Pathology - Research and Practice 215 (2019) 121–126

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Loss of BMP-10 is correlated with poor survival in ovarian cancer

Yunfeng Jin[a,1], Wenjie Zheng[b,1], Li Li[a], Guoqin Huang[c], Ya Liu[d], Haiyan Jiang[a], Yuexiang Zhang[c,*], Chunhui Tang[a,*]

[a] Department of Obstetrics and Gynecology, Affiliated Hospital of Nantong University, Nantong, Jiangsu 226001, China
[b] Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, Nantong, Jiangsu 226001, China
[c] Department of Obstetrics and Gynecology, Affiliated Maternal and Child Health Care Hospital of Nantong University, Nantong, Jiangsu 226018, China
[d] Department of Obstetrics and Gynecology, Affiliated Haian People's Hospital of Nantong University, Nantong, Jiangsu 226600, China

ARTICLE INFO

Keywords:
BMP-10
Ovarian cancer
Prognosis
Metastasis
Proliferation

ABSTRACT

Introduction: The expression of bone morphogenetic protein-10 (BMP-10) is downregulated in some cancer types, but its function and mechanism in ovarian cancer remains unclear.

Materials and methods: BMP-10 expression was detected in ovarian cancer tissues and cell lines by using immunochemistry and western blotting. Prognostic value of BMP-10 was evaluated by Kaplan-Meier curve and Cox regression model. Knockdown or overexpression of BMP-10 was conducted by using specific siRNA or pcDNA-BMP-10 in ovarian cancer cell lines. The biological features induced by BMP-10 were observed by MTT assay, wound-healing and transwell assays.

Results: BMP-10 expression in ovarian cancer tissues was significantly lower than that in ovarian tissues. Low BMP-10 expression in ovarian cancer tissues was related to advance FIGO stage, higher histologic grade, lymph node metastasis, and peritoneal fluid. Kaplan-Meier analysis revealed that low BMP-10 expression was significantly associated with poor prognosis of patients with ovarian cancer. BMP-10 overexpression or knockdown significantly inhibited or promoted proliferation, migration, and invasion of ovarian cancer cells, respectively. Moreover, administration of neutralizing antibody or human recombinant BMP-10 would reverse these effects on ovarian cancer cells.

Conclusion: Low BMP-10 expression was associated with poor prognosis and progression of ovarian cancer.

1. Introduction

Epithelial ovarian cancer is the second most common gynecologic malignancy and the fifth leading cause of cancer death in female, with more than 15,000 women dying of this disease annually worldwide [12]. Women are often diagnosed as ovarian cancer at advanced stage, which might lead to the high mortality rate. It is reported that ovarian tumor cells can shed the peritoneal cavity to the layer of mesothelial cells and then invade the inner surface of the peritoneum [16]. Following invading the superficial layers of abdominal organs, cancer cells may subsequently spread to retroperitoneal lymph nodes and the pleural cavity. Despite the initial effects of debulking surgery followed by chemotherapy, the prognosis of ovarian cancer is still poor due to its metastasis, chemoresistance, and recurrence [17]. Thus, finding novel prognosis marker and targeting molecular may benefit for the ovarian cancer therapy [4].

It has been reported that, several growth factors including vascular endothelial growth factor (VEGF) family, epidermal growth factor receptor (EGFR), placental growth factor-2 (PGF-2), play crucial roles in progression of ovarian cancer. Bone morphogenetic proteins (BMPs) are a group of growth factors also known as cytokines and metabolites [2]. BMPs were firstly defined as pivotal morphogenetic signals in charge of formation of bone and cartilage [1]. Recently. increasing studies indicated that the BMPs were involved in cancer development, including esophageal squamous cancer, breast cancer and colorectal cancer [8,9,21]. In ovarian cancer, the BMP2, BMP4, and BMP6 were upregulated and involved in cell proliferation and invasion via activation of PI3K-Akt and Smads signal pathway [10,11,15]. However, BMP-10 was also associated with tumor progression, which might play roles opposite to other members of BMP family [13,18,22]. BMP-10 were decreased in gastric and bladder cancer and functioned as a tumor suppressor via inhibiting cell proliferation and migration. Nevertheless, its expression characteristics and roles in ovarian cancer remain unknown.

