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Expression of Gab1 Is Associated with Poor Prognosis of Patients with Epithelial Ovarian Cancer

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Growth factor receptor-bound protein-2 (Grb2) can act as the scaffold protein recruiting other molecules to the stimulated receptors. Grb2-associated binding protein 1 (Gab1) is involved in cell proliferation, and its expression may enhance the carcinogenesis and cancer progression. However, the function of Gab1 remains to be investigated. Epithelial ovarian cancer (EOC) is the most lethal malignancy in the female reproductive system with increasing incidence and unsatisfied overall survival (OS). We investigated the expression of Gab1 in EOC tissues and the correlations between Gab1 expression and the clinicopathological characteristics of patients with EOC using Spearman rank test. The staining results were evaluated based on both the percentage of Gab1-positive tumor cells and the staining intensity for Gab1 expression. Kaplan-Meier survival analysis and Cox proportional hazards analysis were used to compare the postoperative OS between EOC patients with high Gab1 expression and those with low Gab1 expression. The high expression of Gab1 was positively correlated with advanced FIGO stage and lymph node metastasis of EOC. Univariate analysis showed that advanced FIGO stage, pathological grade, lymph node metastasis or Gab1 expression were associated with poor OS. Moreover, multivariate analysis revealed that Gab1 expression could be an independent prognostic factor for the poor OS of EOC patients ($P = 0.042$). We propose that Gab1 expression is correlated with poor prognosis of EOC patients and may act as an independent prognostic indicator.

Keywords: epithelial ovarian cancer; FIGO stage; Gab1; lymph node metastasis; prognosis
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Introduction

Ovarian cancer is the most lethal malignancy in the female reproductive system (Wingo et al. 1998; Jemal et al. 2007). The incidence of the disease is steadily increasing in Asian countries in recent years (Lynch et al. 1998), and there are more than 200,000 newly diagnosed cases occurs yearly worldwide (Ferlay et al. 2010). Overall survival (OS) rate of ovarian cancer is elevating thanks to the improving surgical techniques and medical managements, but the long-term prognosis of patients remains unsatisfied. Most patients with epithelial ovarian cancer (EOC) are in advanced clinical stage at the time of diagnosis, resulting in that the 5-year OS rate is only approximately 50% (Heintz et al. 2006). The main reasons for this unsatisfied prognosis of EOC include the silent clinical features, early lymph metastasis and easy recurrence, etc. So new predictive, prognostic and therapeutic biomarkers in EOC are still in urgent need for improving EOC survival rate.

The Grb2-associated binding protein (Gab) family is a kind of adapter proteins which can couple with growth factor receptor binding protein 2 (Grb2). Grb2-associated binding protein 1 (Gab1) is widely expressed in mammals and mainly located in the cytoplasm (Gu and Neel 2003). It can be recruited to the cell membrane and activated once the specific receptors were stimulated (Nishida and Hirano 2003), thus exerting numerous biological effects such as regulating cell proliferation, differentiation and migration (Wohrle et al. 2009). As reported, Gab1 can up-regulate the proto-oncogene expression through interactions with Grb2, protein-tyrosine phosphatase SHP2, phosphatidylinositol 3-kinase (PI3K) and phosphatidylinositol (3,4,5)-trisphosphate (PIP3), therefore it plays an important role in the tumor development and progression (Oka et al. 2008; Wohrle et al. 2009; Felici et al. 2010). Although there have been several studies demonstrated that Gab1 is involved in the development of chondrosarcoma (Fan et al. 2016), cholangiocarcinoma (Sang et al. 2013, 2015), colorectal cancer (Seiden-Long et al. 2008) and mammary tumor (Gillgrass et al. 2003), there are no published reports describing the correlation between Gab1 and the biological behaviors of EOC.

In this study, we explored the role of Gab1 in EOC by detecting Gab1 in 124 samples of EOC with immunohisto-

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chemistry and subsequently analyzed its association with clinicopathological characteristics. In addition, we evaluated the prognostic value of Gab1 in EOC with univaraite and multivariate analyses.

Patients and Methods

Patients and Follow-up

All clinical samples were obtained from patients (n = 124) hospitalized in the Department of Gynecology, Linyi People's Hospital, China, between January 2001 and January 2012. None of the patients received pre-operative immunotherapy, chemotherapy or radiotherapy. The diagnosis of EOC was confirmed with pathological analysis and clinical findings. After surgical resection, the clinical specimens were fixed and then embedded in paraffin. Patients were followed up for medical evaluation either in our hospital or by telephone. Deaths caused by EOC were considered as the ending of their information collection in this study. OS time was defined as the period from the disease diagnosis to the date of death or to the last clinical follow-up time (April 2014). This study was permitted by the ethical committee of Linyi People's Hospital, and all the informed consents from the patients were collected. The clinicopathological features of the EOC patients were described in Table 1.

