ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

The value of PAX8 and WT1 molecules in ovarian cancer diagnosis

LUDMILA LILIAC[1,2], MARIA LUISA CARCANGIU[3], SILVANA CANEVARI[2], IRINA-DRAGA CĂRUNTU[1], DELIA GABRIELA CIOBANU APOSTOL[1], M. DANCIU[1], M. ONOFRIESCU[4], CORNELIA AMĂLINEI[1]

[1]*Department of Morphofunctional Sciences – Histology, Morphopathology, "Grigore T. Popa" University of Medicine and Pharmacy, Iassy, Romania*

[2]*Department of Experimental Oncology and Molecular Medicine – Unit of Molecular Therapies, Istituto dei Tumori, Milan, Italy*

[3]*Department of Anatomic Pathology, Istituto dei Tumori, Milan, Italy*

[4]*Department of Mother and Child Medicine – Obstetrics and Gynecology, "Grigore T. Popa" University of Medicine and Pharmacy, Iassy, Romania*

Abstract

PAX8 and WT1 are transcription factors, each of them with distinct roles in organogenesis, morphogenesis, cell growth, and differentiation. Recently, their expression was also confirmed in a variety of malignancies, being included in the antibodies panel recommended for the female genital tract pathology. The aim of our study was to evaluate PAX8 and WT1 in different types of ovarian cancer (OC) with focus on (i) the completion of evidences of the Müllerian origin and (ii) the establishment of primary ovarian tumor status *vs.* metastasis. The study group consisted of 86 cases, with histopathological diagnosis covering the main subtypes of OC (low- and high-grade serous, low- and high-grade endometrioid, clear cell, mucinous, malignant Brenner tumor, malignant mixed Müllerian tumor, undifferentiated, and borderline). The investigation was based on immunohistochemical examination, performed by using specific antibodies applied on blocks obtained through Tissue MicroArray technique, and interpreted by scores assessing the nuclear positivity of tumoral cells. One case was not valuable due to technical difficulties. PAX8 expression was positive in 70 (81.39%) cases, the remaining 15 (17.44%) negative cases suggesting a non-Müllerian origin. WT1 expression was positive in 61 (71%) cases, mainly expressed in serous carcinoma, regardless of their differentiation degree, and negative in 24 (27%) cases. Our study provide supplementary evidences to support the association of PAX8 and WT1 immunostaining in the investigation of the complex biology of OC, PAX8 confirming the ovarian primary and WT1 allowing the refinement of the diagnosis in phenotype overlapping cases.

Keywords: ovarian cancer, immunohistochemistry, PAX8, WT1.

Introduction

Ovarian carcinoma is the fifth most frequent death cause for women [1]. Among all gynecological cancers, ovarian carcinoma has the highest aggressivity [2] and an elevated rate of mortality as compared with its incidence rate [3]. Both the lack of characteristic symptoms in early stages and of specific markers useful in effective screening program results in a high mortality [4]. The ovarian cancer diagnosis is usually delayed until advanced stages (II–IV), which are characterized by carcinomatous invasion beyond the ovarian surface and pelvis and peritoneal cavity dissemination [5]. In these circumstances, according to the new concept of ovarian carcinogenesis [2, 6], which supports the extraovarian origin, an ideal screening program should be oriented towards possible targets located in the pelvis [7, 8], thus completing the genetic screening that can be currently applied only to the patient subpopulation with family medical history [9].

The tubal secretory cells may be considered as the initiation situs of ovarian carcinogenesis according to the new ovarian carcinogenesis concept [7, 8]. The identification of specific markers of the precursor Müllerian ducts cells from the coelomic epithelium within normal endometrial and tubal mucosa provides strong evidence that supports the new carcinogenic hypothesis [10]. These markers maintain their expression not only in endometrial or tubal tumors, but also in certain types of ovarian and peritoneal tumors [11].

Within this context fall the reports on PAX8 protein [12], a product of PAX gene family. PAX8 belongs to a family of 9 proteins (PAX1–PAX9) where each member is directly implicated in the transcription of various genes, involved in organogenesis, morphogenesis, thyroid, renal and Müllerian cell differentiation [12]. This marker, initially identified in normal cells originating in Müllerian ducts, is also present in ovarian neoplasias [8, 13] and is characteristic for the epithelial phenotypes (serous, clear cell, and endometrioid). Consequently, the positive expression of PAX8 represents a strong argument for the confirmation of the origin of ovarian carcinoma in the fimbrial area of Fallopian tubes or in endometriosis foci

[11]. Moreover, PAX8 allows the differentiation between Müllerian and non-Müllerian origin in the case of an ovarian metastatic carcinoma that could derive from a primary tumor in pancreas, colon or mammary gland [11].

Another marker inserted in the panel of antibodies recommended for the diagnosis of primary tumor of female genital tract [14] is WT1. The tumor suppressor gene WT1 was identified for the first time in the genito-urinary system (kidney, ovary, and testes) [15] and is responsible for the coding of a transcription factor of 52–54 kDa important in cell growth and differentiation [16]. WT1 is responsible for the development of hereditary and sporadic types of Wilms tumors within renal parenchyma. Regarding WT1 role in the female reproductive system, WT1 is involved in structural and functional development of the gonads and is over-expressed in primordial and primary ovarian follicles [17]. In the normal mature ovary, WT1 is expressed in the ovarian surface epithelium and in stromal and granulosa cells [14]. In the tumoral ovary, WT1 is characteristic for the serous subtype of ovarian carcinoma and is rarely found in the non-serous subtypes [14].

The aim of our study was to evaluate the immuno-histochemical (IHC) expression of PAX8 and WT1 in various types of ovarian malignancies with focus on (i) the completion of evidences of the Müllerian origin and (ii) the establishment of primary ovarian tumor status *vs.* metastasis. The motivation for this investigation is supported by the fact that these markers represent for the pathologist valuable instruments for the refinement of the diagnosis in a context where the morphologic profile is characterized by phenotype overlaps. The originality of this research is demonstrated by the scarce information found by a thorough review of the mainstream publications and is furthermore amplified by the two markers complementary expression pattern. To our knowledge, PAX8 and WT1 experience in Romanian histopathology is extremely limited. Therefore, this study is completing the literature data that support the implementation of these markers in the recommended panel in ovarian cancer diagnosis.

Materials and Methods

Case selection

The study group consisted of 86 cases of malignant ovarian tumors diagnosed between January 1st, 2006 and December 31st, 2011 in "St. Spiridon" Emergency Clinical Hospital and "Cuza Vodă" Obstetrics and Gynecology Clinical Hospital from Iassy, Romania.

The study was approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy, Iassy, based on the patients' informed written consent for the usage of the biologic material.

The 86 cases were histopathologically classified according to the carcinogenesis mechanism and based on that proposed by Kurman RJ [7], as follows:

- 23 cases (26.5%) as type I category, *low-grade*: two cases low-grade serous carcinoma (LGSC), 13 cases mucinous ovarian carcinoma (MOC), five cases low-grade endometrioid carcinoma (EOC), two cases clear cell ovarian carcinoma (CC-OC), and one case malignant Brenner tumor (BM);
- 60 cases (73.49%) as type II category, *high-grade*: 52 cases high-grade serous carcinoma (HGSC), three cases high-grade endometrioid carcinoma (HGEC), four cases undifferentiated carcinoma (UNDIFF), one case malignant mixed Müllerian tumor (MMMT);
- three cases as borderline ovarian tumor (BOT).

The correspondence between the histopathological diagnosis, the grade, the FIGO stage and the current status of patients (alive or dead) is illustrated in Table 1.