* Corresponding authors.
E-mail addresses: zhang_yx@163.com (Y. Zhang), ntfytch@163.com (C. Tang).
[1] Common first.

https://doi.org/10.1016/j.prp.2018.10.025
Received 24 August 2018; Received in revised form 7 October 2018; Accepted 20 October 2018

In this study, we investigated the expression of BMP-10 in ovarian cancer tissues. Besides, we also discovered its associations with clinical and pathologic factors, as well as the prognostic implications. Further, we detected the effects of knockdown of BMP-10 on biological behaviors. All these studies will provide new sight for ovarian cancer therapy.

2. Materials and methods

2.1. Samples

All investigations in this study were approved by Ethic Committee at Affiliated Hospital of Nantong University. Informed consent was obtained from each patient. Ten normal human ovarian tissue samples were obtained from patients who underwent gonadectomy. Sixty ovarian cancer tissues were obtained from the pathology files of the Department of Pathology at Affiliated Hospital of Nantong University from 2002 to 2005 under the auspices of an institutional review board approved human subjects study protocol. All tumors were from patients newly diagnosed with ovarian cancer, who had received no therapy before sample collection but received postoperative platinum-based chemotherapy. The follow-up time was 5 years for 56 patients ranging from 1 to 60 months. Besides, 8 pairs of fresh ovarian cancer tissues and adjacent tissues were obtained for western blotting.

2.2. Immunohistochemistry

Formalin-fixed, paraffin-embedded sections were deparaffinized using a graded ethanol. Then 0.3% hydrogen peroxide was used to block endogenous peroxidase activity. The sections were treated with 10 mM citrate buffer (pH 6.0) and heated to retrieve the antigen at 121 °C. Following rinsed in PBST, sections were blocked for 2 h and then incubated overnight at 4 °C with anti-human BMP-10 polyclonal antibody (diluted 1:100; Santa Cruz Biotechnology, Santa Cruz, CA) and anti-Ki67 monoclonal antibody (diluted 1:100; Santa Cruz Biotechnology). Negative control slides were also included using a nonspecific immunoglobulin IgG (diluted 1:100; Santa Cruz Biotechnology). All slides were processed using the anti-peroxidase method (Dako, Hamburg, Germany). After rinsing in water, the sections were counterstained with hematoxylin, dehydrated, and cover slipped. Stained sections were observed under a microscope. All of the scores of staining were blind evaluated by pathologists. For assessment of BMP-10 and Ki67, 5 fields in each specimen were selected randomly, and examined under high-power magnification. More than 500 cells were counted to determine the mean percent, which represented the percentage of positive cells.

2.3. Cell culture

The human ovarian cancer cell line SKOV3 and OVCA3 were obtained from the American Type Culture Collection (ATCC, USA). Omc-3 and Nose007 were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China). Cells were cultured in RMPI-1640 or DMEM (Gibco, USA) with 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin and streptomycin (Sigma, USA) at 37 °C and 5% CO2 in a humidified atmosphere.

2.4. Transfection

cDNA fragments encoding BMP-10 were amplified by polymerase chain reaction (PCR) and purified by gel extraction kit (QIAGEN, USA). Then production was connected with pcDNA vector by T4 DNA ligase. Following transformation and screening, plasmid was extracted by a plasmid extraction kit (QIAGEN, USA) and confirmed by sequencing analysis. Then pcDNA-BMP-10 plasmids and blank vector were transfected to ovarian cancer cells by using Lipofectamine Plus (Invitrogen, USA) according to the manufacturer's instruction. The primers for pcDNA-BMP-10 plasmid were as follows: sense:5′- CCGGAATTCGCAG GCAGTTCCACCGAGCAG -3′; antisense: 5′- CCGCTCGA GGCGTCGCTA TGGCTGCACACC -3′.

