• Clinical Research •

Expression and significance of P53, P21^{WAF1} and CDK1 proteins in epithelial ovarian cancer

Hui-Rong Shi and Rui-Tao Zhang

[Abstract] **Background and Objective**: P53, P21^{WAF1} and CDK1 are key factors in the "P53 pathway" of G_2/M phase DNA damage checkpoint in cell cycle. This study was to investigate the expression and significance of P53, P21^{WAF1} and CDK1 proteins in epithelial ovarian cancer. **Methods**: The expression of P53, P21^{WAF1} and CDK1 proteins in 20 specimens of normal ovarian tissues, 20 specimens of benign epithelial ovarian tumor and 76 specimens of epithelial ovarian cancer was detected by immunohistochemistry. Their correlations to the clinicopathologic characteristics of epithelial ovarian cancer and their interrelationships were analyzed. **Results**: Significant differences were noted in the positive rates of P53, P21^{WAF1} and CDK1 proteins between epithelial ovarian cancer and normal ovarian tissues, benign ovarian tumors ($P < 0.05$). In epithelial ovarian cancer, up-regulation of P53 protein was associated with advanced FIGO stage and poor differentiation; down-regulation of P21^{WAF1} protein was associated with advanced FIGO stage; CDK1 showed no association with any clinicopathologic factors. P53 and CDK1 expression were negatively correlated to P21^{WAF1} expression ($r = -0.388$, $P = 0.001$; $r = -0.282$, $P = 0.014$); P53 expression was positively correlated to CDK1 expression ($r = 0.263$, $P = 0.022$). **Conclusions**: P53 protein is related to the malignancy of epithelial ovarian cancer, P53 and P21^{WAF1} protein may be related to the malignant development of epithelial ovarian cancer. CDK1 detection may be helpful in the early diagnosis of epithelial ovarian cancer.

Key words: ovarian neoplasm, epithelial cancer, G_2/M phase checkpoint, P53, P21^{WAF1}, CDK1, immunohistochemistry

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Ovarian cancer is one of the three major malignancies of female genital organs, with an incidence lower than those of cervical cancer and endometrial cancer. The 5-year survival rate of early stage patients is 90%, whereas that of advanced stage patients is less than 30%; about 70% of patients are diagnosed at advanced stage; its mortality is the highest among all gynecologic malignancies.[1] At present, the molecular biological mechanisms in the carcinogenesis of ovarian cancer remain uncertain. Recent researches suggest that the DNA damage checkpoints at G_2/M phase are involved in the carcinogenesis of epithelial ovarian cancer.[2,3] P53, P21^{WAF1} and CDK1 are key factors of "P53 pathway" of the DNA damage

checkpoint at G_2/M phase. We detected the expression of P53, P21WAF1 and CDK1 in normal ovarian tissue, benign ovarian tumor and epithelial ovarian cancer by immunclinicopathologic features of ovarian cancer and their interrelationships, and explored their roles in the carcinogenesis of ovarian cancer and their clinical significance.

Materials and Methods

Samples and patients clinical data. Paraffin tissue blocks from 116 patients, treated at the First Affiliated Hospital of Zhengzhou University from February 2004 to September 2007, were collected. Of the 116 specimens, 20 were normal ovarian tissues, 20 were benign epithelial ovarian tumors (10 were serous papillary cystadenoma, 10 were mucous papillary cystadenoma), 76 were epithelial ovarian cancers. Patients with epithelial ovarian cancer aged 19 to 82 (median, 52). Regarding histological grade, 14 patients were at grade G1, 22 at grade G2 and 40 at grade G3. According to 2000 FIGO classification of clinical stage, 10 patients were at stage I, 14 at stage II, 48 at stage III and 4 at stage IV. Of the 76 epithelial ovarian cancer patients, 54 had serous carcinoma and 22 had cases of mucous carcinoma; 24 had lymph node metastasis (11 with pelvic lymph node metastasis, 8 with para-aortic lymph node metastasis and 5 with both metastases), and 52 had no lymph node metastasis. All diagnoses were confirmed by pathology. All patients with epithelial ovarian cancer were initially diagnosed, and none received preoperative radiotherapy or chemotherapy.

