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Evaluation of the *BRCA*1/2 mutation as a prognostic marker in primary peritoneal serous cancer

Naoto Furukawa[1*], Sumire Ohno[1], Takahiko Kasai[2], Sachiko Morioka[1], Fuminori Ito[1], Yasuhito Tanase[1], Shoji Haruta[1], Seiji Kanayama[1], Ryuji Kawaguchi[1], Shozo Yoshida[1], Hiroshi Kobayashi[1]

[1]Department of Obstetrics and Gynecology, Nara Medical University, Kashihara, Japan
[2]Diagnostic Pathology, Nara Medical University, Kashihara, Japan
Email: [*]furunao0813@gmail.com

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ABSTRACT

Introduction: The present study was a retrospective investigation of the relation between immunohistochemical *BRCA*1/2 status and prognosis in patients with primary peritoneal serous cancer (PPSC). Materials and Methods: We retrospectively evaluated 14 consecutive patients diagnosed with PPSC other than hereditary breast and ovarian cancer between 2005 and 2010. All patients had serum CA125 levels >40 U/mL prior to starting first-line chemotherapy with paclitaxel and carboplatin. Paclitaxel was administered as a 3-hour intravenous infusion at a dose of 175 mg/m^2 on day 1, and carboplatin was delivered at an area under the curve of 5 based on the Calvert method. Patients received six cycles of first-line chemotherapy, except patients whose disease was determined to be progressive during the chemotherapy regimen. *BRCA*1/2 and *p*53 protein expression was determined by immunohistochemistry of patient tissue samples. The Cox proportional hazards model was used to evaluate univariate and independent multivariate associations with the effect of clinical parameters, such as age at diagnosis; tumor histology; tumor grade; and rate of change in CA125, and *BRCA*1/2, *p*53 status on overall survival. Probability values of less than 0.05 were considered to indicate statistical significance. Results: Two cases (14%) had the *BRCA*1 mutation, and none had the *BRCA*2 mutation. Eleven cases (79%) were positive for *p*53. In the univariate analysis, factors significantly associated with overall survival were (pre-chemotherapy CA125-pre-2nd chemotherapy CA125)/pre-chemotherapy CA125 (*p* = 0.0034) and (pre-chemotherapy CA125-pre-3rd chemotherapy CA125)/pre-chemotherapy CA125 (*p* = 0.0245). *BRCA*1 and *p*53 status were not predictors of overall survival. Multivariate analysis performed with overall survival as an endpoint revealed that none of the factors examined was significant. Median survival rate of patients without a *BRCA*1 mutation was 23.5 months (2 - 82 months), and all died. By contrast, one patient with a *BRCA*1 mutation remains alive at 85 months, and the other patient died at 64 months. Conclusion: *BRCA*1 might be a predictor of overall survival in patients with PPSC receiving chemotherapy.

Keywords: *BRCA*1; Primary Peritoneal Serous Cancer; Prognosis

1. INTRODUCTION

Ovarian cancer is increasing annually in Japan [1], and the total number of ovarian cancer cases in 2006 was 7913 [2]. Standard therapy for ovarian cancer comprises primary surgical cytoreduction followed by platinum-based chemotherapy, but 25% to 90% of cases of ovarian cancer are diagnosed during progression [3]. Paclitaxel and carboplatin as first line chemotherapy are administered to patients with ovarian cancer, and overall response rates of 59% [4] and 68% [5] are reported in studies in which paclitaxel (175 - 185 mg/m^2) and carboplatin (area under the curve of 5 - 6) are administered every 3 weeks. Despite a favorable initial response rate to treatment, early recurrences and platinum resistance are frequently encountered. Several reports have discussed the diverse pathogenesis of ovarian cancers. Low-grade serous carcinomas are associated with mutations in *KRAS*/*BRAF* and evolve slowly [6]. High-grade serous carcinomas are, in contrast, associated with mutations in

[*]Corresponding author.

*p*53, *BRCA*1, or *BRCA*2, and evolve rapidly [6,7]. *BRCA*1 and *BRCA*2 act as tumor suppressors involved in repairing double-strand DNA breaks by homologous recombination. *BRCA*1/2 germline mutations occur in 11% to 15.3% and *BRCA*1/2 somatic mutations occur in 19% of women with unselected ovarian cancers [8]. Patients with *BRCA*1 and *BRCA*2 mutations have increased sensitivity to platinum chemotherapy and a better prognosis [9]. Primary peritoneal serous carcinoma (PPSC) is histologically and clinically similar to stage III-IV ovarian high-grade serous carcinoma, and is rare. The frequency of *BRCA*1/2 germline mutations in PPSC is reported to be 15.8% [10]. Another group reported that germline-*BRCA*1 mutations are found in 26% of PPSC patients [11]. Few reports, however, describe the frequency of somatic mutations of *BRCA*1/2 in patients with PPSC, or the relation between *BRCA*1/2 status with chemosensitivity and prognosis in patients with PPSC. *BRCA* immunohistochemistry identifies *BRCA* mutations with a sensitivity of 80% and a specificity of 93%, suggesting that *BRCA* immunohistochemistry is a promising screening method for *BRCA* mutation detection [12].

