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Anti-Hsp20 antibody concentrations inversely correlated with tumor progression in ovarian cancer

Yanhui Zhu[1], Qingchao Tian[2], Naian Qiao[3], Yin Cheng[4], Haiying Li[5]

[1]*Medical Informatics Center, Peking University, Beijing*
[2]*Department of Obstetrics and Gynecology, Jinan third People's Hospital, Jinan*
[3]*Department of Radiotherapy, Shandong University Qilu Hospital, Jinan,*
[4]*Department of Science and Education, Jinan third People's Hospital, Jinan*
[5]*Division of Ultrasonography, Shandong University Qilu Hospital, Jinan, (China)*

Summary

Purpose: To investigate the serum concentrations of anti-heat shock protein 20 (anti-Hsp20) antibodies in women with ovarian cancer at different clinical stages, and the relationship between these concentrations and tumor progression. *Materials and Methods:* Blood samples were obtained from 72 patients undergoing surgery for ovarian cancer, 21 women with ovarian carcinoid, and 42 healthy women. Anti-Hsp20 antibody concentrations were determined by enzyme-linked immunosorbent assay. *Results:* Mean anti-Hsp20 antibody concentrations were significantly lower in patients with ovarian cancer than in the control group. The anti-Hsp20 antibody concentrations were negatively correlated with ovarian cancer malignancy. *Conclusions:* The present findings suggest that anti-Hsp20 antibodies may play a protective role against ovarian cancer progression, and that anti-Hsp20 antibodies may be a new index for the early diagnosis and treatment of ovarian cancer.

Key words: Heat shock protein; Hsp20; Ovarian cancer.

Introduction

Heat shock proteins (HSPs) are a subset of molecular chaperones best known for their rapid and abundant induction following stress. HSPs, classified by their molecular weight, are highly expressed in many malignant tumors—including ovarian cancer—and most seem to play roles in many aspects of tumor progression and response to therapy, probably due to their antiapoptotic properties [1, 2]. Hsp27 typically functions as a chaperone, but previous studies indicate that it also plays fundamental roles in maintaining intracellular redox potential and stabilizing the cytoskeleton [3, 4]. High Hsp27 expression induced by chronic cellular stress may lead to apoptosis suppression, thus facilitating malignant transformation [5].

The present authors' previous studies of HSP20 revealed its antiapoptotic effect on cardiomyocytes [6, 7]. They also found that Hsp20 expression levels decrease with tumor progression in ovarian cancer patients, suggesting that HSP20 could have a suppressive effect on ovarian cancer progression [8]. In the present study, the authors investigated the relationship between the serum concentration of anti-Hsp20 antibodies in women with ovarian cancer and the progression of ovarian cancer malignancy.

Materials and Methods

Subjects

The study subjects included 145 randomly selected women who were hospitalized for a suspected ovarian tumor between April 2011 and May 2013 and who intended to undergo surgical intervention. Another group of 42 healthy women of similar age were recruited as a control group. All participants were ethnic Han Chinese, and lived in the area of the Shandong Province in the middle-eastern region of China. Table 1 presents the demographics and clinical characteristics of the study population. The Ethics Committee of Jinan Third People's Hospital approved the research protocol, and written informed consent was obtained from each patient and control participant.

Women with ovarian cancer were excluded if they had received hormone therapy or chemotherapy, or if their condition occurred in combination with other malignancies. After screening, the ovarian cancer group included 72 patients: six (18%) International Federation of Gynecology and Obstetrics (FIGO) Stage I cases, ten (29%) FIGO Stage II cases, 12 (35%) FIGO Stage III cases, and six (18%) FIGO Stage IV cases. The histological types were as follows: serous papillary carcinoma (n = 50), mucinous carcinoma (n = 10), clear cell carcinoma (n = 6), endometrioid carcinoma (n = 4), and mixed cystadenocarcinoma (n = 2). Twenty-one women were diagnosed with benign ovarian carcinoid, with the different histological types including serous cystadenoma (n = 6), mucinous cystadenoma (n = 3), mixed cystadenoma (n = 3), and simple ovarian cyst (n = 6).