Table 1 – ***Synoptic presentation of the main characteristics of the cases***

Tumor type	Tumor subtype	Total No. of cases	Other characteristics								
			Age median [years]	*Grade*			*FIGO Stage*			*Status*	
				G1	*G2*	*G3*	*I*	*II*	*III*	*Alive*	*Dead*
				No. of cases							
I	LGSC	2	59	2	0	0	0	0	III C – 2	2	0
	LGEC	5	54	5	0	0	I A – 2	II A – 1	III A – 2	5	0
	CO-CC	2	57	2	0	0	I A – 1	0	III C – 1	1	1
	MOC	13	52	7	4	2	I A – 5 I C – 3	II A – 1	III A – 1 III B – 2 III C – 1	9	4
	BM	1	42	0	0	1	0	0	III A – 1	0	1
II	HGSC	52	61	0	19	33	I A – 2 I C – 4	II A – 2 II B – 2 II C – 1	III A – 7 III B – 6 III C – 28	29	23
	HGEC	3	57	0	1	2	I B – 2	0	III B – 1	2	1
	MMMT	1	81	0	0	1	0	0	III A – 1	0	1
	UNDIF	4	57	0	2	2	I C – 1	II B – 1	III C – 2	3	1
BOT	BOT	3	49	2	1	0	I A – 1	II C – 2	0	2	1

Tissue MicroArray and immunohistochemistry

The immunohistochemical investigation was performed in the Unit of Molecular Therapies, Department of Experimental Oncology and Molecular Medicine, Istituto dei Tumori, Milan, Italy; the PAX8 and WT1 antibodies (Table 2) were applied on blocks obtained through the Tissue MicroArray (TMA) technique.

Table 2 – *Antibodies characteristics*

Antibody	Clone	Dilution	Expression
PAX8	Polyclonal, code 10336-1-AP, ProteinTech, Chicago	1:400	Nuclear
WT1	Clone WT49, code MONX 11064, Monosan, Netherlands	1:80	Nuclear

For each selected case, a representative block was chosen and designated as "donor" block.

TMA was performed with a semi-automated machine, GALILEO TMA CK 3500. From each "donor" block were extracted 1–4 cores of tumoral tissue with a diameter of 1.5 mm, which were incorporated into new "recipient" blocks. Four TMA blocks, each containing 50–60 cores, have been constructed. From the recipient blocks, serial sections were cut at 4 μm in order to perform the IHC coloration with Autostainer Link 48 Dako.

The sections were extracted from paraffin, rehydrated and treated with the HIER (heat-induced epitope retrieval) technique with PT Link Dako using EDTA high pH (15 minutes for PAX8, 30 minutes for WT1).

After blocking endogenous peroxidase, slides were incubated with the primary antibodies for 30 minutes, followed by 15 minutes incubation with the appropriate secondary antibody (EnVision FLEX, anti-rabbit for PAX8, and anti-mouse for WT1, Dako) and 20 minutes with the enzyme (EnVision FLEX/HRP, Dako). The reaction was developed using the 3.3'-diaminobenzidine tetrahydrochloride chromogen (DAB, Dako), for 10 minutes. The slides were counterstained with Hematoxylin. Positive and negative controls were performed concomitantly.

Consequently, the slides were scanned with the APERIO SCAN SCOPE xT. The APERIO IMAGE SCOPE / SCAN SCOPE IMAGE were used for image acquisition. The program facilities allow the visualization and image acquisition with ×4, ×5, ×10, and ×20 magnification, after scanning. The entire tissular area corresponding to each TMA core has been analyzed, the histopathologist progressively using all the program functions, allowing the evaluation using the entire magnification spectrum.

The IHC reaction was interpreted according to the scores reported in the literature as follows:

- PAX8 positive: reaction present in >5% of tumoral cells nuclei [13];
- WT1 positive: reaction present in >50% of tumoral cells nuclei [18].

Results

The two cases diagnosed as LGSC showed a micropapillary pattern, exhibiting delicate central fibrous cores or sometimes absent and tumoral cells with a tendency toward anastomosing arrangements, responsible for a labyrinthic appearance. Psammoma bodies have been identified in a single case. The homogenous cytology showing small, bland nuclei, with regular, rounded contour resulted in both tumors grading as G1.

The pattern of the five cases diagnosed as LGEC was categorized as glandular, with tumoral glands lined by endometrioid-like epithelium, sharp lumens, back-to-back orientation and intervening fibro-cellular stroma. G1 grade attributed to all cases was based on the lack of cellular atypia and of mitotic figures.

Architectural characteristics of the two cases CO-CC were of tubulo-cystic type (cystically dilated glands lined by flattened epithelium), tumoral component being situated within a hyalinized, eosinophilic, and fibroblastic stroma. The tumoral cells showed clear cytoplasm and hyperchromatic nuclei, without atypia, showing the characteristic surface bulge (hobnail pattern). Both cases have been classified as G1.

The 13 cases diagnosed as MOC revealed an expansile pattern, glandular or papillary growth, forming structures of variable size, separated by scarce interglandular stroma by confluent and interconnected proliferation. The morphologic evaluation of cells showing similar features as endocervical or gastrointestinal epithelium resulted in their categorization within the following grading categories: G1 – seven cases, G2 – four cases, and G3 – two cases.

Brenner tumor diagnosis established in a case was supported by the presence of nests of round-ovalar tumoral cells similar to the transitional epithelium arranged in a solid pattern, with well-delimited margins, and dispersed within a dense fibrous stroma. The cellular pleomorphism and nuclear atypia were appreciated as G3.

In 52 cases diagnosed as HGSC, the tumoral pattern was predominantly solid, characterized by compact tumoral islands separated by thin connective septae. The solid architectural organization was associated with extensive necrosis and limited areas showing papillary or micropapillary cellular aggregations, or glandular microcystic type growth. A single case exhibited a transitional-like pattern, containing tumoral papillae with smooth surface and central fibrous cores and multiple lining layers of compact cellular appearance, without slit-like or microcystic type spaces. The analysis of tumoral cellularity (cuboidal and/or cylindrical cells, sometimes with clear or signet ring features, atypical, pleomorphic, hyperchromic nuclei, with irregular nuclear membrane, and unevenly chromatin distribution) supported the grading evaluation as G2 in 19 cases and G3 in 33 cases.

The three cases diagnosed as HGEC were characterized by a solid growth pattern, cribriform, with associated squamous differentiation, in a single case. Based on the cyto-nuclear pleomorphism and on the mitotic index evaluation, one case has been graded as G2 and the other two cases have been graded as G3.

Morphologic appearance supporting the MMMT diagnosis (one case) included an undifferentiated epithelial carcinomatous component and a sarcomatous component, homologous to the native ovarian tissue, the cellular changes severity being appreciated as G3.

Undifferentiated carcinoma diagnosis formulated in four cases was based on the absence of any specific structural elements for epithelial ovarian tumors. According to the cytologic features evaluation (undiffer-

entiation, marked cyto-nuclear pleomorphism), two cases have been graded as G2 and the other two cases as G3.

The three cases diagnosed as BOT showed in 10% of tumoral surface a proliferation pattern characterized by hierarchical branching, with gland-like or papillae formation which could result in a cribriform or Roman-bridge pattern by their fusion. In large tumoral glands and/or papillae, lined by a budded stratified epithelium, single or small cluster cellular detachments were noticed. The grading had been established as G1 in two cases and G2 in one case.

The results of the IHC evaluation of the two markers for each histological subtype in the study group are briefly presented in Table 3. The interpretation of the immunoreactivity was performed for 85 cases, one case being not valuable due to technical difficulties.