Cell Culture

The normal ovarian epithelial cells were prepared with the Auersperg methods (Kruk et al. 1990). Briefly, we obtained the specimens of normal ovarian surface from 17 patients (aging 33-54 years old) who were undergoing biopsy and diagnosed with nonmalignant gynecologic diseases. All the tissue specimens were obtained with prior patient consent and the approval of the Institutional Clinical Ethics Review Board of Linyi People's Hospital. After washed with D-Hanks for 3 times, the specimens were cut into pieces and digested with collagenase for 3 h. Then cultured the cells with RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. The ovarian cancer cell lines OVCAR3 and SKOV3 were purchased from the Cell Bank of the Chinese Academy of Sciences and cultured in the same medium. The cells were harvested for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis to determine the expression level of Gab1.

RT-qPCR

Total RNA was extracted from the cells using the RNAsimple Total RNA kit (#DP419, Tiangen, Beijing, China) and 0.5 μg total RNA of each sample was reverse transcribed to synthesis the cDNA with the reverse transcription kit (Takara, Otsu, Japan). RT-qPCR was performed using the SYBR Premix Ex Taq kit (Takara, Otsu, Japan) and analyzed with the $2^{-\Delta\Delta Ct}$ method on a CFX Connect Real-Time PCR system (Bio-Rad, CA, USA). The reaction conditions were as follows: 95°C for 5 min; 40 cycles of 95°C for 15 sec, 55°C for 20 sec and 72°C for 20 sec. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the normalization internal control. The primer sequences were as followed:

Gab1: Forward, 5′-CCTGTTGCTCATCAACTGTCAAAGC-3′;
Reverse, 5′-CTACA CTCGATGTCCCAGATGGG-3′) (Wickrema et al. 1999).

GAPDH: Forward, 5′-CATTGCCCTCAACGACCACTTTGT-3′
Reverse, 5′-TCTCTCTCTTCCTCTTGTGCTCTTGC-3′.

Immunohistochemistry

Gab1 expression in paraffin-embedded clinical samples was detected through immunohistochemistry. EOC tissues were sliced into 4 μm thick tissue sections and then mounted on the specific slide. The slides were deparaffinized and rehydrated through graded alcohol using standard procedures. Then the slides were incubated in 3% H_2O_2 at room temperature for 10 min to remove endogenous peroxidase. Subsequently, the retrieved Gab1 antigen was obtained with citrate buffer incubation (pH 6.0) under microwave. The slides were then incubated with anti-Gab1 antibody (dilution 1:200; ab59362 Abcam) at 4°C overnight. After sufficient incubation with the primary Gab1 antibodies, the slides were rinsed three times (5 min each) with PBS and incubated with the secondary antibody. After the DAB staining and the hematoxylin counter-staining, the samples were dehydrated with graded alcohol, soaked in xylene, and finally mounted. 5% FBS were used as negative control.

Assessment of Immunohistochemical Staining

Two independent pathologists that were blinded with respect to this study performed the immunoreactivity scoring for Gab1 expression (Fig. 1). The staining results were evaluated based on both the percentage of positive tumor cells and the staining intensity. The positive percentage was scored as 0 (no staining), 1 (1%-10% of the cells stained positive), 2 (11%-50% of the cells stained positive), 3 (51%-75% of cells stained positive), and 4 (76%-100% of cells stained positive). The signal intensity was defined as 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The final staining score was evaluated by multiplying the intensity score with the score for the positive percentage (range: 0-12). And the samples were rescored if the variance between the two pathologists was more than 3. The cut-off of the final score was set as the point with highest sensitivity plus specialist, calculated by receiver operating characteristics (ROC) curve analysis. This cut-off divided our cohort into high-expression group and low-expression group. High-expression group was the group with IHC score 0-2, while low-expression group was defined as score from 3-12.

Statistical Analysis

Statistical analysis was realized using SPSS 17.0 software (SPSS, USA). The correlation between Gab1 expression and clinical parameters was achieved with Spearman's correlation analysis. The Kaplan-Meier survival analysis was performed to determine the survival curves, and the differences were identified using the log-rank test. The Cox proportional hazards multivariate analysis was also performed to identify the independent prognostic factors on the OS of EOC patients. In all cases, P value less than 0.05 was considered as statistical significant.

