcinomas. Am J Clin Pathol. 2015; 144(3):452-63.
21. Xue Q, Lin Z, Cheng YH et al. Promoter methylation regulates estrogen receptor 2 in human endometrium and endometriosis. Biol Reprod. 2007; 77:681-87.
22. Monsivais D, Dyson MT, Yin P et al. ERb- and prostaglandin E2-regulated pathways integrate cell proliferation via Ras-like and estrogen-regulated growth inhibitor in endometri¬osis. Mol Endocrinol. 2014; 28(8): 1304-1315.
23. Chae U, Min JY, Kim SH et al. Decreased progesterone receptor B/A ratio in endometrial cells by tumor necrosis factor-alpha and peritoneal fluid from patients with endometriosis. Yonsei Med J. 2016; 57(6): 1468-74.
24. Aghajanova L, Velarde MC, Giudice LC. Altered gene expression profiling in endometrium: evidence for progesterone resistance. Semin Reprod Med. 2010; 28(1):51-8.
25. Kurman RJ, Carcangiu ML, Herrington CS, Young RH. Tumours of the ovary. In: Kurman RJ, Carcanjiu ML, Herrington CS, Young RH (Editors). WHO Classification of Tumours of Female Reproductive Organs. Lyon: IARC Press, 2014. p. 11-86.
26. Shen F, Gao Y, Ding J, Chen Q. Is the positivity of estrogen receptor or progesterone receptor different between type 1 and type 2 endometrial cancer? Oncotarget. 2017;8(1):506-11.
27. Carcangiu ML, Chambers J T, Voynick IM, Pirro M, Schwartz PE. 183 patients with endometrial carcinoma. Part I: Clinical and histologic correlations. Am J Clin Path.1990; 94, 247-54.
28. Shabani N, Kuhn C, Kunze S et al. Prognostic significance of oestrogen receptor alpha (ERalpha) and beta (ERbeta), progesterone receptor A (PR-A) and B (PR-B) in endometrial carcinomas. Eur J Cancer. 2007; 43:2434-44.
29. Mylonas I, Jeschke U, Shabani N et al. Normal and malignant human endometrium express immunohistochemically estrogen receptor alpha (ER-alpha), estrogen receptor beta (ER-beta) and progesterone receptor (PR). Anticancer Res. 2005;25(3A):1679-86.
30. Sieh W, Kobel M, Longacre TA et al. Hormone-receptor expression and ovarian cancer survival: an Ovarian Tumor Tissue Analysis consortium study. Lancet Oncol. 2013; 14:853-62.
31. Rambau P, Kelemen LE, Steed H et al. Association of hormone receptor expression with survival in ovarian endometrioid carcinoma: biological validation and clinical implications. Int J Mol Sci. 2017; 18:515-20.
32. Worley MJ, Welch WR, Berkowitz RS, Ng S-W. Endometriosis-associated ovarian cancer: a review of pathogenesis. Intern J Mol Sci. 2013;14(3):5367-79.
33. Worley MJ, Liu S, Hua Y et al. Molecular changes in endometriosis-associated ovarian cell carcinoma. Eur J Cancer. 2015; 51(13):1831-42.
34. Kreizman Shefer H, Pricop J, Goldman S et al. Distribution of estrogen and progesterone receptors isoforms in endometrial cancer. Diagn Pathol. 2014; 9:77-85.
35. Kim JJ, Kurita T, Bulun SE. Progesterone action in endometrial cancer, endometriosis, uterine fibroids, and breast cancer. Endocr Rev. 2013; 34(1):130-62.
36. Xiao W, Awadallah A, Xin W. Loss of ARID1A/BAF250a expression in ovarian endometriosis and clear cell carcinoma. Int J Clin Exp Pathol. 2012;5(7):642-50.
37. Munksgaard PS, Blaakaer J. The association between endometriosis and ovarian cancer: a review of histological, genetic and molecular alterations. Gynecol Oncol. 2012; 124: 164-9.
38. Prefumo F, Venturini PL, Fulcheri E. Analysis of p53 and c-erbB-2 expression in ovarian endometrioid carcinomas arising in endometriosis. Int J of Gynecol Pathol. 2003; 22:83-8.
39. Erzen M, Rakar S, Klancar B et al. Endometriosis associated ovarian carcinoma (EAOC): an entity distinct from other ovarian carcinomas as suggested by a nested case-control study. Gynecol Oncol. 2001; 83:100-8.
40. Modesitt SC, Tortolero-Luna G, Robinson JB et al. Ovarian and extraovarian endometriosis-associated cancer. Obstet Gynecol. 2002; 100:788-95.
41. Mahadevappa A, Krishna SM, Vimala MG. Diagnostic and prognostic significance of Ki-67 immunohistochemical expression in surface epithelial ovarian carcinoma. J Clin Diagn Res. 2017;11(2):EC08-EC12.
42. Kuhn E, Kurman RJ, Shih IM. Ovarian cancer is an imported disease: Fact or fiction? Curr Obstet Gynecol Rep. 2012; 1:1-9.
43. Nezhat F, Cohen C, Rahaman J et al. Comparative immunohistochemical studies of bcl-2 and p53 proteins in benign and malignant ovarian endometriotic cysts. Cancer. 2002; 94:2935-40.
44. Nezhat F, Datta M S, Hanson V et al. The relationship of endometriosis and ovarian malignancy: a review. Fertil Steril. 2008; 90:1559-70.
45. Alduaij A, Hansen K, Karim TA et al. Clear cell carcinomas of the mullerian system: does the pathogenesis vary depending on their nuclear grade and their association with endometriosis? An immunohistochemical analysis. Path Res Intern. Volume 2012, Article ID 674748.
46. Huniadi CA, Pop OL, Antal TA et al. The effects of ulipristal on Bax/Bcl-2, cytochrome C, Ki-67 and cyclooxygenase-2 expression in a rat model with surgically induced endometriosis. Eur J Obstet Gynecol Reprod Biol. 2013; 169:360-5.
47. Kahyaoglu I, Kahyaoglu S, Moraloglu O et al. Comparison of Ki-67 proliferative index between eutopic and ectopic endometrium: a case control study. Taiwan J Obstet Gynecol. 2012;51(3):393-6.

47. Kahyaoglu I, Kahyaoglu S, Moraloglu O et al. Comparison of Ki-67 proliferative index between eutopic and ectopic endometrium: a case control study. Taiwan J Obstet Gynecol. 2012;51(3):393-6.

48. Suzuki A, Horiuchi A, Oka K et al. Immunohistochemical detection of steroid receptor cofactors in ovarian endometriosis: involvement of down-regulated src-1 expression in the limited growth activity of the endometriotic epithelium. Virchows Arch. 2010;456:433-41.

49. Calcagno A, Grassi T, Mariuzzi L et al. Expression patterns of Aurora A and B kinases, Ki-67 and the estrogen and progesterone receptors determined using an endometriosis tissue microarray model. Hum Reprod. 2011; 26(10):2731-41.