Table 3 – ***The expression of PAX8 and WT1 in different subtypes of epithelial ovarian carcinomas***

Tumor type	Tumor subtype	No. of cases	PAX8			WT1		
			Positive	*Negative*	*NV*	*Positive*	*Negative*	*NV*
			No. of cases (%)			*No. of cases (%)*		
I	LGSC	2	2 (100%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)
	LGEC	5	4 (80%)	0 (0%)	1 (20%)	1 (20%)	3 (60%)	1 (20%)
	CO-CC	2	2 (100%)	0 (0%)	0 (0%)	1 (50%)	1 (50%)	0 (0%)
	MOC	13	4 (30.77%)	9 (69.23%)	0 (0%)	1 (7.69%)	12 (92.31%)	0 (0%)
	BM	1	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
II	HGSC	52	49 (94.23%)	3 (5.77%)	0 (0%)	50 (96.15%)	2 (3.85%)	0 (0%)
	HGEC	3	3 (100%)	0 (0%)	0 (0%)	1 (33.33%)	2 (66.67%)	0 (0%)
	MMMT	1	1 (100%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
	UNDIF	4	2 (50%)	2 (50%)	0 (0%)	1 (25%)	3 (75%)	0 (0%)
BOT	BOT	3	3 (100%)	0 (0%)	0 (0%)	3 (100%)	0 (0%)	0 (0%)

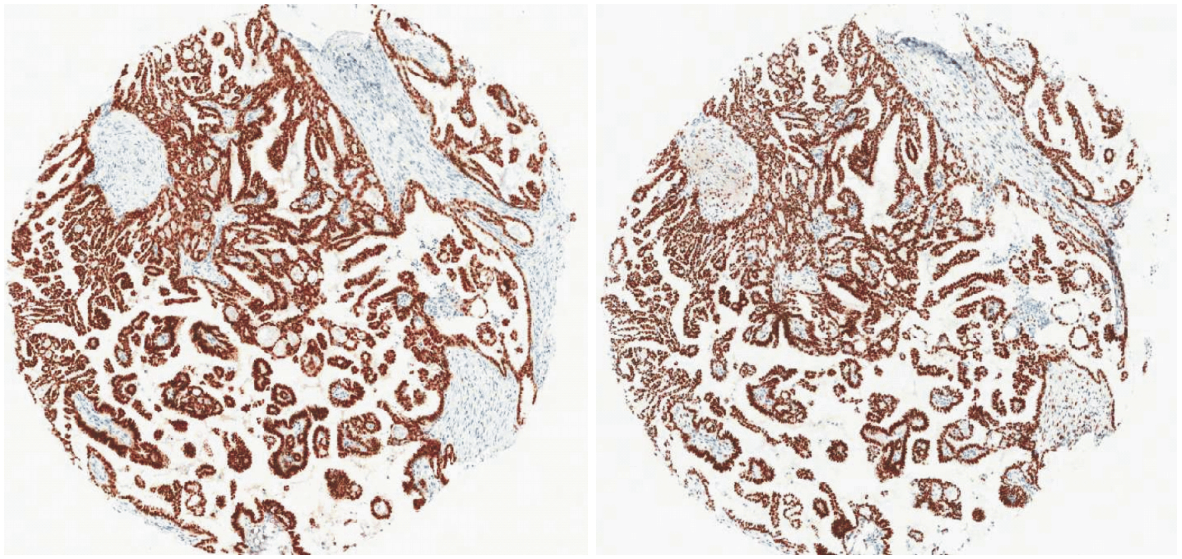
NV – Not valuable cases: including histological, technical, and staining difficulties.

The expression of PAX8 was positive in 70 (81.39%) cases, the remaining 15 (17.44%) cases being negative. Among these, nine (10.46%) cases were later re-diagnosed as metastatic mucinous ovarian carcinomas (based on supplementary IHC exams using CK7 and CK20 as markers).

The expression of WT1 was positive in 61 (71%) cases, the remaining 24 (27%) cases being negative. According to the histologic subtypes, WT1 was mainly expressed in serous carcinoma, regardless of their differentiation degree.

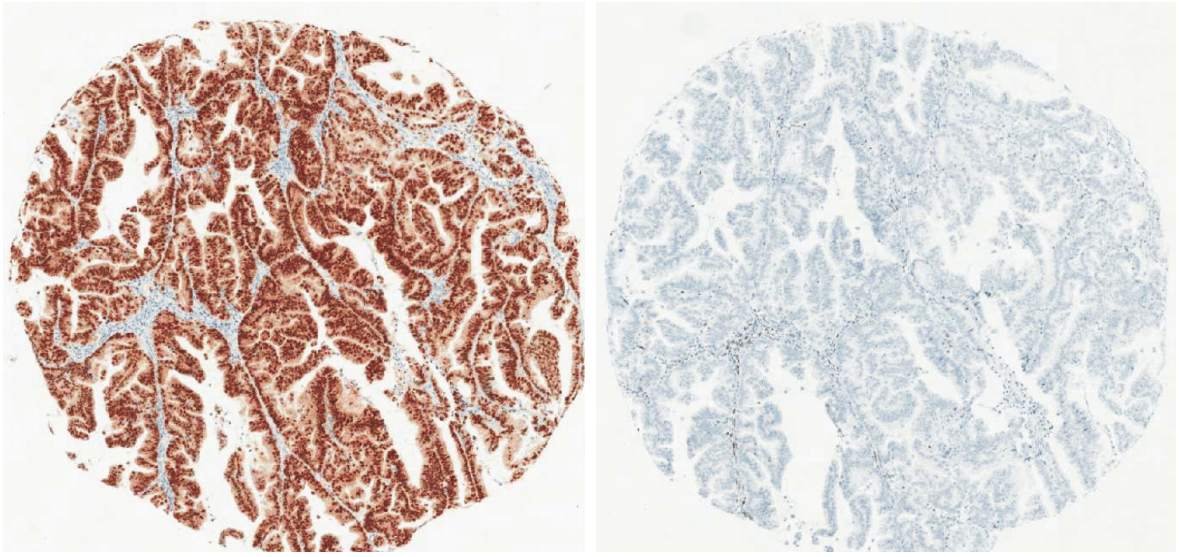
Representative images show the nuclear immuno-localization of PAX8 and WT1 in different histological subtypes of epithelial ovarian cancer (Figures 1–10).

A special mention should be added regarding the PAX8 and WT1 quantification. Although their expression have been quantified according to the positive nuclei percent, the immunohistochemical intensity being considered as irrelevant (based on the operational scores), TMA spots analysis corresponding to the 85 investigated cases revealed different staining intensities (from weak to strong), as showed in the above-mentioned figures.



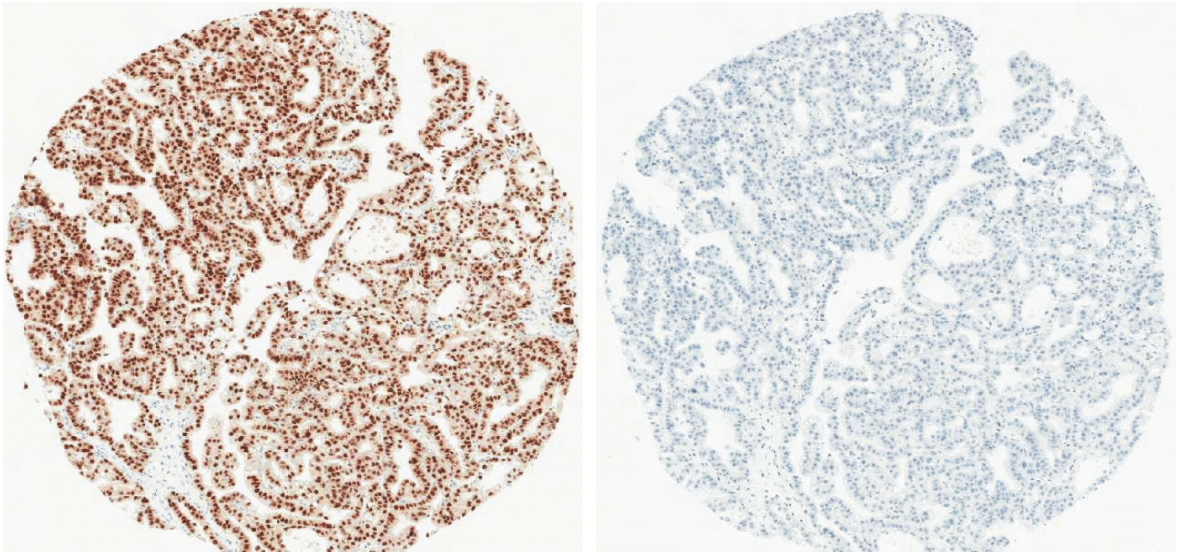
(a) **(b)**

Figure 1 − ***Intense positive reaction for PAX8 (a) and WT1 (b) in low-grade serous carcinoma (IHC, anti-PAX8, anti-WT1, ob. ×4).***



