Expression of p16 in serous ovarian neoplasms

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Summary

Purpose: We aimed to examine p16 protein expression in ovarian serous neoplasms along with normal ovarian tissues. *Materials and Methods:* p16 expression was immunhistochemically evaluated in 86 ovarian serous neoplasms (21 cystadenomas, 20 borderline tumors and 45 carcinomas) and 21 non-neoplastic ovarian tissue. The results were also compared with histopathological grade in serous adenocarcinomas. *Results*: p16 expression rates for benign, borderline ovarian tumors and ovarian cancer were 14.2%, 85% and 86.6%, respectively. It was significantly higher in carcinomas ($p < 0.001$) and borderline tumors ($p < 0.001$) compared to cystadenomas. No immunoreactivity was found in the non-neoplastic ovarian surface epithelial cells. The percentage of p16 expression did not change significantly with histological grade in carcinomas. *Conclusion*: p16 expression is strong and widespread involving most tumor cells in serous papillary ovarian carcinomas, and is probably an early event.

Key words: p16; Immunohistochemistry; Ovarian serous carcinoma; Borderline tumor; Cystadenoma.

Introduction

p16 is an important tumor suppressor gene located on chromosome 9p21. The p16 protein binds specifically to CDK4 and CDK6 inhibiting the formation of the CDK/cyclin D complex and resulting in cell cycle arrest at the G1 phase [1, 2]. The aberrant p16 protein caused by point mutations, small deletions, large hetero- and homozygous deletions, and silencing by methylation of CpG islands in the promoter region has been found in many kinds of human tumors, indicating that these factors are closely related to tumorigenesis [3-5].

In the ovary, as in other human tumors, accumulation of genetic alterations occur during malignant transformation [6]. p16 expression has not been extensively investigated in ovarian neoplasms. Serous type ovarian carcinomas appear to express p16 more commonly than other morphologic subtypes. This is analogous to the situation in uterine serous carcinomas, and suggests that p16 may be involved in the pathogenesis of serous carcinomas within the female genital tract [7].

The aim of our study was to investigate p16 expression in ovarian serous tumors. We attempted to determine the p16 expression among benign, borderline, and malignant ovarian serous tumors; and the correlation between p16 expression and tumor grade in ovarian serous carcinomas.

Materials and Methods

Tissue Specimens

A total of 86 ovarian serous tumor specimens (21 cystadenomas, 20 borderline tumors, and 45 carcinomas) and 21 non-neoplastic normal ovaries were evaluated for p16 expression by immunohistochemistry. Formalin-fixed and paraffin-embedded tissues were retrieved from the files of the Department of Pathology, Uludag University Hospital during the period of 1997-2007. The cases were reviewed by two pathologists involved in the study and the histological diagnoses were confirmed. The tissues were used with the approval of the ethical committee of Uludag University.

Grading of tumors

Malignant tumors were graded according to the World Health Organization (WHO) criteria [8]. Using both architectural and cytological features, tumors were graded as 1, 2, or 3 corresponding to well, moderately or poorly differentiated.

Immunohistochemical staining and analysis

Formalin-fixed, parafin-embedded tissue blocks from each case were cut in 4 μm sections. Antigen retrieval was performed by incubating the sections in EDTA buffer (pH: 9) in a microwave oven (800 W for 5 min + 400 W for 15 min). Endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 15 min at room temperature. Primary antibody for p16 ($p16I^{NK4a}$ Ab-7, clone 16P04, Neomarkers, Lab Vision, Fremont, CA, USA) was diluted at 1:100. The streptavidin-biotin peroxidase complex kit (Lab Vision, Fremont CA, USA) was used for antibody detection. DAB was used as the chromogen. Cells with brown-colored nuclear or cytoplasmic staining were considered positive. Human cervical adenocarcinoma sections were used as positive controls. For negative controls, sections were treated similarly with the exception of the primary antibody. Scoring of immunohistochemistry results was performed on the basis of both the staining intensity and the percentage of immunoreactive epithelial cells. The scoring criteria for p16, as shown in percentages (%): no expression (–); < 20, weak staining (±); 20-30, weak or moderate staining (+); 31-50, moderate or strong staining (++); > 50 strong staining (+++). The scores +, ++ and +++ were considered positive for p16 [9]. Kruskal-Wallis and Mann-Whitney tests were used to compare the p16 stainings of benign, borderline and malignant tumor groups. Spearman's rho correlation coefficient was used to determine the association between tumor grades and p16 staining.