Surgical specimens

Ovarian tissues for histological type analysis were obtained from patients by surgical resection at the Department of Obstet-

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Table 1. — *Demographics and clinical characteristics of the study population.*

Characteristics	Ovarian Cancer Stage I (n = 13)	Ovarian Cancer Stage II (n = 21)	Ovarian Cancer Stage III (n = 25)	Ovarian Cancer Stage IV (n = 13)	Normal carcinoid (n = 42)	Ovarian (n = 21)
Age, years						
Mean (SD)	54.3 (2.0)	55.4 (1.9)	56.5 (2.1)	58.29(1.6)	53.0 (2.3)	51.8 (3.1)
Range	46–59	46–60	51–62	54–63	44–58	43–61
Age distribution, *n* (%)						
≤ 55	6 (46%)	9 (43%)	10 (40%)	5 (38%)	24 (57%)	13 (62%)
>55	7 (54%)	12 (57%)	15 (60%)	8 (62%)	18 (43%)	8 (38%)
Race, *n* (%)						
Asian Chinese	13 (100%)	21 (100%)	25 (100%)	13 (100%)	42 (100%)	21 (100%)
Ovarian cancer stage						
Stage I	13 (18%)					
Stage II		21 (29%)				
Stage III			25 (35%)			
Stage IV				13(18%)		
Histology						
Serous	10 (14%)	16 (22%)	15 (21%)	9 (13%)		
Mucinous	3 (4%)	3 (4%)	4 (6%)	0		
Clear cell	0	0	2 (3%)	4 (6%)		
Endometrioid	0	2 (3%)	2 (3%)	0		
Mixed cystadenocarcinoma	0	0	2 (3%)	0		

rics and Gynecology, Jinan Third People's Hospital. Resected tissue was divided such that part was examined by intraoperative frozen section analysis and the rest was snap-frozen in liquid nitrogen and stored at −80°C.

Serum samples

Patient blood samples were collected from the median cubital vein at 8:00 am after fasting. Blood was collected in a clotting tube. Within four hours of collection, clotted blood was centrifuged at 2000 × *g* for ten minutes, and then serum was aliquoted and stored at −80°C until assay.

Enzyme-linked immunosorbent assay (ELISA)

In the serum samples, the concentration of IgG antibodies against Hsp20 was determined by ELISA. Human recombinant Hsp20 from Prospec was used as an antigen. The ELISA plates were coated with the antigen solution at a concentration of two mg/ml in 50 mmol/L of carbonate buffer at pH 9.6. The tested serum samples were diluted 400-fold using 0.5% bovine serum albumin in phosphate-buffered saline with Tween-20. Horseradish peroxidase-conjugated goat anti-human IgG was used to detect the bound IgG antibodies. Tetramethylbenzidine solution was used as the enzymatic reaction substrate. The Power Wave XS plate reader was used to read the absorbance at 450 nm (reference wavelength, 630 nm), and the results were calculated using KC Junior software.

Calibration was performed using pooled sera obtained from 50 healthy blood donors. Dilution of 1:400 was considered as 100 arbitral units (AU/ml). The optimal sera dilution was chosen experimentally; the 1:400 dilution produced an optimal sample absorbance to background absorbance ratio for most of the studied samples. The calibration curve consisted of six standards with concentrations ranging from 0 to 400 AU/ml. The coefficient of the intra-assay variation was 8%. Determinations were performed during a single series. The sensitivity was about 1 AU. The obtained results were presented using basic parameters of descriptive statistics.

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Differences were analyzed for significance by one-way repeated-measures ANOVA, with further analyses performed using the Newman–Keuls test for multiple comparisons between treatment groups. The results were considered significant at $p < 0.05$. Analyses were performed using GraphPad Prism version 4.0 for Windows.