For gene knockdown, siBMP-10 and control were purchased from Santa Cruz (USA). Transfection with Lipo2000 (Invitrogen, USA) was performed according to the manufacturer's instructions. Sequences for siRNA and negative control were as follows: Sense, 5′-GATCCGGAGA TGTCCATGTCCAA; antisense, 5′-TTGAACTTGGAC ATGGACATCT CCG-3′; negative control: sense, 5′-UUCUCCGATCGUCG CACGUTT-3′; antisense, 5′ACGUGATACGUACGGAGAATT-3′

2.5. Wound-healing assay

Cell motility was assessed by wound-healing assay. Cells of each group were plated in 6-well plates and grown to 90% confluence. Cells were pretreated with Mitomycin C (Sigma, USA) to avoid the interference of cell proliferation. Each well was scratched with 10 μL pipette tips, washed with PBS, and incubated with serum-free medium. The wound-healing was observed under a microscope (Leica, USA) at 0 h and 24 h.

2.6. Transwell assay

Cell invasion was assessed by 8-μm Transwell chambers (Corning, USA). 5×10^4 cells in 200 μL of serum-free medium were added into the upper chambers pre-coated by Matrigel (BD, USA), and 600 μL of culture medium containing 20% FBS was added into the lower chambers. Following incubated for 24 h, chambers were fixed in ethanol and stained with 0.1% crystal violet. Then, each sample were observed and counted at 5 random views under microscope (Leica, USA).

2.7. Cell proliferation assays

Cell proliferation was detected by MTT assay. The transfected cells or control group were seeded in 96-well plates at the density of 5×10^3 cells/well. Then, a total of 20 μL MTT solution was added to each well at different time point. Following incubation of 2 h, 150 μL DMSO was added to dissolve the formazan crystals. At last, plates were detected at wavelength of 570 nm by using a microplate reader (BioTek Instruments, USA).

2.8. Western blotting

Proteins from tissues or cells were extracted with RIPA buffer. Following quantification by BCA kit (Thermo, USA), samples were separated by SDS-PAGE for 1.5 h and transferred onto PVDF membrane for 2 h. Then, the membranes were blocked and incubated with primary antibody of BMP-10 and GAPDH (1: 1000, (BMP-10, Santa Cruz Biotechnology, USA) at 4 °C overnight. After washed by PBST, the membranes were incubated with secondary antibodies (1:1000, Thermo, USA) at room temperature for 2 h. Following washed three time with PBST, samples were visualized by enhanced chemiluminescence solution (Merck Millipore, Germany).

2.9. Statistical analysis

Association between BMP-10 and clinicopathological variables were evaluated using the Mantel-Haenzel W test or Fisher exact test. Survival estimates were computed using the Kaplan-Meier method and multivariable cox regression assay. Comparisons between groups were analyzed using the log-rank test. P value less than 0.05 was considered statistically significant.