Results

Characteristics of EOC Patients

The 124 EOC patients enrolled in our cohort aged from 35 to 76 years (median 53 years). Sixteen cases (12.9%) were diagnosed as histology mucinous carcinoma and others were all serous carcinoma. In our cohort, 39 (31.5%) and 85 (68.5%) were classified as grade G1 and G2/G3, respectively (Table 1). A total of 42 patients (33.9%) were in FIGO stage I/II, while 82 patients (66.1%) were in FIGO stage III/IV. In our study, a total of 74

Table 1. Clinicopathologic characteristcs of the EOC patients and their correlation with Gab1 expression.

Variables	Cases	Gab1 expression		P value[#]
	(n = 124)	Low (n = 80)	High (n = 44)	
Age (years)				0.579
≤ 55	69	46 (66.7%)	23 (33.3%)	
> 55	55	34 (61.8%)	21 (38.2%)	
FIGO stage				0.006*
I ~ II	42	34 (81.0%)	8 (19.0%)	
III ~ IV	82	46 (56.1%)	36 (43.9%)	
Histology type				0.352
Serous	108	68 (63.0%)	40 (37.0%)	
Mucinous	16	12 (75.0%)	4 (25.0%)	
Pathological grade				0.051
G1	39	30 (76.9%)	9 (23.1%)	
G2 ~ G3	85	50 (58.8%)	35 (41.2%)	
Lymph node metastasis				0.028*
No	50	38 (76.0%)	12 (24.0%)	
Yes	74	42 (56.8%)	32 (43.2%)	
Pre-operative CA-125				0.158
≤ 800	67	47 (70.1%)	20 (29.9%)	
> 800	57	33 (57.9%)	24 (42.1%)	

[#]P value was generated by comparing all subgroups and analyzed by Spearman rank correlation test.
*P < 0.05 was considered as statistically significant.
FIGO, International Federation of Gynecology and Obstetrics; Gab1, Grb2-associated binding protein 1.

patients (59.7%) had lymph node invasion and 50 patients (40.3%) had no lymph node invasion found in routine pathology.

Gab1 Expression and Clinicopathologic Parameters

To investigate the clinical significance of Gab1 in EOC, we detected the expression of Gab1 in ROC tissues and cells with IHC and real time RT-qPCR respectively. With RT-qPCR detailed described in Materials and Methods, Gab1 expression in the normal ovarian epithelial cells was demonstrated to be significantly lower than that in ovarian cancer cell lines, which were OVCAR3 and SKOV3 cells (Fig. 1A). In addition, we investigated Gab1 expression in EOC tissues with IHC. Gab1 expression was mainly observed in tumor cell cytoplasm (Fig. 1B, C), which is in accordance with its function for binding Grb2 and interact with downstream proteins. As described in Materials and Methods, our cohort was divided into high-expression group and low-expression group according to the cut-off of IHC score. In all the 124 EOC cases, 80 samples (64.5%) were defined as Gab1 low-expression while 44 (35.5%) cases were considered as Gab1 high-expression.

The correlation between Gab1 expression and the clinical characteristics was analyzed by Spearman rank correlation and exhibited in Table 2. Gab1 expression was significantly higher in the EOC tissues with lymphatic invasion than those with no lymphatic invasion (P < 0.001) (Table 2), indicating that Gab1 may participate in the invasion and metastasis of ovarian cancer. Moreover, patients in advanced stage had higher Gab1 expression by comparing patients in different FIGO stage, and the difference was statistically significant (P < 0.001). We found that Gab1 high expression was also closely correlated with advanced pathological grade (P = 0.026), while it showed no correlation with patients' age (P = 0.307), histological type (P = 0.077), or CA-125 level (P = 0.326).

Overall Survival Analysis of EOC Patients

The survival rate was evaluated to screen prognostic factors in our cohort by univariate analysis with Kaplan-Meier method. Survival information of 124 patients with EOC was obtained through hospital follow-up. The 5-year OS for the overall cohort was 49.33%, with the median survival time 60 months. As shown in Fig. 2, the univariate survival analysis with Kaplan-Meier method demonstrated that the advanced FIGO stage (P < 0.001), higher pathological grade (P = 0.026), positive lymph node invasion (P < 0.001), and Gab1 high-expression (P = 0.001) were all significantly associated with poorer OS of EOC patients, respectively. Patients with Gab1 low-expression had more favorable prognosis compared with those showed high Gab1 expression level (67.1 months vs. 48.9 months; P = 0.001) (Table 2). The 5-year OS rate of the Gab1 low-expression and high-expression group was 53.55% and

