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ORIGINAL PAPER

Prognostic Significance of Phosphatase of Regenerating Liver-3 Expression in Ovarian Cancer

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Abstract Phosphatase of regenerating liver-3 (PRL-3) is overexpressed in several human cancers and associated with tumor progression, invasion and metastasis. However, the correlation between PRL-3 expression and clinical outcome in ovarian cancer has not been studied. In the present study, we investigated the expression of PRL-3 in 119 ovarian cancers and 30 normal ovarian tissues by immunohistochemistry with an anti-PRL-3 mouse monoclonal antibody 3B6, and analyzed its relationship with clinicopathologic factors and survival. The results demonstrated that PRL-3 expression was significantly higher in ovarian cancers compared to normal ovarian tissues ($P<0.001$). PRL-3 expression is not correlated with patient age, menstruation, tumor size, histological type, residual tumor, or other clinical findings. The patients with PRL-3-positive tumors had a significant poor prognosis than those with PRL-3-negative tumors. Univariate analysis identified PRL-3 expression as a poor outcome predictor (HR 1.925, 95% CI, 1.046–3.544, $P=0.035$). Multivariate analysis indicated that PRL-3 expression was an independent prognostic factor of borderline significance (HR 1.695, 95% CI, 0.914–3.145, $P=0.094$). Our results suggest that PRL-3 may serve as a valuable marker for diagnosis of ovarian cancer and as a potential independent prognostic factor for ovarian cancer.

Keywords PRL-3 · Expression · Ovarian cancer · Immunohistochemistry · prognosis

Abbreviations

PRL-3	phosphatase of regenerating liver-3
OS	overall survival
HR	hazard ratio
TP	TP based chemotherapy (combination of taxol and cisplatin)
CAP	CAP based chemotherapy (combination of cyclophosphamide, cisplatin and adriamycin)

Introduction

Ovarian cancer is a common gynecological malignancy worldwide. The 5-year survival rate for this disease did not exceed 40% with relatively little improvement in last decade [1]. The high mortality is largely because of advanced stage at presentation and limited response to current cancer therapies. In addition to the ongoing efforts to develop more effective treatment, it is necessary to identify new biomarkers for predicting the prognosis. The glycoprotein CA125 plays an important role in the diagnosis and follow-up of ovarian cancer [2]. However, the use of CA125 is hindered by its low specificity and

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limited prognostic value [3,4]. Therefore, more reliable biological markers are required for earlier diagnosis, prognosis and follow-up.

Protein phosphorylation is one of the most important post-translational modifications affecting apoptosis, cell cycle progression, protein degradation, and oncogenesis [5]. PRL-3 (Phosphatase of regenerating liver-3), also known as PTP4A3, is a member of small class of tyrosine phosphatases. PRL-1 (Phosphatase of regenerating liver-1) and PRL-2 (Phosphatase of regenerating liver-2) are the other two members of this family [6]. Many studies have shown that PRLs are involved in regulating cell proliferation, oncogenic transformation, migration, and metastasis [7–13]. Cell cycle analysis verified that PRL-1 or PRL-2 overexpression accelerates entry from G1 to S phase [8]. More recently, PRL-3 antibody was shown to dramatically inhibit metastatic tumor formation of A2780 PRL-3-positive human ovarian cancer cells [14]. However, the exact biological function and cellular substrates of the PRLs remain largely unclear.

In our previous studies, we generated a specific monoclonal antibody 3B6 and applied it for immunohistochemical staining to detect PRL-3 expression in colorectal cancer and breast cancer tissues [15–17]. The results showed that PRL-3 expression is associated with liver metastasis and a shorter survival time in colorectal cancer [16]. In breast cancer, PRL-3 was an unfavorable prognostic marker especially for node-negative patients [17]. Other studies showed that PRL-3 expression is correlated with lymph node metastasis in colonic adenocarcinoma and may participate in the progression and metastasis of gastric carcinoma [18–20]. Moreover, Polato *et al.* reported that PRL-3 mRNA expression was higher in the late stage than in the early stage of ovarian cancer [9]. However, the protein expression level of PRL-3 and its prognostic value in ovarian cancer tissues remain unknown. Therefore, in this study, we detected PRL-3 with specific monoclonal antibody 3B6 by immunohistochemical method to investigate whether PRL-3 is associated with the prognosis of patients with ovarian cancer (Table 1).