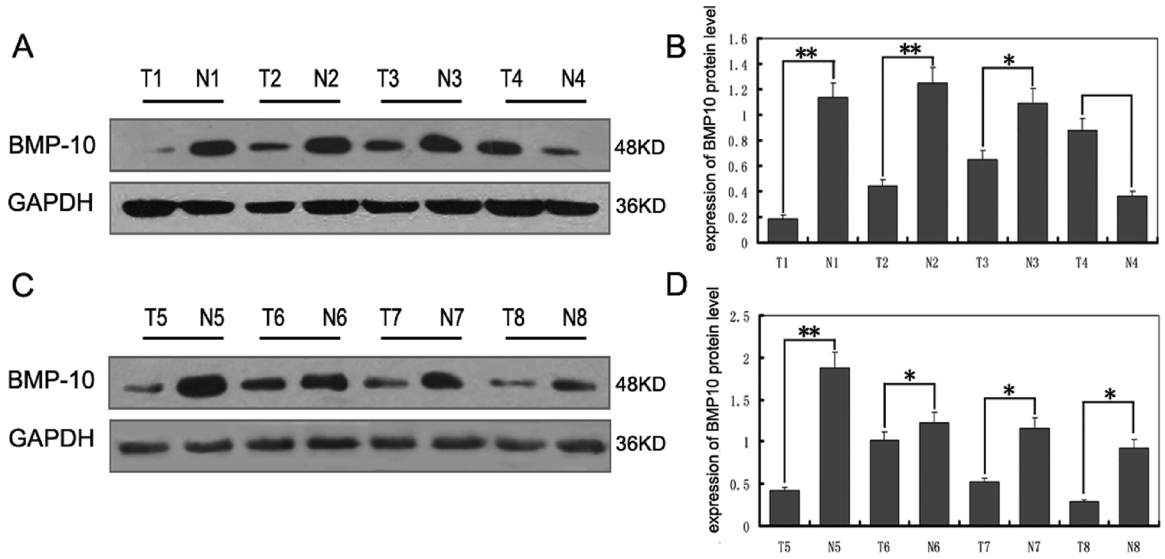

Fig. 1. BMP-10 expression in ovarian cancer tissues and adjacent tissues.
Western blotting was conducted to detect BMP-10 expression in ovarian cancer tissues and self-matched tissues. **(A, C)** BMP-10 expression was determined in 8 pairs of ovarian cancer tissues and adjacent tissues using western blotting. **(B, D)** Semi-quantification of intensity in A and C. **BMP-10**, bone morphogenetic protein-10. *, $P < 0.05$; **, $P < 0.01$.

3. Results

3.1. Expression of BMP-10 in ovarian cancer tissues and adjacent tissues

BMP-10 expression in 8 pairs of fresh cancerous tissues and adjacent tissues is shown in Fig. 1. Protein was extracted from fresh tissues and prepared for protein quantification. Then, results of western blotting showed that, in most pairs of samples, the BMP-10 expression was lower in cancerous tissues in contrast to the adjacent tissues (Fig. 1A & B). However, in T4 and N4 case, higher BMP-10 expression was observed in tumor tissue rather than normal tissue, which could be estimated as an individual difference. Furthermore, semi-quantification indicated that BMP-10 protein expression was significantly lower than that in adjacent tissues (Fig.1C &D, $P < 0.01$).

3.2. BMP-10 expression in ovarian tissues with different histological grade

The BMP-10 expression in ovarian tissues with different grades is demonstrated in Fig. 2. Western blotting results showed that BMP-10 was overexpressed in normal ovarian tissues, while lower BMP-10 expression was observed in ovarian cancer tissues, especially in tissues of advanced grade (Fig. 2A). Consistently, immunochemical results of 60 ovarian cancer tissues also indicated that advanced grade tissues had stronger staining intensity than that of low grade tissues. However, Ki-67 had an opposite expression characteristic with BMP-10 (Fig. 2B). The mean percentage of Ki67- positive tumor cells was 25.12% (ranged from 0.85% to 84.38%) in ovarian cancer tissues, while mean percentage of BMP-10 was 4.38% (ranged from 2% to 17%). As shown in Fig. 2C, a negative correlation between BMP-10 and Ki67 expression was found based on proliferative activity ($r^2 = -0.383$, $P < 0.01$).

3.3. Correlation between BMP-10 Expression and clinicopathological parameters in ovarian cancer

The association between BMP-10 expression and clinicopathological features is summarized in Table 1. For statistical analysis, the expression of BMP-10 in the carcinoma specimens were divided into 2 groups, namely, high and low expressions, according to the percentage of BMP-10-positive cells, using a cutoff level of 30% representing the mean value of BMP-10 expression. BMP-10 expression was significantly correlated with International Federation of Gynecology and Obstetrics (FIGO) stage ($P = 0.08$) and histologic grade ($P = 0.018$), lymph node