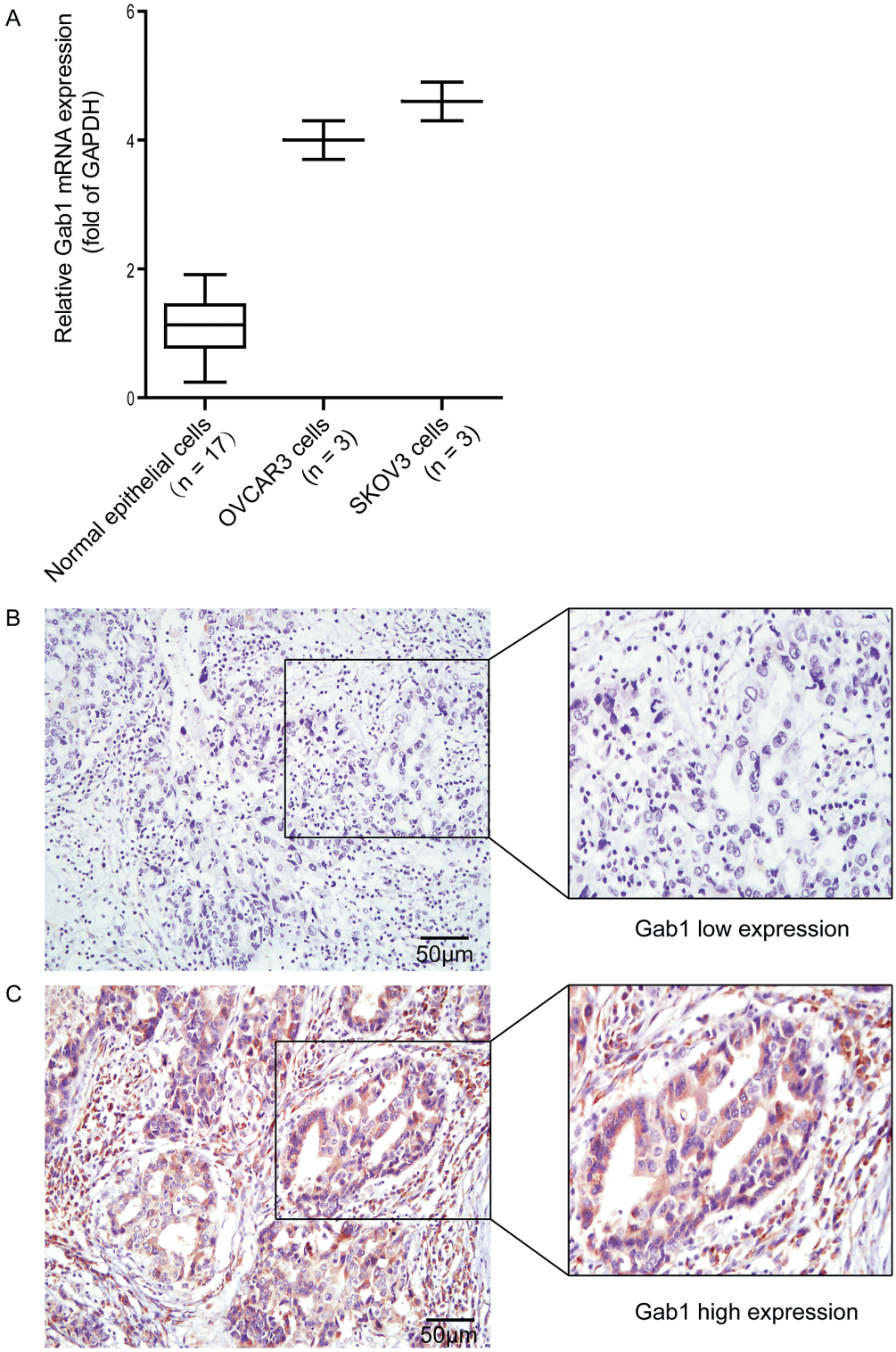

Fig. 1. Expression pattern of Gab1 in EOC cells and tissues.
(A) RT-qPCR assay showed high expression of Gab1 mRNA in EOC cells than that in normal epithelial ovarian cells. (B) Low Gab1 staining in serous EOC tissues. The score of staining intensity is 0 and the score of positive cell percentage is 0. Total score is 0 (calculated by score of staining intensity × the score of positive cell percentage) and defined as Gab1 low-expression. (C) Representative high Gab1 staining in serous EOC tissues. In this case, the score of staining intensity is 3 and the score of positive cell percentage is 3. So the total score is 9 and defined as Gab1 high-expression.

Table 2. Kaplan-Meier survival analysis (log-rank test) of EOC patients according to Gab1 expression.