Materials and Methods

Patients

A study of 119 patients with ovarian cancer, undergoing surgery at Beijing Cancer Hospital in the period of 2000–2005, was performed. The median age at diagnosis was 55 years (range 19–84). 100 patients were followed until death or more than 3 years after diagnosis. Primary histological examination was performed at the Department of Pathology, Beijing Cancer Hospital. Histologic tumor slides were reevaluated by two pathologists. All tumors were staged according to the International Federation of Gynecology and Obstetrics (FIGO) classification. Details concerning patient age, menstruation, clinical stage, tumor size, histological type, and other clinicopathologic factors are given in Table 2. After ovarian surgery, 101 patients received adjuvant chemotherapy as summarized in Table 2. Another 30 normal ovarian tissue samples were obtained from women undergoing surgery for endometrial carcinoma or ovarian cancer in the contralateral ovary. Informed consent was obtained and the study had the approval from the Hospital Research Ethics Committee.

Immunohistochemistry

The specificity of the mouse monoclonal antibody 3B6 against PRL-3 was characterized in our previous study [15–17]. For immunohistochemical staining, all the specimens were fixed in freshly prepared 10% neutral buffered formalin, embedded in paraffin wax, and cut into 5 μm sections. After baked at 60°C overnight, sections were dewaxed and rehydrated. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide for 10 min at room temperature. After being blocked with 5% skim milk, sections were incubated with the 3B6 antibody (2.5 μg/ml) at 4°C overnight followed by the incubation with the second antibody from EnVisionTM kit (Dako Cytomation) for 30 min at room temperature. Reaction product was visualized with diaminobenzidine (DAB, Sigma) for 5 min at room temperature. Sections were counterstained with hematoxylin. Purified IgG from normal mouse serum was used as negative control. Evaluation of PRL-3 immunoreactivity was carried out independently by two experienced pathologists without any knowledge of the clinical data. Tissue samples were estimated in a consecutive analysis to ensure maximal internal consistency. The analysis was assessed according to both the percentage of positive cells and the intensity of the cytoplasmic staining in ten randomly chosen microscopic fields. Assays were scored as negative if <10% of tumor cells were stained and positive if ≥10% of tumor cells were stained. The staining

Table 1 Frequency of different levels of PRL-3 expression

Tissue type	0 *n* (%)	1+ *n* (%)	2+ *n* (%)	3+ *n* (%)	Negative *n* (%)	Positive *n* (%)	*P*
Normal ovarian tissue	30 (100)	0 (0)	0 (0)	0 (0)	30 (100)	0 (0)	<0.001
Ovarian cancer	76 (63.9)	30 (25.2)	13 (10.9)	0 (0)	76 (63.9)	43 (36.1)	

Table 2 Correlation between PRL-3 expression and clinicopathologic characteristics in ovarian cancer

Variables	Cases (*n*)	PRL-3 expression		χ^2	*P*
		Negative	Positive		
Age					
<50	40	27	13	0.345	0.687
≥50	79	49	30		
Menopause					
no	37	25	12	0.319	0.363
yes	82	51	31		
FIGO stage					
I	16	14	2	6.436	0.092
II	13	8	5		
III	60	39	21		
IV	30	15	15		
Tumor size					
≤5 cm	23	15	8	0.023	0.542
>5 cm	96	61	35		
Histotype					
Serous	88	55	33	7.128	0.129
Mucinous	13	11	2		
Clear cell	8	4	4		
Endometrioid	8	6	2		
Other	2				
Residual tumor					
≤2 cm	59	37	22	0.589	0.532
>2 cm	40	22	18		
Unknown	20				
Celiac node status					
Absent	37	26	11	0.954	0.221
Present	82	50	32		
Chemotherapy					
TP	43	28	15	0.137	0.934
CAP	37	23	14		
other	21	14	7		

intensity was classified as the following: no staining or staining observed in <10% of tumor cells was given a score 0; a weak staining detected in ≥10% of tumor cells was scored as 1+; and a moderate or strong complete staining observed in ≥10% of tumor cells was scored as 2+ or 3+, respectively.

Statistical Analysis

The association between PRL-3 expression and clinicopathologic variables was analyzed with the Chi-square test. The impact of PRL-3 on patient overall survival (OS) was assessed with the hazard ratio (HR; relative risk of death in the PRL-3-positive group) calculated with the Cox univariate and multivariate proportional hazard regression model. Survival curves were estimated using the Kaplan-Meier method and compared with the log-rank test. All statistical analyses were performed with the SPSS statistical software package 13.0 (SPSS, Inc., Chicago, IL). *P* values less than 0.05 were considered statistically significant.