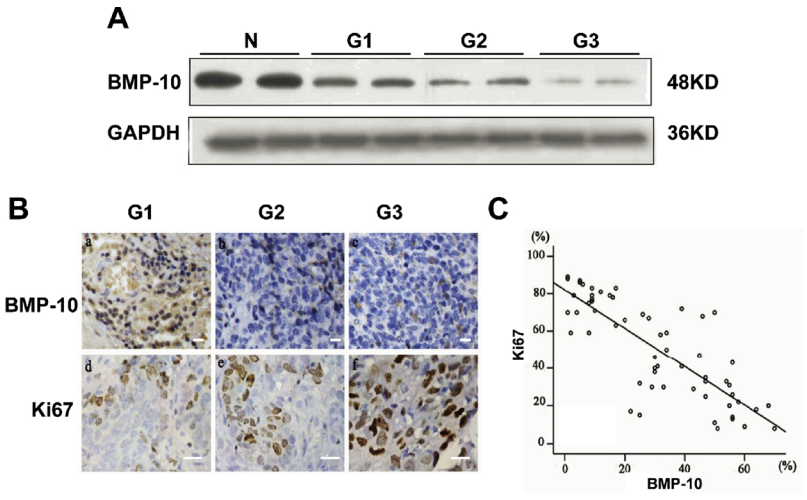

Fig. 2. BMP-10 expression in ovarian cancer with different histological grade.
(A) Expression of BMP-10 was detected in ovarian cancer tissues with different histological grade and adjacent tissues. **(B)** Paraffin-embedded tissue sections were stained with antibodies of BMP-10 and Ki67 and counterstained with hematoxylin (×400), both of BMP-10 and Ki-67 were distributed in nucleus. **(C)** Scatterplot of Ki67 versus BMP-10 with regression line showing a correlation of them using the Spearman correlation coefficient. Scale bar, 100 μM. **BMP-10**, bone morphogenetic protein-10; **G1**, Histologic grade 1; **G2**, Histologic grade 2; **G3**, Histologic grade 3.

Table 1
Correlation of BMP-10 expression with clinical parameters in ovarian cancer.

Parameters	n	BMP-10 expression		P
		–	+	
Age				0.921
≤55	27	14	13	
> 55	33	17	16	
FIGO stage				0.018*
I	15	2	13	
II	6	3	3	
III	31	20	11	
IV	8	6	2	
Histological subtype				0.213
Serous adenocarcinoma	42	20	22	
Endometrioid adenocarcinoma	7	3	4	
Clear cell carcinoma	3	2	1	
Mucinous adenocarcinoma	8	4	4	
Histological grade (Silverberg)				0.008*
G1	20	4	16	
G2	16	4	12	
G3	24	20	4	
Residual tumor size				0.675
≤1 cm	45	25	20	
>1 cm	15	5	10	
Lymph node metastasis				0.004*
Yes	22	18	4	
No	38	10	28	
Peritoneal fluid				0.032*
Yes	24	19	5	
No	36	10	26	
Cancer cells in ascites				0.122
Yes	31	18	13	
No	29	11	18	

BMP-10, bone morphogenetic protein-10; **FIGO**, International Federation of Gynecology and Obstetrics.
* $P < 0.05$.

metastasis ($P = 0.004$) and peritoneal fluid ($P = 0.032$). However, no significant correlation was found with age, histologic subtype, residual tumor size, and tumor cells in the peritoneal fluid.

3.4. BMP-10 expression as a prognostic marker in ovarian cancer

The overall survival curve according to BMP-10 expression is shown in Fig. 3. The ovarian cancer patients with low BMP-10 expression had significant shorter overall survival than patients with high BMP-10 expression ($P < 0.01$). Furthermore, multivariate Cox regression analysis indicated that BMP-10 expression (HR:4.834; 95%CI: 1.97–9.757; $P = 0.002$) was an independent prognostic factor in ovarian cancer, along with factors like FIGO stage, the absence of peritoneal fluid, and lymph node metastasis (Table 2).

3.5. BMP-10 negatively correlated with proliferation of ovarian cancer cells

To examine the role of BMP-10 in ovarian cancer, in vitro experiment was further conducted (Fig. 4). BMP-10 expression varied among different ovarian cancer cell lines, with higher expression in Nose007 and Skvo3 and lower expression in Ovca-3 and Omc-3 (Fig. 4A). Thus, knockdown or overexpression of BMP-10 was performed to discover its effects on biological behaviors of ovarian cancer cells (Fig. 4B&C). Results of MTT demonstrated that overexpression of BMP-10 obviously inhibited the proliferation of Ovca3 cells. However, neutralizing antibody abrogated the proliferation repression induced by BMP-10 upregulation (Fig. 4D). Consistently, BMP-10 silencing could significantly promote the proliferation of Skvo3 cells, while continuous administration of rhBMP-10 inhibit cell proliferation (Fig. 4E). These results indicated that BMP-10 might negatively regulate the proliferation of ovarian cancer cells.