Variables	Cases	Overall survival time (months)		P value
	(n = 124)	Mean	Median	
Age (years)				0.307
≤ 55	69	63.08	55	
> 55	55	60.11	62	
FIGO stage				< 0.001*
I ~ II	42	85.65	92	
III ~ IV	82	48.29	50	
Histology type				0.077
Serous	108	62.46	62	
Mucinous	16	46.97	52	
Pathological grade				0.026*
G1	39	67.78	-	
G2 ~G3	85	57.61	59	
Lymph node metastasis				< 0.001*
No	50	80.71	80	
Yes	74	46.16	49	
Preoperative CA-125				0.326
≤ 800	67	60.88	72	
> 800	57	58.93	59	
Gab1 expression				0.001*
Low expression	80	67.09	72	
High expression	44	48.94	48	

*statistically significant.
FIGO, International Federation of Gynecology and Obstetrics; Gab1, Grb2-associated binding protein 1.

46.07%, respectively. No other clinicopathologic factors were observed to be significantly correlated with prognosis in our study.

Gab1 as an Independent Prognostic Factor in EOC

Based on the results of univariate analysis, we further performed the multivariate analysis to identify the independent prognostic factor of EOC with Cox proportional hazards model. The FIGO stage, pathological grade, lymph node metastasis and Gab1 expression level were enrolled in Cox proportional hazards model. With multivariate analysis, Gab1 high-expression was identified as an independent prognostic factor for EOC (hazard ratio [HR] = 1.629, 95% confidence interval [CI] = 1.018-2.606, P = 0.042) (Table 3), which could predict poorer prognosis of EOC independently. In addition, advanced FIGO stages (HR = 2.946, 95% CI = 1.125-7.713, P = 0.028) and positive lymph node invasion (HR = 2.315, 95% CI = 1.025-5.226, P = 0.043) were also confirmed as independent prognostic factors of EOC. Results obtained from multivariate analysis suggests that advanced FIGO stage, positive lymph node invasion as well as Gab1 high-expression are all risk factors and can predict poorer prognosis of EOC (Table 3).

Discussion

EOC accounts for about 80%-90% of primary ovarian cancers and is the most lethal gynecological tumor (Siegel et al. 2014). The lack of specific symptoms and absence of reliable early diagnostic methods result in approximately 70% of patients are at an advanced stage when diagnosed (Lan et al. 2009). Despite the improvement of new therapeutic methods and surgery procedures, the 5-year survival rate of EOC patients remains unsatisfying (Anuradha et al. 2014; Colombo et al. 2014). Therefore, understanding the molecular mechanisms underlying the tumorigenesis and tumor progression and searching for novel biomarkers to predict the prognosis of EOC is of great value for providing new therapeutic targets and improving the survival rate of patients.

Gab1, which is mainly located in the cytoplasm, can be regulated by various growth factors such as VEGF, HGF, NGF, PDGF, EGF and other stimuli (Liu and Rohrschneider 2002; Gu and Neel 2003; Lemarie and Lehoux 2011), thereby propagating signal pathways which are essential for cell proliferation. Gab1 lacks enzymatic activity but it can be rapidly phosphorylated on its tyrosine residues, providing binding sites for various SH2 domain-containing proteins including phospholipase C (PLC), SHP2 and PI3K