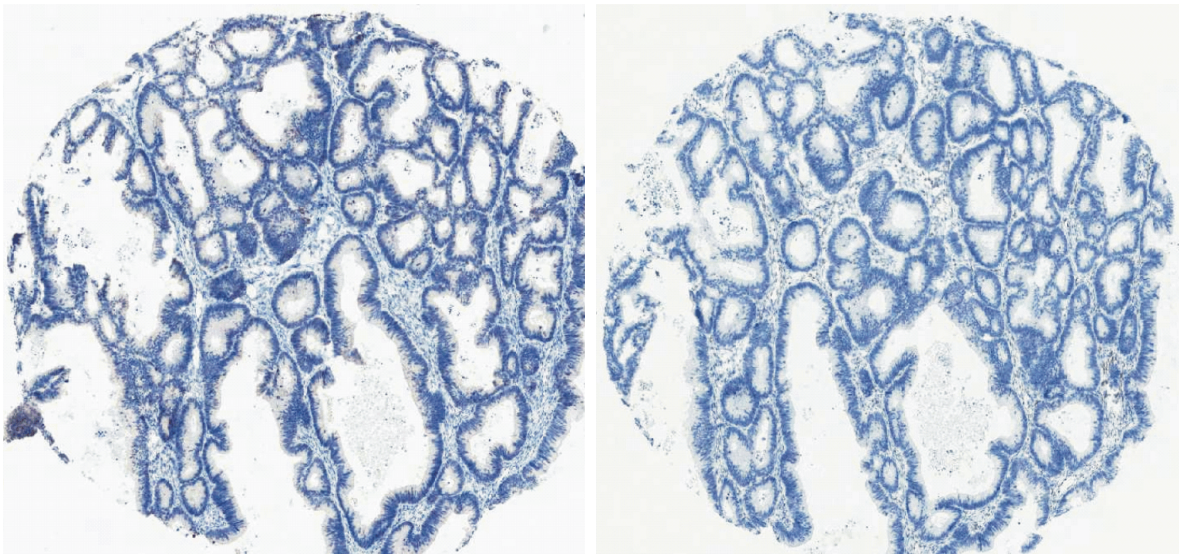
(a) (b)

Figure 2 − ***Intense and homogenous positive reaction for PAX8 (a), negative reaction for WT1 (b) in low-grade endometrioid carcinoma (IHC, anti-PAX8, anti-WT1, ob. ×4).***



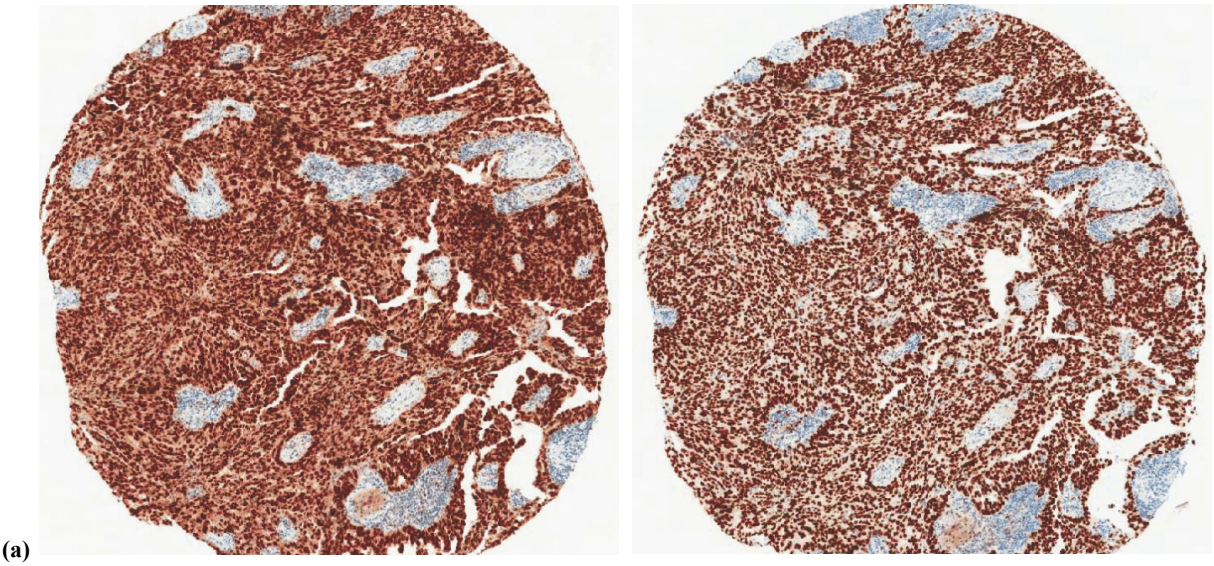
(a) (b)

Figure 3 − ***Intense positive reaction for PAX8 (a) and negative reaction for WT1 (b) in clear cell ovarian carcinoma (IHC, anti-PAX8, anti-WT1, ob. ×4).***



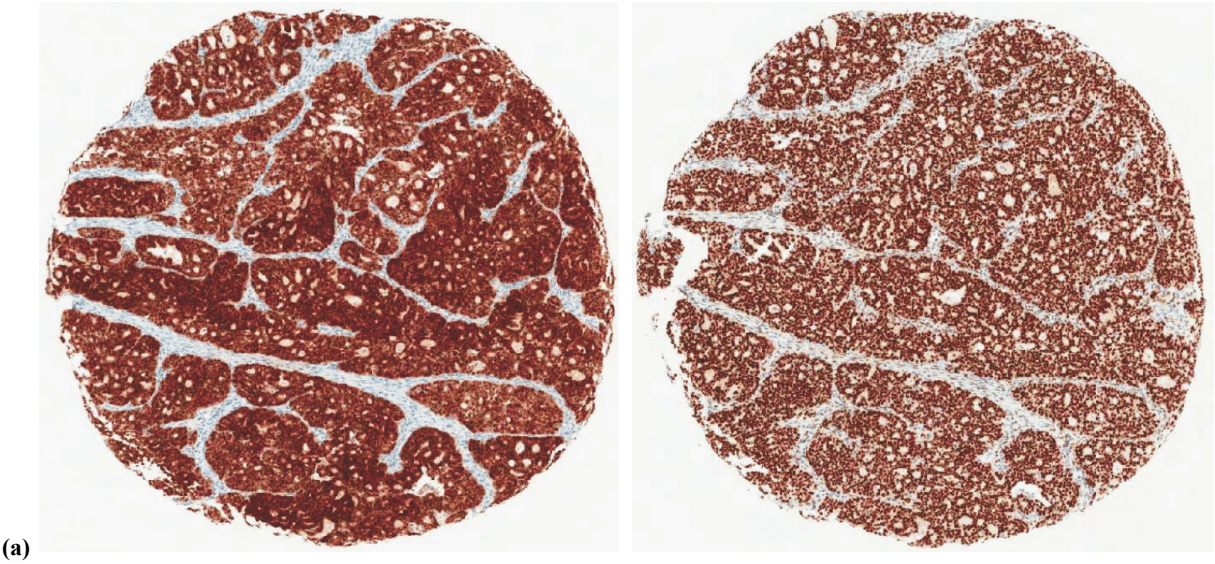
(a) (b)