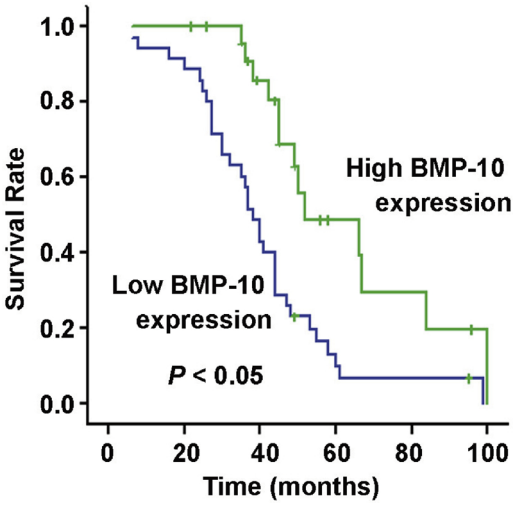

Fig. 3. Kaplan-Meier survival curves.
Kaplan-Meier method was used to evaluate the prognostic value of BMP-10 in 56 patients with ovarian cancer. Overall survival curve showed that patients with higher BMP-10 expression had a longer overall survival. **BMP-10**, bone morphogenetic protein-10.

Table 2
Multivariate analysis in ovarian cancer patients.

Factors	HR	95%CI	P
Age	0.934	0.492–1.772	0.833
FIGO stage	3.211	1.967–4.324	0.021*
Histological classification	0.976	0.644–1.408	0.911
Histological grade	2.129	1.206–3.761	0.009*
Residual tumor size	0.873	0.395–1.936	0.739
Lymph node metastasis	0.413	0.195–0.875	0.021*
Peritoneal fluid	2.871	1.231–3.421	0.020*
Cancer cells in ascites	0.736	0.342–1.231	0.211
BMP-10	4.834	1.97–9.757	0.002*

HR, hazard ratio; **CI**, confidence interval; **BMP-10**, bone morphogenetic protein-10; **FIGO,** International Federation of Gynecology and Obstetrics.
* $P < 0.05$.

3.6. BMP-10 downregulated ability of migration and invasion of ovarian cancer cells

The effects of BMP-10 on migration and invasion are presented in Fig. 5. Each groups of cells were pre-treated (transfection, neutralizing antibody or rhBMP-10 administration) according to the study design, respectively. Compared with blank and normal group, BMP-10 overexpression significantly downregulated the ability of wound-healing and invasion in Ovca3 cells, but neutralizing antibody could reverse the repression effects (Fig. 5A&C). Similarly, Knockdown of BMP-10 could promote migration and invasion of Skvo3 cells, which could be abolished by rhBMP-10 administration (Fig. 5B&D). It suggested that BMP-10 could inhibit the migration and invasion of ovarian cancer cells.

4. Discussion

Ovarian cancer is a lethal gynaecological malignancy with different genetical, biochemical and morphological types. About 70% of patients worldwide, diagnosed with advanced-stage ovarian cancer, suffer a poor survival due to recurrence, metastasis and chemoresistance [6]. Given the poor overall survival in advanced ovarian cancer, it is of great significance to find therapeutically relevant targets to improve patient prognosis.

BMP family has recently emerged as a group of cancer related proteins. It is reported that BMP-2 [9], BMP-4 [5] and BMP-9 [7] could