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Results

Using a cutoff value of ≥ 20% positivity, p16 expression was detected in 14.2% (3/21) of benign tumors, 85% (17/20) of borderline tumors, and 86.6% (39/45) of ovarian carcinomas. In general, all the p16 immunoreactive cells exhibited both nuclear and cytoplasmic staining. In normal ovaries, no immunoreactivity was found. In serous cystadenomas, only three cases showed positivity; expression was weak in 33.3% (1/3) and moderate in 66.7% (2/3). In borderline tumors, 17/20 showed positivity, and the percentage of weak-moderate and strong expression were 29.4% (5/17), 52.9% (9/17), and 17.6% (3/17), respectively. In serous carcinomas, 39/45 showed positivity and the majority (33/39, 84.6%) were strongly positive (Figure 1, Table 1).

Table 1. — *Expression of p16 in serous ovarian neoplasms compared to normal ovaries*.

	Total cases	Immunointensity 0	1+	2+	3+	Total
Normal ovary	21	21 (100)	0	0	0	0
Cystadenoma	21	18 (85.7)	1 (4.8)	2 (9.5	0	3/21 (14.2)
Borderline	20	3 (15)	5 (25)	9 (45)	3 (15)	17/20 (85%)
Adenocarcinoma	45	6 (13.3)	3 (6.7)	3 (6.7)	33 (73.3)	39/45 (86.6%)
Total staining	107	48	10	15	36	

Values in parenthesis are in percentages.

Significantly higher p16 levels were detected in all tumors compared to the control group ($p < 0.001$). Significantly high p16 expression was observed in serous carcinomas compared to the normal ovarian tissues, benign and borderline tumors ($p < 0.001$); and in borderline tumors compared to the benign group ($p < 0.001$).

Using a cutoff value of > 50% positivity, p16 expression was detected in 15% (3/20) of borderline tumors, and 73.3% (33/45) of ovarian carcinomas, and the difference between the borderline tumors and ovarian carcinomas was statistically significant ($p < 0.001$).

Of the 45 serous carcinomas, two were well (G1), 30 moderately (G2), and 13 poorly differentiated (G3). Grade 1 tumors were very few in number (2 cases), so they were taken into account together with Grade 2 tumors. No significant correlation was found between the grades of the malignant tumors and p16 expression (r = 0.084; $p = 0.582$). p16 protein expression in ovarian serous carcinomas by tumor grade is shown in Table 2.

Table 2. — *p16 immunopositivity in ovarian serous carcinomas compared to tumor grade*.

Grade	Total number	p16 IHC immunostaining pattern negative	(+)	(++)	(+++)	Total staining
1-2	32	4 (12.5%)	3 (10.7%)	2 (7.1%)	23 (82.1%)	28 (87.5%)
3	13	2 (15.4%)	0	1 (9.1%)	10 (90.9%)	11 (84.6%)

Discussion

The p16 gene is considered to be a potential tumor-suppressor gene [1]. There have been relatively few studies documenting p16 expression in ovarian cancers and conflicting results have been published. The loss of p16 expression has been reported in 26-37% of ovarian cancers

