

The study of p53 and p16 immunoexpression in serous borderline and malignant ovarian tumors

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Abstract

The study assessed p53 and p16 immunoexpression in 20 cases of ovarian serous carcinomas and four cases of serous borderline tumors, the results being statistically analyzed in relation to clinicopathological data of the cases. The p53 immunoreaction was observed in 85% of cases, the medium percentage of positivity being 15% for borderline tumors, 45% for low-grade carcinomas and 60% for high-grade carcinomas. The p16 immunoreaction was observed in 75% of cases, the medium percentage of positivity being 30% for borderline tumors, 25% for low-grade carcinomas and 62% for high-grade carcinomas. The p53 and p16 reaction was also identified at the tubal epithelium in cases of invasive carcinomas. Statistical analysis indicated significant differences in p53 expression depending on tumor type and for p53 and p16 expression compared to the degree of tumor differentiation. The study indicated a diffuse immunostain for p53 and p16 in high-grade serous ovarian carcinomas. The presence of "p53 signature" and areas with variable tumor differentiation and reactivity, in the case of high-grade carcinomas, supporting the existence of multiple mechanisms of their occurrence and progression.

Keywords: ovarian serous tumors, p53, p16, immunohistochemistry.

Introduction

Ovarian cancer represents 3% of females' malignancies, with over 140 000 worldwide annual associated deaths [1–3].

Epithelial tumors constitute over 90% of ovarian cancers, serous lesions representing 60–80% of them and being among the aggressive, with a poor prognosis and short survival period [4, 5].

To improve the prognosis of these lesions, numerous studies have attempted to determine biomolecular mechanisms involved in ovarian carcinogenesis and to identify potential predictive biomarkers for these lesions. In recent years, several studies have confirmed and introduced the dual theory of ovarian carcinogenesis according to which malignant tumors may arise by different mechanisms, according to their differentiation degree [6–9]. These mechanisms are the result of impaired expression of numerous genes and their protein products.

p16INK4A and TP53 are involved in cell cycle regulation and are considered by many authors as indicators of tumor aggressiveness [5, 10, 11].

The analysis of p16 and p53 immunoexpression in serous ovarian carcinomas revealed in most studies the proteins overexpression for high-grade, aggressive

lesions, supporting the existence of two independent pathogenic pathways [12–15], while other studies invalidate this aspect or prognostic value of the biomarkers [16–18].

In this study, we analyzed the immunohistochemical expression of p16 and p53 in the serous ovarian malignant and borderline tumors.

Materials and Methods

Study cohort characteristics

The study included a total of 24 selected ovarian tumors from casuistry of the Pathology Laboratory of the Romanian Railways Hospital, Craiova, Romania.

The biological material was represented by total and partial hysterectomy pieces, which were processed by common histopathological technique using 10% formalin fixation, paraffin embedding and Hematoxylin–Eosin stain.

Clinical data were analyzed and the histopathological diagnosis was done in conformity with criterions established in 2003 by IARC nominated work group for female genital tract tumors within *World Health Organization* [19].

The immunohistochemical processing was made on

serial sections, using mouse antihuman monoclonal antibodies (Table 1).

Table 1 – The panel of antibodies used

Antibody	Clone/Source	Dilution	Antigen retrieval
P53	DO-7	1:50	Citrate, pH 6
P16	E6H4	Ready to use	Epitope Retrieval Solution/Kit

Immunohistochemical reactions were performed with the LSABTM+ Kit/HRP (DAKO, code K0679) in case of p53 and CINtec[®] Histology Kit (DAKO, code K5334) for p16 expression detecting. For the visualization of reactions we used DAB (3,3'-diaminobenzidine, DAKO), followed by counterstained with Hematoxylin.

For quantification of immunohistochemical results were reported as percentage the number of labeled cells for each case to obtain the positivity index (PI) [12]. Intensity of reaction was assessed as low, moderate or intense.

Negative external control staining was done by omitting primary antibodies. Statistical analysis use ANOVA and Pearson tests (SPSS 10 software).