Main reagents. P53 mouse anti-human monoclonal antibody (ZM-0408) and P21WAF1 mouse anti-human monoclonal antibody (ZM-0206) were produced by Zymed Company, US (both were ready for use), purchased from Beijing Zhongsha Golden Bridge Biotechnology Co., Ltd. CDK1 mouse anti-human monoclonal antibody (MS-110) was produced by Neomarkers Company, US (1:100 dilution), purchased from Fujian Maixin Biotechnology Co., Ltd. General type of SP immunohistochemistry kit (SP-9000) and DAB reagent kit (ZLI-9032) were purchased from Beijing Zhongsha Golden Bridge Biotechnology Co., Ltd.1.3 Immunohistochemical method

Serial sections of the paraffin blocks at the thickness of 3-5 □m were detected by SP immunohistochemistry according to the operation manual. PBS instead of primary antibody was applied as negative control. Breast cancer tissues were applied as positive control for P53 and P21WAF1; HeLa cells were applied as positive control for CDK1. Cell staining was observed under a microscope: brown particles or flakes represented positive staining; P53 and P21WAF1 were expressed in nuclei and CDK1 in cytoplasm (partly in nuclei).

Staining criteria for P53 and P21WAF1 were as follows:[4] ten serial high power fields were selected and 100 cells in each field were counted to calculate positive rate. A specimen with a positive rate of <5% was defined as negative, 5%-49% as positive (+), and >50% as strong positive (++).
Staining criteria for CDK1 were as follows:[5] 20 high power fields were selected and 100 cells in each field were counted. According to the proportion of positive cells, a specimen with a proportion of positive cells of <1/3 was scored 1, 1/32/3 scored 2, >2/3 scored 3, and no positive cells scored 0. According to cell staining intensity, no staining scored 0, light yellow scored 1, brown yellow scored 2 and yellow brown scored 3. Accumulated score was calculated by multiplying staining proportion score and staining intensity score: a score of 0 was defined as negative for 0 score, a score of 1-4 as positive (+),and a score of >4 as strong positive (++).

Statistical analysis. SPSS 13.0 statistical software package was used for statistical analysis. Pearson Chi-square test and Fisher precise probability method were applied to test the differences in P53, P21WAF1 and CDK1 expression between various tissues. Spearman correlation test was applied to test the interrelationships of P53, P21WAF1 and CDK1 in epithelial ovarian cancer. Bilateral □ = 0.05 was applied as test standard.

Results

Expression of P53, P21^{WAF1} and CDK1. The positive rates of P53 was 0 in normal ovarian, 0 in benign ovarian tumor and 60.5% in ovarian cancer; those of P21^{WAF1} were 90.0%, 65.0% and 36.8%, respectively; those of CDK1 were 20.0%, 40.0% and 92.1%, respectively (Table 1). The differences between ovarian cancer and normal ovarian or benign tumor were significant ($P < 0.05$), while no significant difference between normal ovarian and benign ovarian tumor was observed ($P > 0.05$).

Correlations of P53, P21waf1 and CDK1 expression to clinicopathologic features of ovarian cancer. P53 expression was associated with the clinical stage and histological differentiation of epithelial ovarian cancer; P21^{WAF1} expression was associated with the clinical stage of epithelial ovarian cancer; CDK1 had no relationship with any clinicopathologic parameters of epithelial ovarian cancer (Table 2).

2.3 Interrelationships among P53, P21^{WAF1} and CDK1 expression in epithelial ovarian cancer

P53 expression was negatively correlated to P21^{WAF1} expression in epithelial ovarian cancer ($r = -0.388$, $P = 0.001$), and positively correlated to CDK1 expression ($r = 0.263$, $P = 0.022$); P21^{WAF1} expression was negatively correlated to CDK1 expression ($r = -0.282$, $P = 0.014$).

Table 1 The expression of P53, P21^{WAF1} and CDK1 proteins in different ovarian tissues

Group	Cases	P53				P21^{WAF1}				CDK1			
		−	+	++	Positive rate(%)	−	+	++	Positive rate(%)	−	+	++	Positive rate(%)
Normal ovarian tissues	20	20	0	0	0	2	18	0	90.0	16	4	0	20.0
Benign epithelial tumor	20	20	0	0	0	7	13	0	65.0	12	8	0	40.0
Epithelial ovarian cancer	76	30	12	34	60.5[a]	48	19	9	36.8[a]	6	20	50	92.1[a]

[a]$P<0.05$, vs. normal ovarian tissues and benign epithelial tumor.