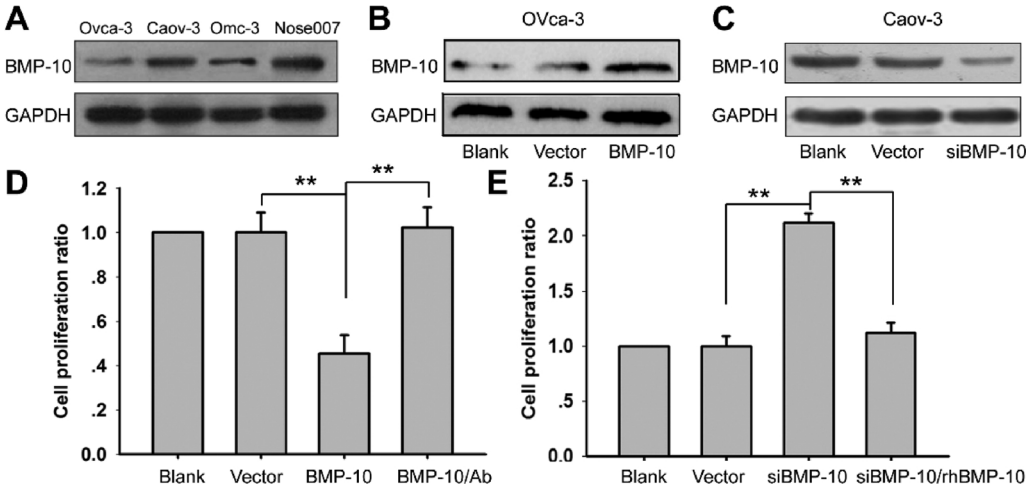

Fig. 4. BMP-10 inhibited the proliferation of ovarian cancer cells.
(A) BMP-10 expression in ovarian cancer cell lines was detected by western blotting. **(B)** BMP-10 was overexpressed in Ovca3 cells by using pcDNA-BMP-10 plasmid. **(C)** siRNA was transfected into Skvo3 cells to knock down BMP-10. **(D)** Effects of overexpression of BMP-10 on proliferation of Ovca3 cells. **(E)** Effects of BMP-10 silencing and rhBMP-10 administration on proliferation of Skvo3 cells. **BMP-10**, bone morphogenetic protein-10; **rhBMP-10**, recombinant human BMP-10; **ab,** neutralizing antibody. *, $P < 0.05$; **, $P < 0.01$.