The acquisition of the images was done with Nikon Eclipse E600 and software program Lucia 5.

Results

Histopathological analysis indicated the presence of serous malignant tumors in 20 (83.3%) cases and serous non-invasive borderline tumors in four (16.7%) cases (Table 2).

Table 2 – Clinico-pathological parameters of the studied cases

Parameters	Variables	Borderline tumors (No.)	Malignant tumors (No.)
Age [years]	<50	1	2
	>50	3	18
Tumor size [cm]	<10	–	5
	>10	4	15
Histopathological appearance	–	MP (4)	WD (12)
			PD (8)
FIGO stage	IA	–	13
	IB		5
	IIA		2
P53 expression	Negative (No. of cases)	–	3
	Intensity	moderate	variable
	Medium labeling index	15%	45% (WD) 60% (PD)
P16 expression	Negative (No. of cases)	1	5
	Intensity	strong	strong
	Medium labeling index	30%	25% (WD) 62% (PD)

MP: Micropapillary; WD: Well-differentiated; PD: Poorly differentiated.

Malignant ovarian serous tumors were represented by adenocarcinomas in four (20%) cases and cystadenocarcinomas in 16 (80%) cases, most being well-differentiated (60%), unilateral (55%), over 10 cm in size (75%) and diagnosed in patients over 50 years (90%) (Table 2).

In 14 cases, tumors were in stage IA, five cases in stage IB and two cases in stage IIC, which present invasion in the fallopian tube (Table 2).

Serous borderline tumors presented a micropapillary growth pattern, more frequent unilateral and size over 10 cm, being diagnosed mainly in patients over 50 years (75%) (Table 2).

The p53 immunoexpression was observed in all borderline tumors at nuclear level, with mild intensity stain and medium PI of 15% (Figure 1A).

In carcinomas, the reaction was present in 17 (85%) cases, negative cases belonging to low grade tumors. Carcinomas indicated low/moderate intensity of reaction and medium PI of 45% (Figure 1B). High-grade carcinomas had moderate/strong reaction and medium PI of 60% (Figure 1C).

In cases with fallopian tube invasion, we observed focal stain with increased intensity for 20% of tubal epithelial cells. For cases with variable areas of differentiation, the stain was intense, with PI of 60–75% for well-differentiated areas and 80–90% for the poorly differentiated ones (Figure 1D).

Analysis of p16 expression indicated the positivity at nuclear and cytoplasmic level in three (75%) cases of borderline tumors. The stain was heterogeneous, with diffusely positive areas, high intensity and PI of 75–85% and focal positive areas, high intensity and PI of 20–25%, with a medium PI of 30% (Figure 1E).

In case of carcinomas, the stain was positive in 15 (75%) cases, negative cases belonging to low or high-grade tumors. For low-grade carcinomas, the stain was moderate/intense intensity with medium IP of 25% labeled cells (Figure 1F). For high-grade carcinomas the intensity was increased, with diffuse appearance and IP of 90%, including malignant cells exfoliate from the cysts (Figure 1G). Sometimes, in these cases, were present focal areas with PI of 30% positive cells. Medium index of the positivity in these cases was 62%.

In cases with Fallopian tube invasion was observed focal stain with increased intensity in the tubal epithelium and PI of 25% (Figure 1H).

In two cases of poor differentiated carcinomas were present well-differentiated areas with moderate intensity and 25% labeled cells, the pattern being heterogeneous.

One-way ANOVA test indicated significant differences for p53 expression in malignant and borderline tumors [$F(1.22)=37.06$, $p=0.000$]. The same test indicated significant differences for p53 expression [$F(1.18)=155.63$, $p=0.000$] and p16 expression [$F(1.18)=13.27$, $p=0.002$], depending on differentiation degree. Pearson test indicated a positive linear correlation between p53 and p16 stain values [$r(18)=0.664$, $p=0.001$]. There were no statistical associations with other clinico-pathological analyzed parameters.