In the present study, we retrospectively investigated the relation between immunohistochemical *BRCA*1/2 status and prognosis in patients with PPSC.

2. MATERIALS AND METHODS

2.1. Patient Selection

The present study was conducted in accordance with the principles of the Declaration of Helsinki. We retrospectively evaluated 14 consecutive patients diagnosed with PPSC (all stage IIIC) other than hereditary breast and ovarian cancer between 2005 and 2010. Genetic testing was not available during this period, however, so hereditary breast and ovarian cancer were diagnosed by asking patients detailed questions regarding family history. All patients underwent a laparotomy or laparoscopy to obtain a small tissue sample for examination under a microscope, but none of them underwent debulking surgery. Diagnostic criteria include normal sized ovaries; extraovarian site involvement greater than surface involvement of the ovary; an ovarian component of less than 5 × 5 mm within the ovary and otherwise confined to the surface of the ovary; and histologic characteristics predominantly of the serous type [13]. All patients had serum CA125 levels >40 U/mL prior to starting first-line chemotherapy with paclitaxel and carboplatin. Paclitaxel was administered as a 3-hour intravenous infusion at a dose of 175 mg/m^2 on day 1, and carboplatin was delivered at an area under the curve of 5 based on the Calvert method. Patients received 6 cycles of first-line chemotherapy, except patients whose disease was determined to be progressing during the chemotherapy regimen. The retrieved clinical data included patient age at diagnosis, tumor histology, tumor grade, CA125, and overall survival from the medical records. Rates of change in CA125 were calculated as follows: (pre-chemotherapy CA125—pre-2nd chemotherapy CA125)/pre-chemotherapy CA125; and (pre-chemotherapy CA125—pre-3rd chemotherapy CA125)/pre-chemotherapy CA125. Pre-chemotherapy CA125 represents the CA125 level before administering the first dose of chemotherapy, Pre-2nd chemotherapy CA125 represents the CA125 level before administering the second dose of chemotherapy; and Pre-3rd chemotherapy CA125 represents the CA125 level before administering the third dose of chemotherapy.

2.2. Immunohistochemistry

*BRCA*1/2 and *p*53 protein expression was determined by immunohistochemistry of patient tissue samples. Immunohistochemistry staining was performed according to standard techniques. Staining was carried out with a mouse monoclonal IgG antibody, anti-*BRCA*1 (OP92-100UGCN, Merck Chemicals Ltd, Nottingham, UK), and anti-*BRCA*2 (MAB2476, R & D Systems, Minnesota, US). Two independent pathologists blinded to the clinical data and each other's opinion scored the expression based on the staining intensity. Scoring was as follows: 0, 0%; 1, 1 to <10%; 2, 10 to <50%; 3, 50 to <90%; 4, if >90% of the cells were positive. The tumor was considered *BRCA*1/2 mutation-positive when less than 10% of the tumor cells had a positive reaction. The tumor was considered *p*53-positive when 50% or more of the tumor cells had a positive reaction.

2.3. Statistical Analysis

All statistical analyses were conducted using SPSS software Version 17 (SPSS Inc., Chicago, IL, USA).The Cox proportional hazards model was used to evaluate univariate and independent multivariate associations with the effect of clinical parameters, and *BRCA*1/2 and *p*53 status on overall survival. Probability values of less than 0.05 were considered to indicate statistical significance.

3. RESULTS

The baseline patient characteristics are shown in **Table 1**. Two cases (14%) had a *BRCA*1 mutation, and none had a *BRCA*2 mutation. Eleven cases (79%) were positive for *p*53. In the univariate analysis, the factors significantly associated with overall survival were (pre-chemotherapy CA125—pre-2nd chemotherapy CA125)/pre-chemotherapy CA125 (p = 0.0034) and (pre-chemotherapy CA125—pre-3rd chemotherapy CA125)/pre-chemotherapy CA125 (p = 0.0287). Neither *BRCA1* nor *p*53 status were detected as predictors of overall survival (**Table 2**).

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Table 1. Patient characteristics.

Median age (range)		58 (37 - 79)
Histology (serous)	Grade 2	6
	Grade 3	8
Median pre-treatment CA125 (range) U/ml		906 (344 - 13998)

Table 2. Univariate analysis of risk factors associated with overall survival.