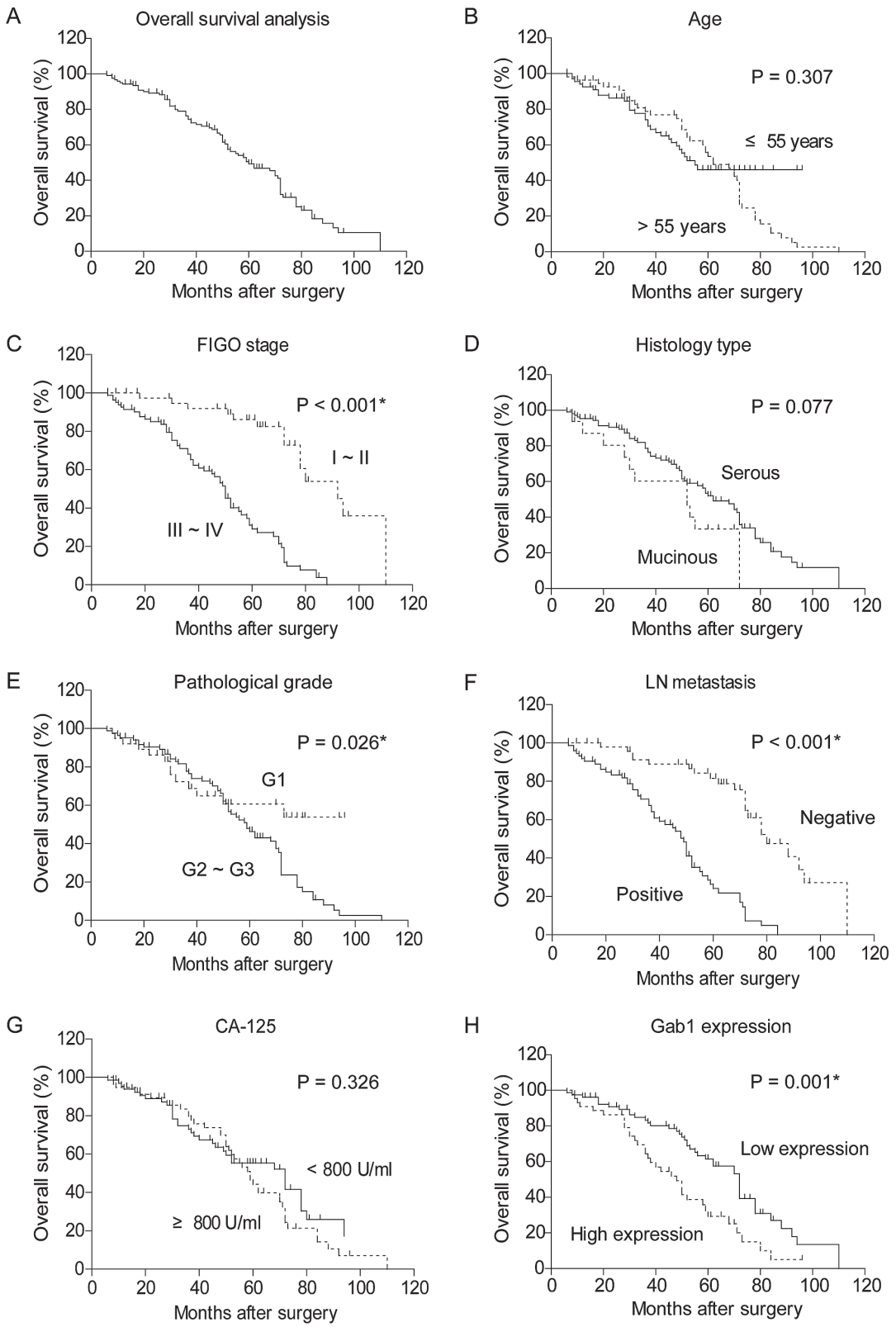

Fig. 2. Correlation between the clinicopathologic parameters and prognosis.
Overall survival curve of the EOC patients was analyzed (A). Kaplan-Meier survival analysis suggests that older age (B), advanced FIGO stage (C), higher pathological grade (E), positive lymph node (LN) metastasis (F) and high Gab1 expression level (H) were significantly associated with poorer prognosis, while the histology types (D) and the CA-125 level (G) showed no significant correlation with prognosis.

Table 3. Cox proportional hazards analysis of the prognostic factors for EOC.

Variables	Hazard ratio	95% Confidence Interval	P value[#]
FIGO stage	2.946	1.125-7.713	0.028*
Pathological grade	0.995	0.517-1.918	0.989
Lymph node metastasis	2.315	1.025-5.226	0.043*
Gab1 expression	1.629	1.018-2.606	0.042*

*statistically significant.
FIGO, International Federation of Gynecology and Obstetrics; Gab1, Grb2-associated binding protein 1.

regulatory subunit p85 (Lemarie and Lehoux 2011). Its association with SHP2 and the p85 regulatory subunit of PI3K are crucial for the activation of ERK1/2 and AKT, respectively (Sarmay et al. 2006; Wohrle et al. 2009). Recently, there are several reports indicating the essential role of Gab1 in regulating angiogenesis using the endothelial cell-specific Gab1 knockout (Gab1-ecKO) mice (Lu et al. 2011; Shioyama et al. 2011). Moreover, Gab1 was also considered to participant in the tumor angiogenesis. It has been reported that the capillary vascular density in tumors that implanted in Gab1-ecKO mice was significantly decreased compared with that in normal mice, as well as the tumor volume and tumor weight (Zhao et al. 2011), indicating that endothelial Gab1 deficiency could inhibit tumor angiogenesis and tumor growth. Collectively, Gab1 functions as a critical molecule that controls both HGF- and VEGF-mediated downstream signaling pathways and regulates cell proliferation, migration and survival.