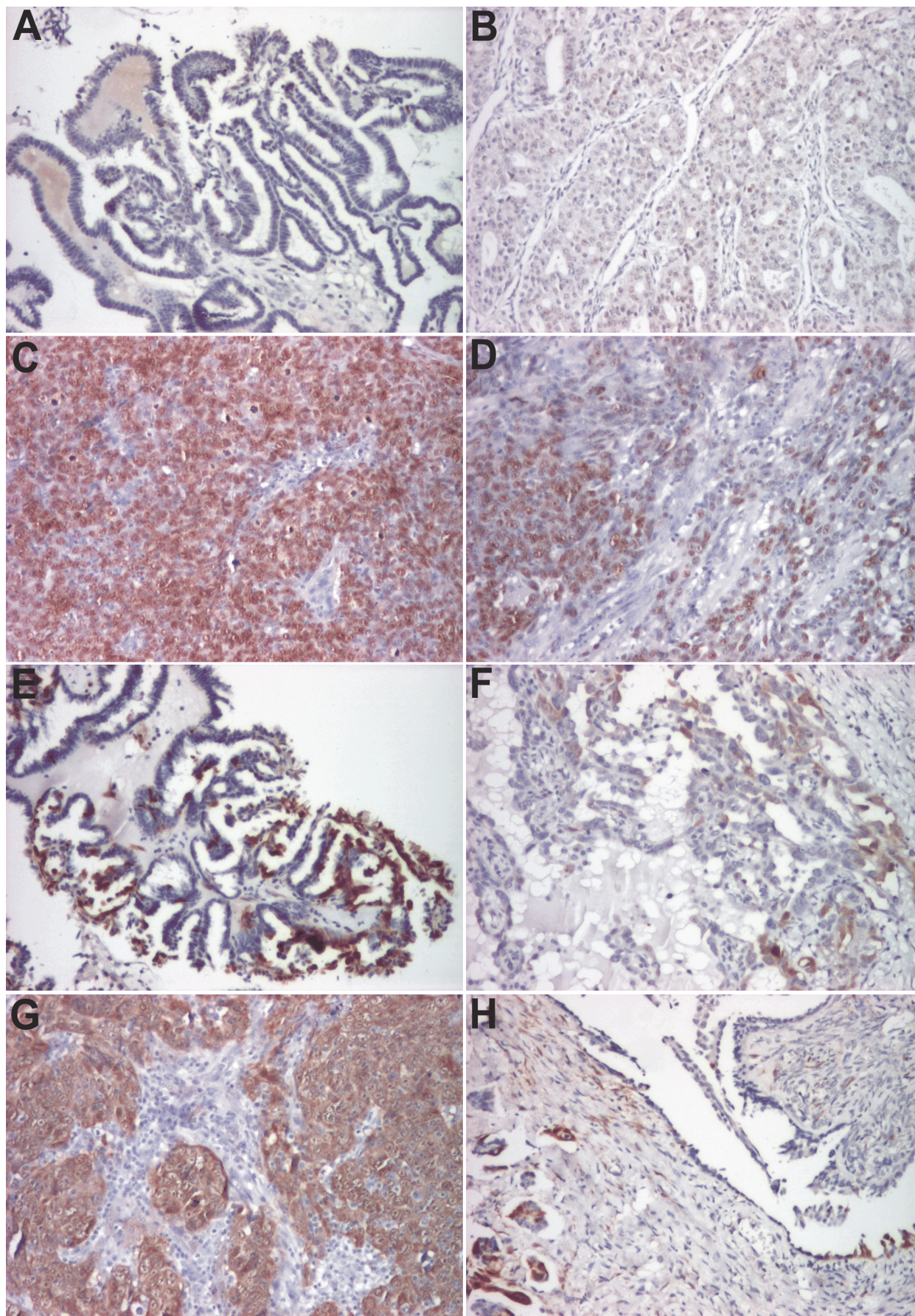


Figure 1 – P53 stain, $\times 100$: (A) Borderline tumor; (B) Low-grade serous carcinoma; (C) High-grade serous carcinoma; (D) High-grade serous carcinoma with well-differentiated areas. P16 stain, $\times 100$: (E) Borderline tumor; (F) Low-grade serous carcinoma; (G) High-grade serous carcinoma; (H) High-grade serous carcinoma with Fallopian tube invasion.