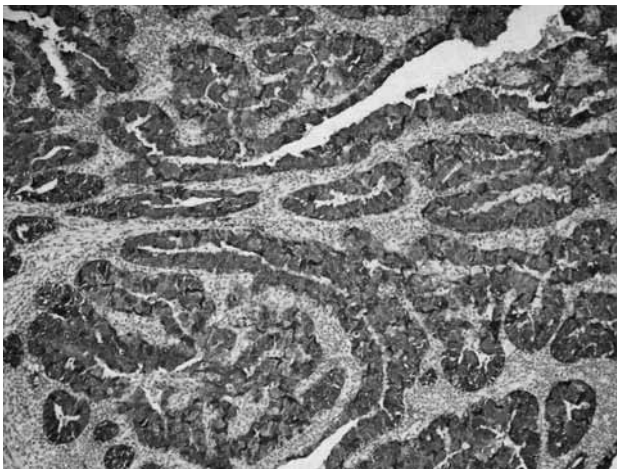

Figure 1. — Strong (+++) p16 expression in serous carcinoma (p16 immunostain x 400).

[10, 11]. However, some studies indicate that p16 overexpression is relatively common in malignancies occurring at this site [12-14]. Armes *et al*. [7] found serous papillary carcinoma immunopositivity for p16 in 9/10 cases using a tissue microarray. Dong *et al*. [15] and Milde-Langosch *et al*. [16] detected the immunohistochemical expression of p16 protein in 89 and 80% of ovarian tumors, and 96% and 90% of serous carcinomas, respectively. Our findings also confirm that a large proportion of ovarian serous carcinomas have strong p16 protein expression. We observed p16 expression in 86.6% of ovarian carcinomas, and noticed that many of these invasive carcinomas had uniform and strongly intense p16 overexpression throughout the majority of tumor cells.

Ovarian cancer is a heterogenous group of neoplasms with several different histologic types, each with its own underlying molecular genetic mechanism [17]. Therefore, immunohistochemical expression of proteins as well as molecular analyses should be evaluated separately by histologic type. However the expression of cell cycle regulatory proteins according to histologic subtypes has received little attention. Recent evidence demonstrated that high p16 expression was associated with serous histology and loss of p16 expression was detected mainly in mucinous and endometrioid types [7, 15-19]. Increased p16 expression is observed in high-grade serous and undifferentiated carcinomas compared with other morphologic types of ovarian carcinoma, and critical molecular abnormalities are present in high-grade serous carcinoma of the ovary [20, 21]. Forty-six ovarian serous papillary carcinomas examined by whole section immunohistochemistry revealed that 31 cases (67.4%) displayed strong nuclear and cytoplasmic staining for p16 in more than 80% of tumor cells [18]. It has been shown that a large proportion of high-grade ovarian serous carcinomas and also uterine serous carcinomas have strong diffuse p16 expression [18]. These findings suggest that p16 expression may be one of the histological type-specific events in ovarian tumorigenesis.

There have been only limited studies concerning p16 expression in serous borderline and benign neoplasms. Armes *et al*. reported 90% of serous adenocarcinomas were positive for p16, while borderline serous tumors showed negative staining, where there were only three borderline cases [7]. In another study, most benign ovarian neoplasms in contrast to 11% of malignant tumors were negative [15]. p16 expression was reported to be decreased from benign and borderline to malignant tumors, and it was concluded that p16 protein was down-regulated in ovarian carcinoma [22]; however in the two latter studies, histopathologic subtypes were not evaluated separately [15, 22]. In another study including only serous type carcinoma and borderline neoplasms, p16 positivity was reported in 93.5% (43/46) of invasive tumors and 88.9% (16/18) of borderline tumors [23]. In the current study, we observed overall positive p16 expression in 85% and 86.6%, with high and widespread strong expression of 15% and 73.3% of serous borderline tumors and carcinomas, respectively.

Studies investigating p16 expression of normal ovarian surface epithelia are few in number with strikingly conflicting results, which may be caused by the use of different p16 antibodies [10, 24]. Many commercially available p16 antibodies show substantial nonspecific binding in normal epithelial cells [25]. We also did not observe any expression in normal ovarian surface epithelium.

In summary, we have demonstrated increased expression of p16 in serous adenocarcinomas compared with serous borderline tumors and serous cystadenomas, and no expression in normal epithelial cells; and confirmed the strong and widespread expression in serous ovarian carcinomas.

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