Figure 4 − ***Negative reaction for PAX8 (a) and WT1 (b) in mucinous ovarian carcinoma (IHC, anti-PAX8, anti-WT1, ob. ×4).***



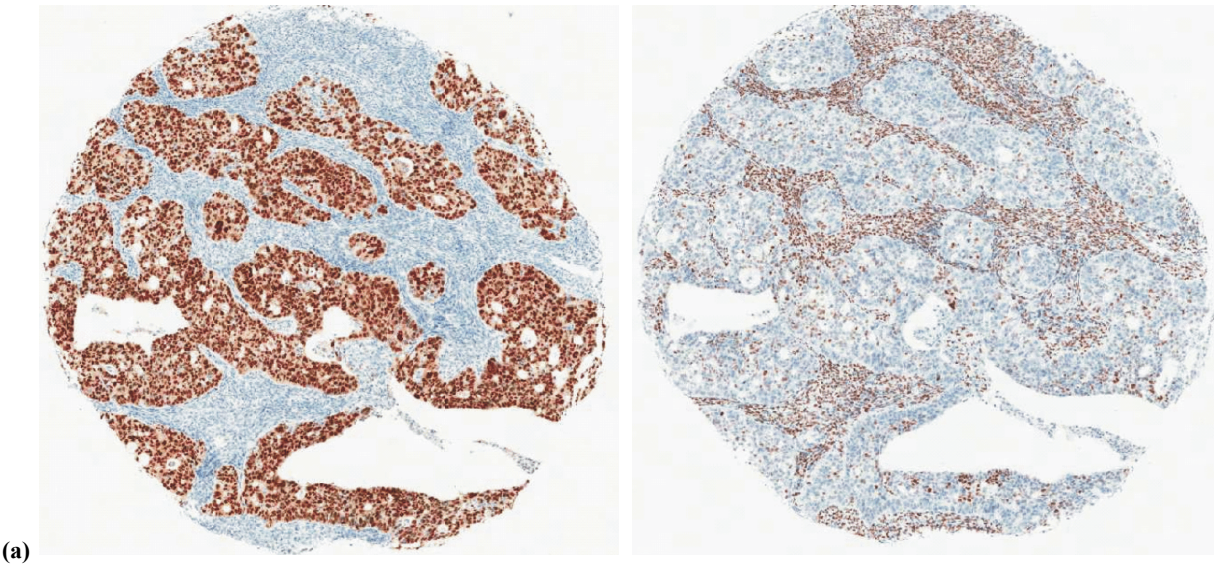
(a) **(b)**

Figure 5 − ***Strong and homogenous positive reaction for PAX8 (a) and WT1 (b) in high-grade serous carcinoma (IHC, anti-PAX8, anti-WT1, ob. ×4).***



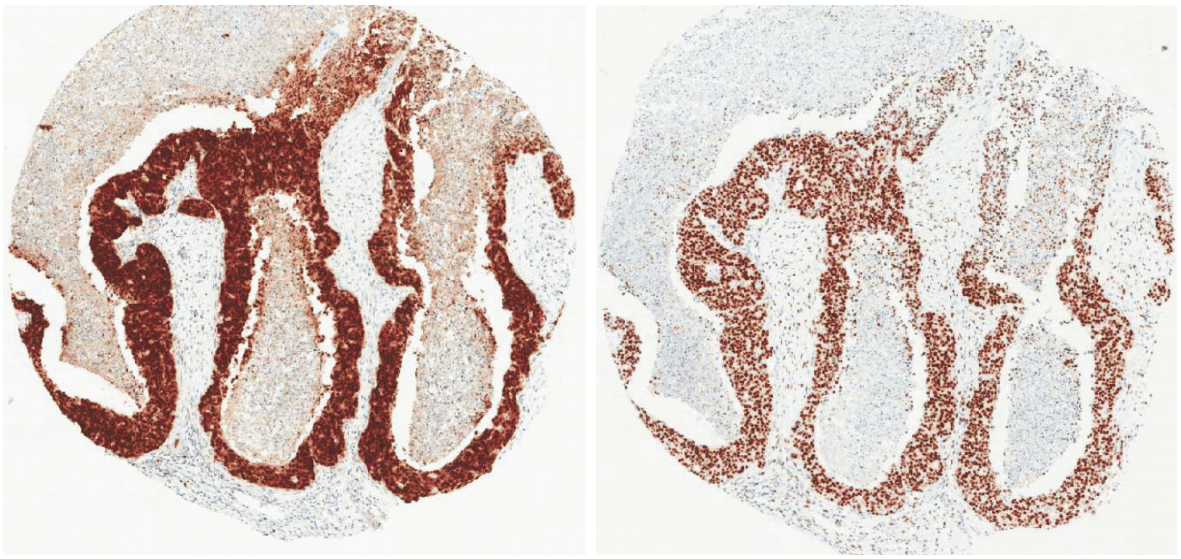
(a) **(b)**

Figure 6 − ***Strong and homogenous positive reaction for PAX8 (a) and WT1 (b) in high-grade endometrioid carcinoma (IHC, anti-PAX8, anti-WT1, ob. ×4).***



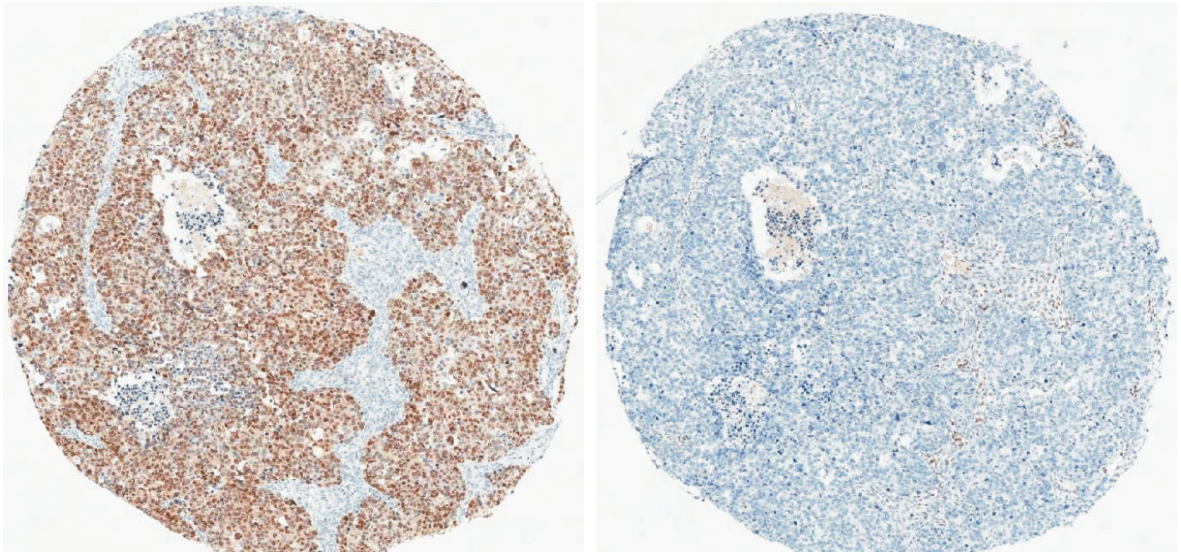
(a) **(b)**

Figure 7 − ***Moderately positive reaction for PAX8 (a) and negative reaction for WT1 (b) in high-grade endometrioid carcinoma (IHC, anti-PAX8, anti-WT1, ob. ×4).***