The function of Gab1 has been partially described in the development of chondrosarcoma, mammary tumor and several digest system cancers. Our results for the first time reveal the correlation between Gab1 and EOC. We detected the expression level of Gab1 in 124 EOC samples, and the Gab1 high expression ratio was 35.5% (44/124). Through clinicopathological parameter analysis, we concluded that high Gab1 expression was significantly correlated with advanced FIGO stage and lymph node metastasis. Therefore, Gab1 may possibly function in tumor development and progression of EOC. In addition, Kaplan-Meier survival analysis and log-rank test results demonstrated that patients with higher Gab1 expression exhibited poorer OS. Further multivariate analysis also indicated that Gab1 expression could serve as an independent prognostic factor for the prognosis evaluation of EOC patients.

We also detected that higher Gab1 expression was significantly correlated with lymphatic invasion; so there is high possibility that Gab1 plays an indispensable role in mediating tumor metastasis. Since Gab1 was reported to activate SHP2 under the regulation of EGFR (Cunnick et al. 2001), it's likely that high Gab1 expression can increase the activity of SHP2 and PI3K, thus performing its function in cancer progression through the downstream AKT and ERK signaling pathways. The oncogenic role of Gab1 in EOC may also be regulated by the growth factors such as VEGF, EGF and HGF. To further illuminate the mechanisms of how Gab1 participates in the tumor invasion may need more fundamental results, such as microarray, co-immunoprecipitation and *in vivo* experiments, etc. This study mainly focused on the clinical significance of Gab1 as a prognostic biomarker in EOC and might be helpful in the novel drug development of the disease.

In conclusion, high Gab1 expression in EOC patients is closely associated with tumor progression and poor prognosis.

Conflict of Interest

The authors declare no conflict of interest.

References

Anuradha, S., Webb, P.M., Blomfield, P., Brand, A.H., Friedlander, M., Leung, Y., Obermair, A., Oehler, M.K., Quinn, M., Steer, C. & Jordan, S.J. (2014) Survival of Australian women with invasive epithelial ovarian cancer: a population-based study. *Med. J. Aust.*, **201**, 283-288.

Colombo, P.E., Fabbro, M., Theillet, C., Bibeau, F., Rouanet, P. & Ray-Coquard, I. (2014) Sensitivity and resistance to treatment in the primary management of epithelial ovarian cancer. *Crit. Rev. Oncol. Hematol.*, **89**, 207-216.

Cunnick, J.M., Mei, L., Doupnik, C.A. & Wu, J. (2001) Phosphotyrosines 627 and 659 of Gab1 constitute a bisphosphoryl tyrosine-based activation motif (BTAM) conferring binding and activation of SHP2. *J. Biol. Chem.*, **276**, 24380-24387.

Fan, Y., Yang, F., Cao, X., Chen, C., Zhang, X., Zhang, X., Lin, W., Wang, X. & Liang, C. (2016) Gab1 regulates SDF-1-induced progression via inhibition of apoptosis pathway induced by PI3K/AKT/Bcl-2/BAX pathway in human chondrosarcoma. *Tumour Biol.*, **37**, 1141-1149.

Felici, A., Giubellino, A. & Bottaro, D.P. (2010) Gab1 mediates hepatocyte growth factor-stimulated mitogenicity and morphogenesis in multipotent myeloid cells. *J. Cell. Biochem.*, **111**, 310-321.

Ferlay, J., Shin, H.R., Bray, F., Forman, D., Mathers, C. & Parkin, D.M. (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int. J. Cancer*, **127**, 2893-2917.

Gillgrass, A., Cardiff, R.D., Sharan, N., Kannan, S. & Muller, W.J. (2003) Epidermal growth factor receptor-dependent activation of Gab1 is involved in ErbB-2-mediated mammary tumor progression. *Oncogene*, **22**, 9151-9155.

Gu, H. & Neel, B.G. (2003) The "Gab" in signal transduction. *Trends Cell Biol.*, **13**, 122-130.

Heintz, A.P.M., Odicino, F., Maisonneuve, P., Quinn, M.A., Benedet, J.L., Creasman, W.T., Ngan, H.Y., Pecorelli, S. & Beller, U. (2006) Carcinoma of the ovary. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. *Int. J. Gynaecol. Obstet.*, **95 Suppl 1**, S161-S192.

Jemal, A., Siegel, R., Ward, E., Murray, T., Xu, J. & Thun, M.J. (2007) Cancer statistics, 2007. *CA Cancer J. Clin.*, **57**, 43-66.

Kruk, P.A., Maines-Bandiera, S.L. & Auersperg, N. (1990) A simplified method to culture human ovarian surface epithelium. *Lab. Invest*., **63**, 132-136.

Lan, C., Li, Y. & Liu, J. (2009) Intraperitoneal access via direct puncture is an alternative way to deliver intraperitoneal chemotherapy in ovarian, fallopian tube and primary peritoneal cancer. *Gynecol. Oncol.*, **114**, 42-47.