	Hazard ratio	95% confidence	95% interval	P value
Age	0.9340	−0.1630	0.0155	
Grade 2/Grade 3	1.4380	−0.7581	0.4333	
Pre-treatment CA125	0.9999	−0.0003	0.0004	
(pre-chemotherapy CA125—pre-2nd chemotherapy CA125)/pre-chemotherapy CA125	0.1619	−3.3304	−0.6100	0.0034
(pre-chemotherapy CA125—pre-3rd chemotherapy CA125)/pre-chemotherapy CA125	0.0307	−6.8083	−0.3960	0.0287
*BRCA*1 mutation +/−	0.1796	−0.00485	2.3284	
*BRCA*2 mutation +/−	-			
*p*53 +/−	0.9511	−0.9317	0.7442	

Multivariate analysis performed with overall survival as an endpoint revealed that neither was a significant factor (**Table 3**). Median survival rate was 23.5 months (2 - 82 months) in patients with no *BRCA*1 mutation, and all had died. By contrast, one patient with a *BRCA*1 mutation remains alive (85 months), and the other patient died at 64 months. Median survival rate was 23 months (2 - 85 months) in patients that were *p*53-positive, and 27 months (22 - 36 months) in patients that were *p*53-negative.

4. DISCUSSION

PPSC is reported to arise from the extra-ovarian peritoneum [14]. A recent study, however, demonstrated an association with tubal intraepithelial carcinoma in 47% of patients with PPSC [15], suggesting the potential role of the distal fallopian tube as an organ of serous carcinogenesis. Symptoms include abdominal distension and pain with ascites caused by peritonitis carcinomatosa. Most patients do not consult a gynecologist until the disease is in an advanced stage, and patients with PPSC have a worse prognosis than those with ovarian cancer.

Table 3. Multivariate analysis of risk factors associated with overall survival.

	Hazard ratio	95% confidence	95% interval
(pre-chemotherapy CA125—pre-2nd chemotherapy CA125)/pre-chemotherapy CA125	0.5597	−3.8685	3.5259
(pre-chemotherapy CA125—pre-3rd chemotherapy CA125)/pre-chemotherapy CA125	0.0913	−10.4273	4.8152

Median survival is 12 to 18 months [16]. PPSC is an ovarian cancer subtype, and therefore the chemotherapy regimen is the same as that for ovarian cancer. Patients with ovarian cancer with a *BRCA* mutation are considered to be platinum-sensitive, and thus have better prognosis than those with no *BRCA* mutation. Overall survival is 52.7 months in patients with low/intermediate *BRCA*1 mRNA expression, which is significantly better than the overall survival of 18.2 months in patients with high *BRCA* expression [9]. In the present study, median survival was 22 months in patients with positive *BRCA*1 expression and 64 months in the 2 patients with negative *BRCA*1 expression. Based on the present study, *BRCA*1 may be also a predictor of the prognosis of PPSC, although the sample size was small. By contrast, no *BRCA*2 mutation was detected in the present study. Some reports documented an overall survival advantage conferred by *BRCA*2 mutations compared with either having no *BRCA* mutation or having a *BRCA*1 mutation in ovarian cancer [17,18]. In these reports, the frequency of the *BRCA*2 mutation was half that of the *BRCA*1 mutation. In the present study, it is therefore not surprising that no cases of *BRCA*2 mutation were detected because the *BRCA*1 mutation was detected in only two cases. No relation between *p*53 status and overall survival was detected in the present study. Over half the PPSC patients were *p*53-positive, and *BRCA*1 mutation carriers had a higher overall incidence of *p*53 mutations than those with wild-type *BRCA*1 [11]. The *p*53 signature, which is defined as a morphologically normal, strongly *p*53-immunopositive segment of tubal secretory (non-ciliated) cells spanning at least 12 consecutive nuclei [19], has been found in patients with *BRCA* germline mutations, with an incidence ranging from 11% to 71% of cases, and also in normal controls (19% - 50%) [20-22]. To our knowledge, however, no reports have described a relation between *p*53 status and overall survival in patients with PPSC.

CA125 is a useful marker for estimating treatment efficacy in patients with ovarian cancer and PPSC. The CA125 regression rate is a predictor of optimal debulking surgery in patients receiving neoajduvant chemo-

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therapy with advanced ovarian cancer [23,24], suggesting that the CA125 regression rate may be predictor of overall survival in patients with PPSC without surgery. In the present study, however, the CA125 regression rate was detected as a factor in univariate analysis but not in multivariate analysis. Possible explanations for CA125 regression rate not being detected as a predictor of overall survival in the present study might be the more aggressive behaviour of PPSC compared with ovarian cancer and the small sample size. Limitations of the present study include the small sample size, the fact that it is a retrospective study, hereditary breast and ovarian cancer might be included because genetic testing was not available.

5. CONCLUSION

In conclusion, the *BRCA*1 mutation was detected in 14% in patients with PPSC and these patients had a better prognosis than patients not having the *BRCA*1 mutation. *BRCA*1 might be a predictor of overall survival in patients with PPSC receiving chemotherapy. There is a need for new biomarkers, however, because most patients with *PPSC* have a poor prognosis and have no *BRCA*1 mutation.

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