Results

Comparison of anti-Hsp20 antibodies among different groups

Figure 1 shows the results of ELISA for anti-Hsp20 expression in serum from patients with ovarian cancer Stages I, II, III, and IV, from ovarian carcinoid patients, and from control subjects with normal ovaries. Serum anti-Hsp20 concentrations were significantly reduced in malignant cases compared to in healthy controls, as well as in malignant cases compared to in benign cases ($p < 0.05$ for both). Concentrations of anti-Hsp20 antibodies also significantly differed between ovarian cancer Stages I and II ($p < 0.05$), with a trend towards an inverse correlation between cancer stage and anti-Hsp20 antibody expression in tumor tissues (Figure 1).

Discussion

The present results showed that the concentrations of anti-Hsp20 antibodies were lower in ovarian tumor tissue than in normal tissue, with a trend toward anti-Hsp20 antibody concentration decreasing in correlation with increasing ovarian cancer progression tissues. These findings are

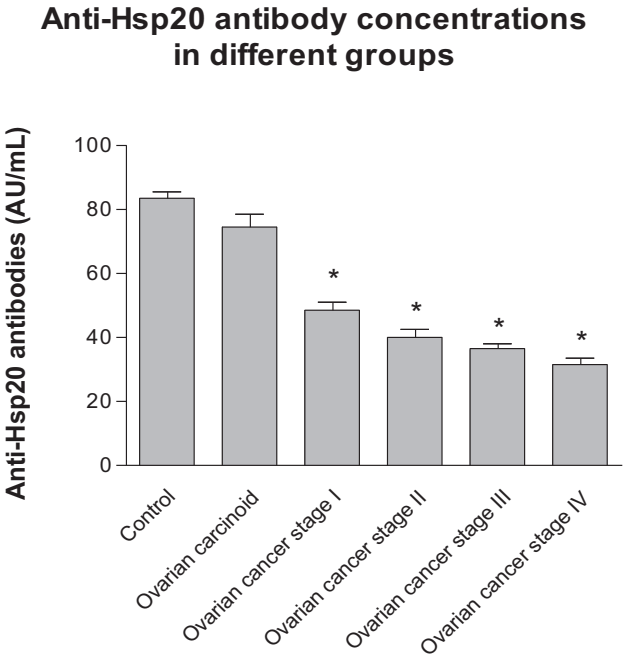

Figure 1. — ELISA was used to analyze the concentration of anti-Hsp20 antibodies in blood samples collected from 72 patients with ovarian cancer, 21 patients with ovarian carcinoid, and 42 control subjects. Values on the vertical axis represent the mean ± standard error of the mean from independent experiments. *$p <$ 0.05 compared to control samples and ovarian carcinoid samples. p was also < 0.05 for comparisons between ovarian cancer Stages I and II.

consistent with those of the authors' previous study, which demonstrated that HSP levels in tumor tissues were attenuated in association with ovarian cancer progression, such that HSP expression was inversely related to the grade of malignancy. To the authors' knowledge, this is the first report of a significant association between concentrations of anti-Hsp20 antibodies and ovarian cancer progression.

Olejek *et al*. previously reported that the mean concentration of anti-Hsp27 antibodies was significantly higher in a group of patients with ovarian carcinoma than in the control group. Their analysis of the association between anti-Hsp27 antibodies and the stage of clinical progression revealed higher concentrations of anti-Hsp27 antibodies in less advanced ovarian carcinoma specimens [8]. Thus, it seems that anti-Hsp20 antibodies and anti-Hsp27 antibodies have different responses to tumor malignancy. Further studies are warranted to investigate the underlying mechanisms behind these associations.

Conclusion

The present results strongly suggest that anti-Hsp20 antibody concentration decreases with tumor progression in ovarian cancer patients, thus indicating a possible suppressive effect of anti-Hsp20 antibodies on ovarian cancer progression. The authors are currently conducting studies to investigate the underlying mechanism of this effect, as well as to optimize the detection of anti-Hsp20 antibodies in serum for the early identification of ovarian cancer.

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Address reprint requests to:
YANHUI ZHU, M.D.
Medical Informatics Center,
Peking Univerisity,
38 Xueyuan road, Beijing 100191 (China)
e-mail: gzyh@hsc.pku.edu.cn