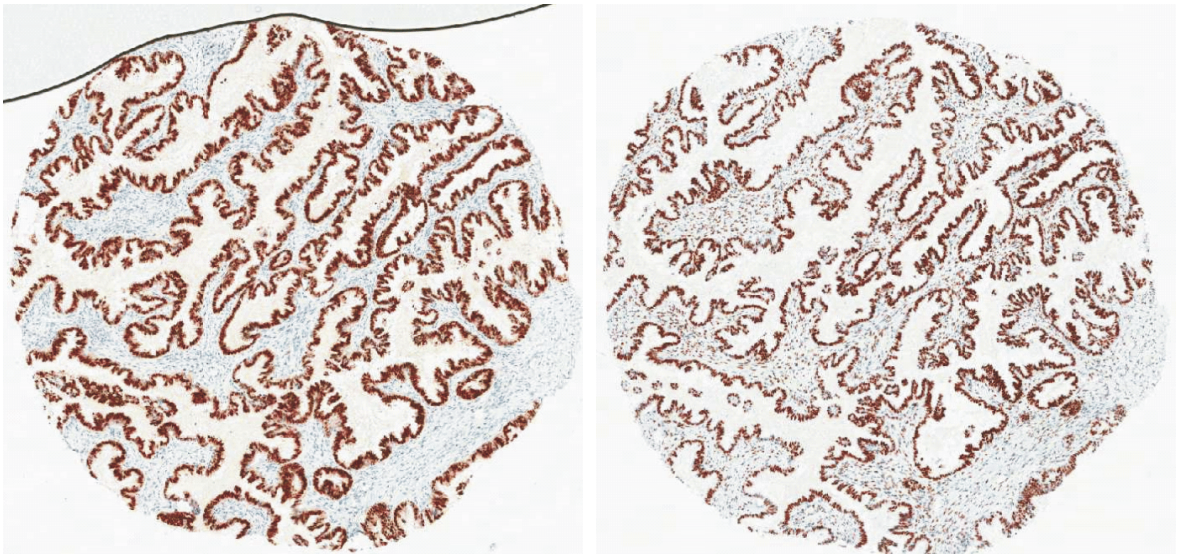
(a) (b)

Figure 8 − ***Strong positive reaction for PAX8 (a) and weak positive reaction for WT1 (b) in malignant mixed Müllerian tumor (IHC, anti-PAX8, anti-WT1, ob. ×4).***



(a) (b)

Figure 9 − ***Weak positive reaction for PAX8 (a) and negative reaction for WT1 in undifferentiated carcinoma (IHC, anti-PAX8, anti-WT1, ob. ×4).***



(a) (b)

Figure 10 − ***Strong positive reaction for PAX8 (a) and moderately positive reaction for WT1 in borderline ovarian tumor (IHC, anti-PAX8, anti-WT1, ob. ×4).***

Discussion

Although the ovary is only 3×2×1 cm in size, it is the organ where the highest number of tumoral histological subtypes may develop related to a certain anatomical location. Excepting metastatic tumors, more than 100 histological subtypes, specifically 115, have been identified in ovary and coded by *WHO* into three main phenotypes: malignant, benign, and borderline, respectively [5].

The origin of the ovarian tumors is still a controversial issue. Several hypotheses and theories supported by embryological and molecular evidences have been advanced at different times. A recent proposal pleads for the replacement of the "ovarian tumor" term with the one of "pelvic serous tumor" that covers all three anatomical locations – ovary, peritoneum and Fallopian tube. The "pelvic serous tumor" term suggests the morphologic similarities, which make sometimes the identification of the primary location impossible [19].

The current paper focuses on the value of PAX8 and WT1 as operational elements in an IHC algorithm that can be applied in the epithelial ovarian carcinomas in positive and differential diagnosis, respectively. The necessity of a diagnostic refinement, which surpasses the phenotypic resemblances falls outside the strict framework of exact pathologic classification. Such a refinement that contributes to the avoidance of diagnostic over- or under-evaluations is vital in the context of a scientific research that centers on the correlation of the molecular profile with prognosis and survival elements.

PAX8 expression value

The research oriented mainly towards the identification of a diagnosis marker with specificity for the malignant ovarian status created the premises for the increase in the value of PAX8 usage, recent studies investigating the expression of PAX8 in the normal and tumoral ovary [8, 11–13, 20–23].

The surface epithelium of the normal ovary does not express PAX8 due to its mesonephric (coelomic) origin [13]. The presence of PAX8 both in normal secretory epithelial cells of the Fallopian tubes and in ovarian tumors supports the Müllerian origin of the ovarian carcinoma [8]. Thus, PAX8 is a valuable marker that supports the hypothesis stating that HGSC originates in the fimbrial area of the Fallopian tubes. According to the mainstream publications reports, almost all serous and non-serous ovarian carcinomas (endometrioid, clear cell or transitional) regardless of their differentiation degree, as well as tubal and peritoneal serous carcinomas are positive for PAX8.

Although literature data shows a low or absent PAX8 immunoreactivity of tumoral cells in mucinous ovarian carcinoma, any degree of PAX8 immunopositivity favors the ovarian primary, with a special comment in cases where PAX8 negativity does not completely exclude an ovarian origin [12].

Due to its Müllerian specificity, this marker is useful for the differentiation of the ovarian carcinomas, especially in the advanced stages, from breast carcinomas or malignant mesotheliomas exhibiting similar histologic features [8]. Moreover, PAX8 has a diagnostic value as Müllerian differentiation marker in pleural and peritoneal serous effusions demonstrating the origin in HGSC or in LGSC [12, 24].

The expression of PAX8 is not different in primary from secondary tumors and consequently being regarded as an important diagnosis marker in metastatic ovarian carcinoma [23].

The positivity of PAX8 is reported not only in the malignant lesions of structures of Müllerian origin but also in the benign ones, such as endometriosis, endosalpingiosis, paraovarian or paratubal Müllerian embryonic remnants [23]. Moreover, the areas of normal ovary adjacent to the benign or malignant tumoral territories do not express PAX8 in stromal cells, a feature, which emphasizes the epithelial specificity of this marker exhibiting an intrinsic embryological control directed exclusively towards the epithelial sublineages [13].

We must stress the fact that to our knowledge the single other study, which correlates the expression of PAX8 to the classic clinical prognosis factors [11], had reported no value in the outcome prediction.

In our study, PAX8 evaluation was based only on its nuclear expression, without taking into account the cytoplasmic immunoreactivity. The immunostaining was regarded as positive if more than 5% of the nuclei showed positivity.

The results allowed a comparative analysis with the recent reports of studies focused on the investigation of PAX8 in different epithelial ovarian tumoral subtypes (Table 4) [8, 12, 13, 20–23].