Table 2 Correlations of P53, P21^{WAF1}, and CDK1 expression to clinicopathologic features of the 76 cases of epithelial ovarian cancer

Item	Cases	P53 [cases (%)]	P_1 value	P21^{WAF1} [cases (%)]	P_2 value	CDK1 [cases (%)]	P_3 value
Age							
<50 years	26	14(53.8)		11(42.3)		23(88.5)	
≥50 years	50	32(64.0)	0.462	17(34.0)	0.617	47(94.0)	0.406
FIGO stage							
Ⅰ/Ⅱ	24	8(33.3)		14(58.3)		23(95.8)	
Ⅲ/Ⅳ	52	38(73.1)	0.002	14(26.9)	0.011	47(90.3)	0.656
Histological grade							
G1	14	4(28.6)		8(57.1)		11(78.6)	
G2	22	13(59.1)		9(40.9)		20(90.9)	
G3	40	29(72.5)	0.015	11(27.5)	0.126	39(97.5)	0.075
Histological type							
Serous	54	36(66.7)		22(40.7)		51(94.4)	
Mucous	22	10(45.5)	0.121	6(27.3)	0.306	19(86.4)	0.348
Ascites							
No	20	9(45.0)		9(45.0)		17(85.0)	
Yes	56	37(66.1)	0.116	19(33.9)	0.427	53(94.6)	0.183
Lymph nodes metastasis							
No	52	29(55.8)		21(40.4)		49(94.2)	
Yes	24	17(70.8)	0.313	7(29.2)	0.446	21(87.5)	0.373

Discussion

Previous studies suggested that cell cycle disorder is involved in the carcinogenesis of ovarian cancer.[6,7] The DNA repair by the DNA damage checkpoints at G_2/M phase is the final occasion before mitosis.[8] "P53 pathway" is one of the two most important molecular pathways of DNA damage checkpoints at G_2/M phase, which functions on CDK1 mainly through P21^{WAF1} to regulate the activity of G_2/M phase checkpoints.[9,10] Investigating the role of "P53 pathway" in ovarian cancer will help to find out the molecular mechanisms in the carcinogenesis of ovarian cancer.

Related researches showed overexpression of P53 and low expression of P21^{WAF1} in poorly differentiated ovarian serous cystadenocarcinoma, and the overall and tumor-free survival of these patients were short, while the patients without P53 expression and with P21^{WAF1} overexpression had a higher 10-year survival rate.[11-13] Barrette et al.[5] found high CDK1 expression in epithelial ovarian cancer, but it had no relationship with histological grade and clinical stage. Welsh et al.[14] found high CDK1 expression in ovarian cancer, both in tissues and in cell lines.

Our study found P53 overexpression, low or loss of P21^{WAF1} expression and CDK1 overexpression in epithelial ovarian cancer; P53 expression was negatively correlated to P21^{WAF1} expression and positively correlated to CDK1 expression; P21^{WAF1} expression was negatively correlated to CDK1 expression. We speculated that the overexpression of P53 suppresses the expression of P21^{WAF1}, which leads to up-regulation of CDK1 expression, indicating that "P53 pathway" is involved in the carcinogenesis of ovarian cancer. Moreover, high P53 expression was more likely presented in advanced and poorly differentiated ovarian cancer and low P21^{WAF1} expression in advanced ovarian cancer. The differences in P53 and P21^{WAF1} expression between early stage and advanced ovarian cancer were significant ($P < 0.05$). These results suggested that P53 is associated with the malignant degree of epithelial ovarian cancer, P53 and P21^{WAF1} might be involved in the malignant progression of epithelial ovarian cancer.

Barrette et al.[5] have suggested that the abnormal expression of CDK1 might be an early event of tumorigenesis. Landen et al.[15] have also proposed similar point of view when investigating early events of the tumorigenesis of epithelial ovarian cancer. In our study, the positive rate of CDK1 was gradually increased with tumor progression; there was no significant difference between normal ovarian tissue and benign ovarian tumor ($P > 0.05$), while the differences between epithelial ovarian cancer and the other two groups were significant ($P < 0.05$). CDK1 was detected in most epithelial ovarian cancer tissues, but it had no correlation with the clinical features such as the histological grade and clinical stage. This can be applied to the early diagnosis of ovarian cancer. The molecular mechanism and clinical application of CDK1 overexpression need to be further explored.

To sum up, our research clarified the relationships between P53, P21^{WAF1} and CDK1 expression and epithelial ovarian cancer: P53 is associated with the malignant degree of epithelial ovarian cancer, P53 and P21^{WAF1} might be involved in the malignant progression of epithelial ovarian cancer. CDK1 detection helps early diagnosis of ovarian cancer. Clarifying the role of P53, P21^{WAF1} and CDK1 in the carcinogenesis and prognosis of epithelial ovarian cancer helps to find out the roles of dysfunction of cell cycle DNA repair checkpoints in the carcinogenesis of ovarian cancer and suggest new targets of biological prevention and treatment.

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