Discussion

Biomolecular mechanisms that control ovarian tumors have been extensively studied on serous carcinomas from this level.

One mechanism regards low-grade carcinomas and requires their development from serous cystadenomas and cystadenofibromas who have progressed to an atypical proliferative tumor, micropapillary noninvasive borderline tumor and then invasive tumor [20]. Similar genetic profile of lesions within the filiation, and the frequent presence of non-invasive lesions associated with high-grade carcinomas support this hypothesis [18, 20, 21]. Also, in low-grade lesions the TP53 mutations are less common, in about 8% of cases [8, 20].

The other mechanism concerns only high-grade carcinomas, which are considered “*de novo*”, also existing other hypotheses such as the presence of high-grade precursor lesions in the tubal epithelium [9, 22]. In these cases, TP53 mutations and protein over-expression occurs in about 80% of lesions [20].

Wild type of p53 plays an important role in arresting cells with damaged DNA that pass from G1 to S phase of cell cycle and apoptosis induction, altered expression being documented in numerous sites of malignant cells.

In our study, p53 immunostaining was present in all borderline tumors and 85% of carcinomas, the highest PI being observed in high-grade carcinomas.

Most studies of the literature indicates p53 positivity in 80% of serous ovarian carcinomas and protein expression differences depending on the degree of differentiation, high-grade tumors being intensively p53 positive [12, 14, 15].

The two pathogenic ways in ovarian carcinogenesis are most often independent [8, 20]. Also, in the case of relapses, most often low grade carcinomas retain their degree of differentiation [8, 20].

Nevertheless, studies have proven the occurrence of high-grade serous ovarian carcinomas from low-grade lesions, being also reported cases of high grade and borderline synchronous tumors or recurrent borderline tumors carcinoma as high-grade carcinomas [17, 18]. In addition, in this study, two cases of carcinomas presented areas with variable tumor differentiation and immunoreaction, which may support this theory.

Recently, it was identified a lesion in the fallopian tube, carcinoma *in situ*, which is cytologically similar with serous ovarian high-grade carcinomas, presenting diffuse expression of p53 [9]. In this study, the presence of “p53 signature” in the tubal epithelium in cases of high-grade carcinomas may support this theory.

P16 is a negative regulator of cell cycle control that ensures passage from G1 on S phase. In our study, p16 was identified in 75% of malignant and borderline tumors. In high-grade carcinomas and tumors borderline, the stain had the highest values.

Literature data indicate that p16 expression is diffuse in high-grade carcinomas, representing an early event of ovarian carcinogenesis [10, 12–14]. However, there are studies that found other results. Thus, in 2010, Nazlioglu HO *et al.* found no differences in p16 expression depending on the degree of tumor differentiation in serous ovarian carcinomas [16].

Skirnisdóttir I *et al.* analyzed the expression of EGFR and p53 on a group of 226 surface ovarian carcinomas and proposed their stratification into three groups – low risk (well-differentiated, and negative for p53 and EGFR), intermediate risk (well-differentiated, p53/EGFR positive or poorly differentiated and p53/EGFR negative) and high-risk (poorly differentiated and p53/EGFR positive) [23].

As already mentioned in a previous study, EGFR and HER2/neu expression is increased in high-grade serous ovarian carcinomas. Thus, a diffuse positive profile of EGFR/HER2neu/P53/P16 characterized aggressive serous ovarian carcinomas and indicated a poor prognosis. Also, non-invasive borderline tumors with a such focal or diffuse profile indicate an increased risk for invasion and poor outcome.

Conclusions

The study indicated a diffuse p53 and p16 immunostain in high-grade serous ovarian carcinomas. The presence of “p53 signature” and areas with variable tumor differentiation and reactivity in high-grade carcinomas cases, support the existence of multiple mechanisms for their occurrence and progression.

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