Table 4 – ***PAX8: literature data vs. personal results***

PAX8 expression	Serous carcinoma	Endometrioid carcinoma	Clear cell carcinoma	Mucinous carcinoma
	No. of positive cases from the total No. of investigated cases (%)			
Bowen NJ *et al.*, 2007 [20]	15/19 (79%)	9/14 (64%)	2/2 (100%)	2/18 (11%)
Köbel M *et al.*, 2008 [21]	No data	48/125 (38.4%)	100/132 (75.75%)	2/31 (6.45%)
Nonaka D *et al.*, 2008 [22]	81/84 (96.4%)	16/18 (88.9%)	10/10 (100%)	1/12 (8.3%)
Laury AR *et al.*, 2011 [12]	164/165 (99%)	11/12 (92%)	2/2 (100%)	10/25 (40%)
Ozcan A *et al.*, 2011 [23]	101/102 (99%)	72/77 (93%)	16/16 (100%)	4/10 (40%)
Tacha D *et al.*, 2011 [13]	101/109 (93%)	49/59 (83%)	3/3 (100%)	27/54 (50%)
Tong GX *et al.*, 2011 [8]	14/16 (87.5%)	4/5 (80%)	3/3 (100%)	0/2 (0%)
Personal results	49/52 (94%) HGSC 2/2 (100%) LGSC	3/3 (100%) HGEC 4/5 (80%) LGEC	2/2 (100%)	4/13 (31%)

Our results accomplish the few previous studies and support the role of PAX8 in the pathological evaluation of the tumoral specimens both for diagnostic classification of the ovarian carcinomas and for confirmation of the primary location of undifferentiated or metastatic tumoral subtypes.

PAX8 expression both in secretory cells of the Fallopian tubes and in HGSC confirms the relationship between the Fallopian tube and the ovary in the carcinogenesis process sequences and underlines the role of PAX8 as a specific oncogene of the Müllerian cell lineage [23].

WT1 expression value

WT1 is recommended in the differential diagnosis of primary ovarian tumors exhibiting non-specific morphological features or in exclusion of uterine, breast, pancreatobiliary or gastrointestinal metastases, exhibiting similar morphologic phenotype [14, 25]. Moreover, co-expression of WT1 and PAX8 has been recently demonstrated as a valuable association in confirming the ovarian origin of malignant effusions [24].

WT1 is extremely useful in synchronous endometrial and ovarian serous tumors frequently encountered in gynecologic pathology [15]. In these synchronous tumors, the primary origin is essential in selection of a targeted chemotherapeutic management of the ovarian tumor with endometrial metastasis or of an association chemotherapy–radiotherapy in endometrial tumor with ovarian metastasis. Furthermore, the current research trend centered on ovarian cancer is focused on the correlation of the ovarian tumoral biology with a personalized therapy in accordance with the molecular background [14].

WT1 is generally consistently negative in the endometrial serous tumors as compared with the ovarian counterpart and thus contributes to the differentiation between the two serous tumoral entities [15], each of them being associated to specific pathogenic mechanisms. However, in cases when both entities are WT1 positive for thorough investigations are necessary to identify supplementary data of the dissemination pathway (either ovarian-endometrial or endometrial-ovarian sequence) [26].

WT1 expresses a different pattern in different histological subtypes of ovarian carcinomas. Serous ovarian carcinomas – HGSC, LGSC – that originate in the Fallopian tubes, in the peritoneum and in the ovarian cortical inclusion cysts are always WT1 positive [27]. A major issue is related to the ovarian endometrioid subtype, which has a heterogeneous WT1 expression: WT1 positivity reflects *de novo* ovarian tumor or originating in the Fallopian tube or peritoneum, while WT1 negativity indicates an ovarian tumor with origin in endometriosis foci [14]. Thus, the difference in WT1 expression translates distinct pathogenic events [14]. WT1 differentiates serous ovarian carcinomas exhibiting similar morphology to that of pure clear cell ovarian carcinoma, as WT1 is negative [28]. WT1 does not have a differential diagnosis value in ovarian HGSC *vs.* high-grade serous peritoneal carcinoma *vs.* high-grade serous tubal carcinoma, as all these three entities express WT1 in a diffusely and evenly pattern [14].

WT1 is overexpressed not only in ovarian and endometrial tumors but also in the mesothelial ones and in the intraabdominal desmoplastic small cell tumors.

In order to increase the diagnosis safety, WT1 is useful in association with others IHC markers [28]. For example, in case of an ovarian histological phenotype with transitional cells, WT1 is not effective on its own because both HGSC and transitional cell carcinoma are positive for WT1 [14]. Nevertheless, WT1 is effective in the differentiation of a transitional cells ovarian carcinoma from a metastatic tumor from the urinary tract, which is usually negative for WT1 [14].

There is a general literature consensus of WT1 expression being associated to an unfavorable prognosis and an accelerated tumoral progression in patients diagnosed with HGSC [29]. Major concerns arise when the carriers of BRCA1/2 gene with previous history of breast tumor are diagnosed with serous ovarian cancer – which can be de novo or a metastasis from the mammary gland [14]. WT1 is useful in this direction, as the WT1 gene is negative in breast pathology and uniformly positive in the ovarian tumors [14].

WT1 quantification was accomplished by taking into account only the nuclear immunoreactivity for at least 50% of the tumoral cells, although diffuse positivity is typical in serous subtypes [18]. The WT1 expression was evaluated only in circumstances where it appeared in the tumoral ovarian epithelium and not in the tumoral stroma. According to the IHC evaluation, we have to point out the fact that WT1 has a tendency towards expression in the advanced stages of the serous subtypes as compared with the non-serous ones.

Our results have been compared with the main recent studies focused on the investigation of WT1 in different tumoral subtypes of the ovarian epithelial category (Table 5) [15, 29–31].

Table 5 – *WT1: literature data vs. personal results*

WT1 expression	Serous carcinoma	Endometrioid carcinoma	Clear cell carcinoma	Mucinous carcinoma	Borderline tumor
	No. of positive cases from the total No. of investigated cases (%)				
Acs G *et al.*, 2004 [30]	24/28 (86%)	0/12 (0%)	4/18 (22.22%)	0/11 (0%)	No data
Al-Hussaini M *et al.*, 2004 [15]	36/38 (94.7%)	0/13 (0%)	No data	No data	14/16 (87.5%)
Hylander B *et al.*, 2006 [31]	66/74 (89.18%)	0/4 (0%)	1/5 (20)%	1/3 (33.33%)	No data
Høgdall EV *et al.*, 2007 [29]	75/343 (22%)	4/83 (5%)	0/46 (0%)	0/50 (0%)	36/94 (36%)
Personal results	50/52 (96.15%)	2/5 (40%)	1/2 (50%)	1/13 (7.69%)	3/3 (100%)

Conclusions

There is an increasing demand in translation of PAX8 and WT1 research in the complex biology of ovarian carcinomas into current diagnostic practice. In our experience, histopathologists are currently confronted to frequent diagnostic difficulties and dilemmas. The high diversity of ovarian cancers patterns are certainly linked to the variability of tumor origin and dual mechanism of ovarian carcinogenesis. Consequently, our study adds supplementary evidence in demonstrating PAX8 and WT1 value as effective markers in diagnosis refinement. PAX8–WT1 association increases the diagnosis specificity and sensitivity and opens up new perspectives for optimal supervision of disease evolution. Further monitoring is necessary to validate the prognosis value of these two markers.

Acknowledgments

The first author acknowledges the support of the POSDRU/88/1.5/S/58965 project, financed by the European Social Fund and the Romanian Government. She is also greatly indebted to the research group of Unit of Molecular Therapies, Department of Experimental Oncology and Molecular Medicine, Istituto dei Tumori, Milan, Italy.

References

[1] American Cancer Society, *Cancer Facts & Figures*, 2009, www.cancer.org/downloads/STT/500809web.pdf.

[2] Kurman RJ, Shih IeM, *The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory*, Am J Surg Pathol, 2010, 34(3):433–443.

[3] Li J, Fadare O, Xiang L, Kong B, Zheng W, *Ovarian serous carcinoma: recent concepts on its origin and carcinogenesis*, J Hematol Oncol, 2012, 5:8.