Lemarie, C.A. & Lehoux, S. (2011) The gift of Gab1 (Grb-2-associated binder 1). *Arterioscler. Thromb. Vasc. Biol.*, **31**, 956-957.

Liu, Y. & Rohrschneider, L.R. (2002) The gift of Gab. *FEBS Lett.*, **515**, 1-7.

Lu, Y., Xiong, Y., Huo, Y., Han, J., Yang, X., Zhang, R., Zhu, D.S., Klein-Hessling, S., Li, J., Zhang, X., Han, X., Li, Y., Shen, B., He, Y., Shibuya, M., Feng, G.S. & Luo, J. (2011) Grb-2-associated binder 1 (Gab1) regulates postnatal ischemic and VEGF-induced angiogenesis through the protein kinase A-endothelial NOS pathway. *Proc. Natl. Acad. Sci. USA*, **108**, 2957-2962.

Lynch, H.T., Casey, M.J., Lynch, J., White, T.E. & Godwin, A.K. (1998) Genetics and ovarian carcinoma. *Semin. Oncol.*, **25**, 265-280.

Nishida, K. & Hirano, T. (2003) The role of Gab family scaffolding adapter proteins in the signal transduction of cytokine and growth factor receptors. *Cancer Sci.*, **94**, 1029-1033.

Oka, M., Kikkawa, U. & Nishigori, C. (2008) Protein kinase C-betaII represses hepatocyte growth factor-induced invasion by preventing the association of adapter protein Gab1 and phosphatidylinositol 3-kinase in melanoma cells. *J. Invest. Dermatol.*, **128**, 188-195.

Sang, H., Li, T., Li, H. & Liu, J. (2013) Down-regulation of Gab1 inhibits cell proliferation and migration in hilar cholangiocarcinoma. *PLoS One*, **8**, e81347.

Sang, H., Li, T., Li, H. & Liu, J. (2015) Gab1 regulates proliferation and migration through the PI3K/Akt signaling pathway in intrahepatic cholangiocarcinoma. *Tumour Biol*., **36**, 8367-8377

Sarmay, G., Angyal, A., Kertesz, A., Maus, M. & Medgyesi, D. (2006) The multiple function of Grb2 associated binder (Gab) adaptor/scaffolding protein in immune cell signaling. *Immunol. Lett.*, **104**, 76-82.

Seiden-Long, I., Navab, R., Shih, W., Li, M., Chow, J., Zhu, C.Q., Radulovich, N., Saucier, C. & Tsao, M.S. (2008) Gab1 but not Grb2 mediates tumor progression in Met overexpressing colorectal cancer cells. *Carcinogenesis*, **29**, 647-655.

Shioyama, W., Nakaoka, Y., Higuchi, K., Minami, T., Taniyama, Y., Nishida, K., Kidoya, H., Sonobe, T., Naito, H., Arita, Y., Hashimoto, T., Kuroda, T., Fujio, Y., Shirai, M., Takakura, N., et al. (2011) Docking protein Gab1 is an essential component of postnatal angiogenesis after ischemia via HGF/c-met signaling. *Circ. Res.*, **108**, 664-675.

Siegel, R., Ma, J., Zou, Z. & Jemal, A. (2014) Cancer statistics, 2014. *CA Cancer J. Clin.*, **64**, 9-29.

Wickrema, A., Uddin, S., Sharma, A., Chen, F., Alsayed, Y., Ahmad, S., Sawyer, S.T., Krystal, G., Yi, T., Nishada, K., Hibi, M., Hirano, T. & Platanias, L.C. (1999) Engagement of Gab1 and Gab2 in erythropoietin signaling. *J. Biol. Chem*., **274**, 24469-24474.

Wingo, P.A., Ries, L.A., Rosenberg, H.M., Miller, D.S. & Edwards, B.K. (1998) Cancer incidence and mortality, 1973-1995: a report card for the U.S. *Cancer*, **82**, 1197-1207.

Wohrle, F.U., Daly, R.J. & Brummer, T. (2009) Function, regulation and pathological roles of the Gab/DOS docking proteins. *Cell Commun. Signal.*, **7**, 22.

Zhao, J., Wang, W., Ha, C.H., Kim, J.Y., Wong, C., Redmond, E.M., Hamik, A., Jain, M.K., Feng, G.S. & Jin, Z.G. (2011) Endothelial Grb2-associated binder 1 is crucial for postnatal angiogenesis. *Arterioscler. Thromb. Vasc. Biol.*, **31**, 1016-1023.