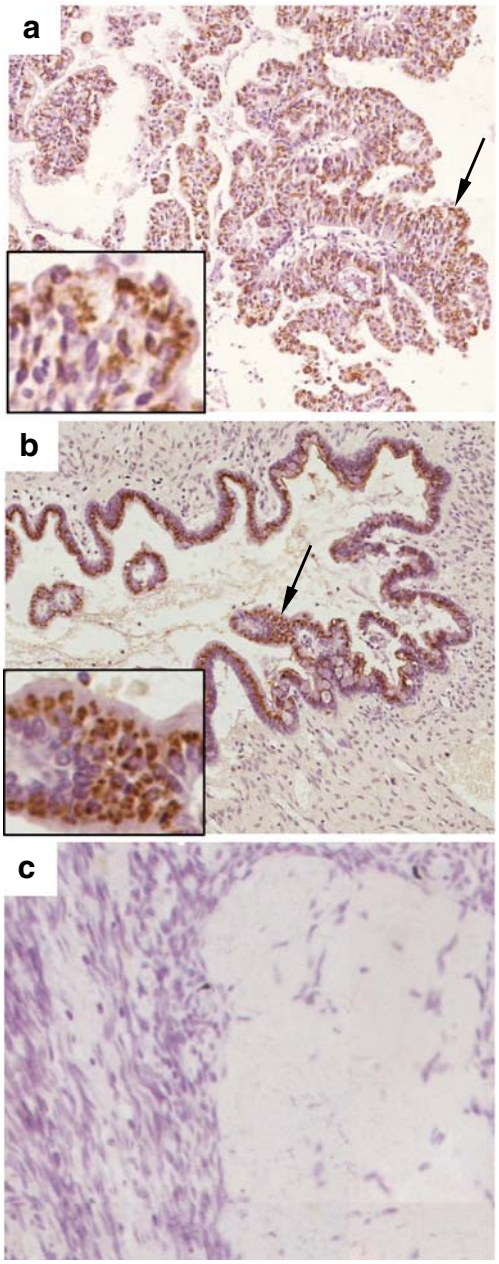

Fig. 1 Immunohistochemistry of PRL-3 expression in ovarian tissues. Immunoreaction with brown color indicated PRL-3 expression in cancer cells. The arrows pointed to positive staining. Insert within **a** and **b** was a high-power magnification to demonstrate cellular staining (magnification 400×). **a** Positive staining of PRL-3 in ovarian serous papillary cancer (magnification 40×). **b** Positive staining of PRL-3 in ovarian mucinous cancer (magnification 40×). **c** Negative staining of PRL-3 in normal ovarian tissue (magnification 100×)

Table 3 Univariate and multivariate analyses of PRL-3 Expression and clinicapathologic findings

Variables		
Univariate	Hazard ratio (95%CI)	*P*-value
PRL-3 expression (+ vs. −)	1.925 (1.046–3.544)	0.035
Age (<50 vs. ≥50)	1.625 (0.798–3.309)	0.181
Menopause (Post vs. Pre)	1.341 (0.659–2.730)	0.419
FIGO stage (III/IV vs. I/II)	3.397 (1.210–9.536)	0.006
Tumor size (>5 cm vs. ≤5 cm)	0.959 (0.471–1.953)	0.908
Multivariate		
PRL-3 expression (+ vs. −)	1.695 (0.914–3.145)	0.094
Age (<50 vs. ≥50)	2.011 (0.597–6.779)	0.260
Menopause (Post vs. Pre)	0.818 (0.243–2.758)	0.746
FIGO stage (III/IV vs. I/II)	3.519 (1.228–10.081)	0.019
Tumor size (>5 cm vs. ≤5 cm)	1.304 (0.630–2.698)	0.475

Results

The expression of PRL-3 in 149 clinical ovarian samples was analyzed by immunohistochemistry. The frequency of PRL-3 expression evaluated is described in Table 1. When stratifying the frequency of expression into two groups—negative (0) and positive (1+, 2+ and 3+ immunopositivity), 36.1% (43 in 119) of the cancer tissues exhibited cytoplasm staining for PRL-3 in >10% of the tumor cells. As shown in Fig. 1 a, b, PRL-3 protein was mainly localized in the cytoplasm of ovarian cancer cells and the staining was often heterogeneous and granular. In 30 cases of normal ovarian tissues, PRL-3 staining was either absent or present in only few cells (Fig. 1 c). Therefore, PRL-3 expression was significantly higher in ovarian cancers compared to normal ovarian tissues ($P<0.001$) (Table 1).

We analyzed the association between PRL-3 expression and clinical characteristics in 119 ovarian cancers by the chi-square test. Except that PRL-3-positive tumors were more likely to be at late stages ($P=0.092$), there was no relationship of PRL-3 expression with those factors, such as age, menstruation, tumor size, histological type, residual tumor, celiac node status or chemotherapy (Table 2).