[4] Ricciardelli C, Oehler MK, *Diverse molecular pathways in ovarian cancer and their clinical significance*, Maturitas, 2009, 62(3):270–275.

[5] Vergara D, Merlot B, Lucot JP, Collinet P, Vinatier D, Fournier I, Salzet M, *Epithelial–mesenchymal transition in ovarian cancer*, Cancer Lett, 2010, 291(1):59–66.

[6] Dubeau L, *The cell of origin of ovarian epithelial tumours*, Lancet Oncol, 2008, 9(12):1191–1197.

[7] Vang R, Shih IeM, Kurman RJ, *Fallopian tube precursors of ovarian low- and high-grade serous neoplasms*, Histopathology, 2013, 62(1):44–58.

[8] Tong GX, Devaraj K, Hamele-Bena D, Yu WM, Turk A, Chen X, Wright JD, Greenebaum E, *Pax8: a marker for carcinoma of Müllerian origin in serous effusions*, Diagn Cytopathol, 2011, 39(8):567–574.

[9] Auersperg N, Wong AS, Choi KC, Kang SK, Leung PC, *Ovarian surface epithelium: biology, endocrinology, and pathology*, Endocr Rev, 2001, 22(2):255–288.

[10] Hirshfield AN, *Development of follicles in the mammalian ovary*, Int Rev Cytol, 1991, 124:43–101.

[11] Mhawech-Fauceglia P, Wang D, Samrao D, Godoy H, Ough F, Liu S, Pejovic T, Lele S, *Pair Box 8 (PAX8) protein expression in high grade, late stage (stages III and IV) ovarian serous carcinoma*, Gynecol Oncol, 2012, 127(1):198–201.

[12] Laury AR, Perets R, Piao H, Krane JF, Barletta JA, French C, Chirieac LR, Lis R, Loda M, Hornick JL, Drapkin R, Hirsch MS, *A comprehensive analysis of PAX8 expression in human epithelial tumors*, Am J Surg Pathol, 2011, 35(6):816–826.

[13] Tacha D, Zhou D, Cheng L, *Expression of PAX8 in normal and neoplastic tissues: a comprehensive immunohistochemical study*, Appl Immunohistochem Mol Morphol, 2011, 19(4):293–299.

[14] Bárcena C, Oliva E, *WT1 expression in the female genital tract*, Adv Anat Pathol, 2011, 18(6):454–465.

[15] Al-Hussaini M, Stockman A, Foster H, McCluggage WG, *WT-1 assists in distinguishing ovarian from uterine serous carcinoma and in distinguishing between serous and endometrioid ovarian carcinoma*, Histopathology, 2004, 44(2):109–115.

[16] Oka Y, Tsuboi A, Oji Y, Kawase I, Sugiyama H, *WT1 peptide vaccine for the treatment of cancer*, Curr Opin Immunol, 2008, 20(2):211–220.

[17] Makrigiannakis A, Amin K, Coukos G, Tilly JL, Coutifaris C, *Regulated expression and potential roles of p53 and Wilms' tumor suppressor gene (WT1) during follicular development in the human ovary*, J Clin Endocrinol Metab, 2000, 85(1):449–459.

[18] Roh MH, Yassin Y, Miron A, Mehra KK, Mehrad M, Monte NM, Mutter GL, Nucci MR, Ning G, Mckeon FD, Hirsch MS, Wa X, Crum CP, *High-grade fimbrial-ovarian carcinomas are unified by altered p53, PTEN and PAX2 expression*, Mod Pathol, 2010, 23(10):1316–1324.

[19] Köbel M, Kalloger SE, Huntsman DG, Santos JL, Swenerton KD, Seidman JD, Gilks CB; Cheryl Brown Ovarian Cancer Outcomes Unit of the British Columbia Cancer Agency, Vancouver BC, *Differences in tumor type in low-stage versus high-stage ovarian carcinomas*, Int J Gynecol Pathol, 2010, 29(3):203–211.

[20] Bowen NJ, Logani S, Dickerson EB, Kapa LB, Akhtar M, Benigno BB, McDonald JF, *Emerging roles for PAX8 in ovarian cancer and endosalpingeal development*, Gynecol Oncol, 2007, 104(2):331–337.

[21] Köbel M, Kalloger SE, Boyd N, McKinney S, Mehl E, Palmer C, Leung S, Bowen NJ, Ionescu DN, Rajput A, Prentice LM, Miller D, Santos J, Swenerton K, Gilks CB, Huntsman D, *Ovarian carcinoma subtypes are different diseases: implications for biomarker studies*, PLoS Med, 2008, 5(12):e232.

[22] Nonaka D, Chiriboga L, Soslow RA, *Expression of PAX8 as a useful marker in distinguishing ovarian carcinomas from mammary carcinomas*, Am J Surg Pathol, 2008, 32(10):1566–1571.

[23] Ozcan A, Shen SS, Hamilton C, Anjana K, Coffey D, Krishnan B, Truong LD, *PAX 8 expression in non-neoplastic tissues, primary tumors, and metastatic tumors: a comprehensive immunohistochemical study*, Mod Pathol, 2011, 24(6):751–764.

[24] Zhao L, Guo M, Sneige N, Gong Y, *Value of PAX8 and WT1 immunostaining in confirming the ovarian origin of metastatic carcinoma in serous effusion specimens*, Am J Clin Pathol, 2012, 137(2):304–309.

[25] Madore J, Ren F, Filali-Mouhim A, Sanchez L, Köbel M, Tonin PN, Huntsman D, Provencher DM, Mes-Masson AM, *Characterization of the molecular differences between ovarian endometrioid carcinoma and ovarian serous carcinoma*, J Pathol, 2010, 220(3):392–400.

[26] Sumathi VP, Al-Hussaini M, Connolly LE, Fullerton L, McCluggage WG, *Endometrial stromal neoplasms are immunoreactive with WT-1 antibody*, Int J Gynecol Pathol, 2004, 23(3):241–247.

[27] Netinatsunthorn W, Hanprasertpong J, Dechsukhum C, Leetanaporn R, Geater A, *WT1 gene expression as a prognostic marker in advanced serous epithelial ovarian carcinoma: an immunohistochemical study*, BMC Cancer, 2006, 6:90.

[28] McCluggage WG, *Immunohistochemical markers as a diagnostic aid in ovarian pathology*, Diagn Histopathol, 2008, 14(8):335–351.

[29] Høgdall EV, Christensen L, Kjaer SK, Blaakaer J, Christensen IJ, Gayther S, Jacobs IJ, Høgdall CK, *Expression level of Wilms tumor 1 (WT1) protein has limited prognostic value in epithelial ovarian cancer: from the Danish "MALOVA" ovarian cancer study*, Gynecol Oncol, 2007, 106(2):318–324.

[30] Acs G, Pasha T, Zhang PJ, *WT1 is differentially expressed in serous, endometrioid, clear cell, and mucinous carcinomas of the peritoneum, Fallopian tube, ovary, and endometrium*, Int J Gynecol Pathol, 2004, 23(2):110–118.

[31] Hylander B, Repasky E, Shrikant P, Intengan M, Beck A, Driscoll D, Singhal P, Lele S, Odunsi K, *Expression of Wilms tumor gene (WT1) in epithelial ovarian cancer*, Gynecol Oncol, 2006, 101(1):12–17.

Corresponding author
Irina-Draga Căruntu, Professor, MD, PhD, Discipline of Histology, Department of Morphofunctional Sciences, "Grigore T. Popa" University of Medicine and Pharmacy, 16 University Street, 700115 Iassy, Romania; Phone +40727–003 700, e-mail: irinadragacaruntu@gmail.com

Received: October 28th, 2012

Accepted: February 5th, 2013