To study whether PRL-3 is a prognostic factor for ovarian cancer, the Cox proportional hazard model and Kaplan-Meier survival curve were used to evaluate the prognosis significance of PRL-3 expression in 100 ovarian cancer patients after surgery with over 36-month follow-up period. The association between PRL-3-positive tumors and survival outcome is presented in Table 3. In a univariate Cox regression analysis, PRL-3-positive patients had a higher risk of death (HR, 1.925; $P=0.035$). A multivariate Cox regression analysis showed that PRL-3 retained a borderline significance (HR, 1.695, $P=0.094$) for correlation with a worse OS in patients with ovarian cancers. This regression model suggests that there is approximately a 69.5% increase in the risk of death in patients with PRL-3-positive tumors, compared to those who are PRL-3-negative. Kaplan-Meier survival curves further showed that patients with PRL-3-positive tumors have substantially shorter OS compared to those with PRL-3-negative tumors (P=0.031) (Fig. 2). As expected, clinical stage was significantly associated with decreased OS in both univariate and multivariate analyses ($P=0.006$ and $P=0.019$, respectively). However, patient age, menstruation, and tumor size were not significant predictors of OS in both univariate and multivariate analyses (P>0.05).

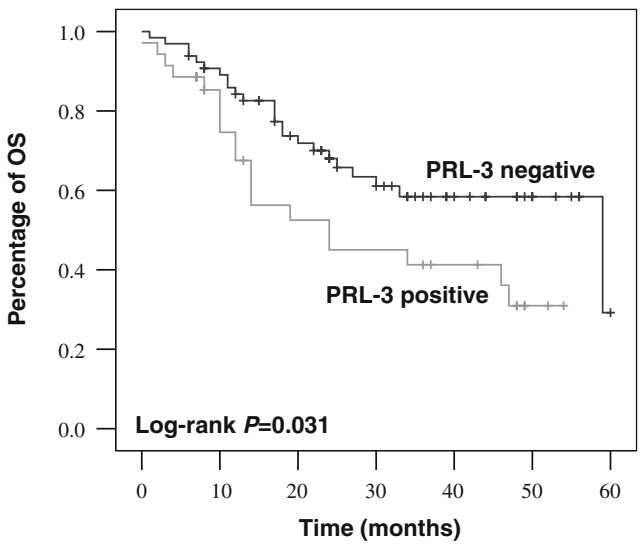

Fig. 2 Overall survival analysis of patients with ovarian cancer using Kaplan-Meier method. The result indicated that PRL-3-positive tumors are associated with a worse prognosis than PRL-3-negative tumors for the patients with ovarian cancer

Discussion

The phosphatases of PRL family, consisting of PRL-1, PRL-2, and PRL-3, have been identified as biomarkers and potential therapeutic targets in cancer. PRL phosphatases regulate key pathways involved in tumorigenesis and metastasis, and are overexpressed in a variety of cancer tissues compared with their normal counterparts [21–23]. Recently, a lot of studies showed that high expression of PRL-3 was associated with invasion, metastasis and poor prognosis in colorectal cancer and gastric cancer [24]. In breast cancer, PRL-3 might serve as a novel prognostic factor which may help to predict an adverse disease outcome [25]. In ovarian cancer, although it has been reported that PRL-3 expression at mRNA level was associated with progression, the prognostic value of PRL-3 expression at protein level has not been evaluated [9].

In the present study, we analyzed the expression of PRL-3 by immunohistochemistry in ovarian cancers and normal ovarian tissues. PRL-3 expression was undetectable in 30 normal ovarian tissues, while 36.1% (43/119) positive expression was observed in ovarian cancer tissues. The results showed that PRL-3 expression was significantly higher in ovarian cancers compared to normal ovarian tissues. We also found that PRL-3-positive tumors were more likely to have late stage disease. Our results of PRL-3 expression at protein level are consistent with the reports of PRL-3 at mRNA level by Polato *et al.* that PRL-3 was associated with ovarian cancer progression [9]. In our present work, the correlation of PRL-3 expression with lymph node metastasis was not analyzed, because only few cases had retrospective records of lymph node metastasis.

Several studies showed that PRL-3 is a valuable prognostic marker for colorectal carcinoma, gastric cancer and breast cancer [16–20, 24, 25]. In this study, we found that patients with PRL-3-positive tumors had substantially shorter OS than those with PRL-3-negative tumors. In addition, PRL-3 expression showed a significant association with worse clinical outcome in univariate analysis, and retained a marginal significance for OS after adjust for other prognostic factors in multivariate analysis. Although more samples should be required to further confirm its clinical significance, our present results suggested that PRL-3 was a potential independent prognostic factor for OS, and a predictor for poor clinical outcome.

In conclusion, our study suggests that PRL-3 was overexpressed in ovarian cancers compared with normal ovarian tissues. Moreover, PRL-3 may serve as a potential independent prognostic factor for overall survival in patients with ovarian cancer. Therefore, PRL-3 is considered to be a marker in ovarian cancer and our data also open the way to the study of the relevance of PRL-3 function in the progression of ovarian cancer.

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