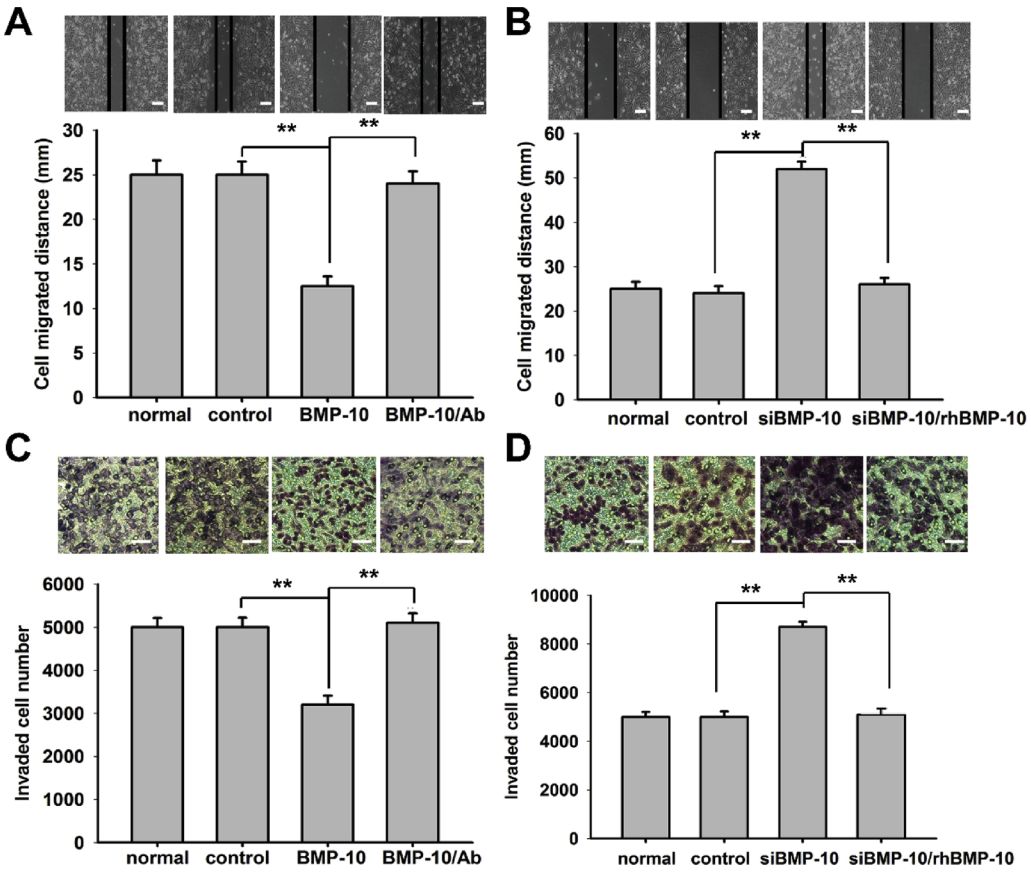

Fig. 5. BMP-10 repressed ability of migration and invasion of ovarian cancer cells.
(A, B) Wound-healing assay was conducted to find the effects induced by BMP-10 overexpression or inhibition (post-treatment of neutralizing antibody or rhBMP-10). **(C, D)** After knockdown or overexpression of BMP-10 in Ovca3 cells and Skvo3 cells, transwell assay was performed to discover the ability of invasion of these groups of cells. **BMP-10**, bone morphogenetic protein-10; **rhBMP-10**, recombinant human BMP-10; **ab,** neutralizing antibody. Scale bar, 100 μM. *, $P < 0.05$; **, $P < 0.01$.

promote progression in lung cancer, liver cancer, and breast cancer. Interestingly, it is different when focusing on BMP-10. There was evidence that BMP-10 was down-regulated in gastric cancer, urothelial cancer, and breast cancer compared to normal tissue. For in vitro performance, BMP-10 could inhibit the cell growth, migration and metastasis of cancer cells by targeting tumor related pathways, indicating a possibly inhibitory role in tumor progression [13,19,22]. However, the expression features and roles in ovarian cancer remained to be elucidated.

In our study, BMP-10 expression was discovered in a cohort of ovarian cancer patients for the first time. Apart from an individual case, BMP-10 expression in adjacent benign tissues was obviously higher than that in ovarian cancer tissues in most pairs of tissues. Besides, advanced ovarian cancer tissues had lower expression of BMP-10, which was consistently confirmed by western blotting and immunochemistry. Notably, a significantly negative correlation was found between BMP-10 and proliferation marker Ki67, suggesting that BMP-10 might act as a tumor suppressor in ovarian cancer. From a clinical perspective, lower BMP-10 expression was correlated with advanced FIGO stage, higher histological grade, lymph node metastasis, but not with histological subtype. It might be attributed to the limited sample size.

As we know, the well-recognized prognostic factors for survival are tumor type, tumor grade, FIGO stage, and distant metastases in ovarian cancer. Therefore, it is of importance to develop reliable biomarkers for the surveillance of the progression and prognosis of ovarian cancer. BMP-10 has been recommended as a candidate prognostic marker for patients with breast cancer, and low BMP-10 expression might lead to

shorter survival of breast cancer [19]. Consistently, we observed that a high BMP-10 expression conferred a better overall survival in patients with ovarian cancer. And it was recommended as an independent factor for prognosis of ovarian cancer patients, along with factors like metastasis, peritoneal fluid, FIGO stage and histological grade. These evidences indicated that BMP-10 expression might be an underlying biomarker in predicting the progression and prognosis of ovarian cancer.

It is well-known that uncontrolled growth and metastasis are important features of malignancies, contributing to main causes of cancer-related death [3]. Thus, to discover the effects of BMP-10 on ovarian cancer, we conducted loss- or gain- of functions experiments in vitro. Results showed that specific siRNA could significantly promote the proliferation, migration and invasion in Skvo3 cells, while overexpression of BMP-10 repressed these malignant behaviors in Ovca3 cells. However, rhBMP-10 or neutralizing antibody abrogated the effects induced by knockdown or overexpression of BMP-10, indicating that BMP-10 might participate in the aggression of ovarian cancer. Previous studies reported that BMP-10 induced apoptosis in prostate cancer cells through a Smad independent pathway by activating BMP receptors XIAP (ILP) and ERK1/2 [20]. In addition, BMP-10 could down-regulated beta-catenin/TCF signaling by up-regulating Axin protein level. Although the possible mechanism was not clarified in this study, it is supposed that BMP could negatively regulate some tumor-related pathways, and subsequently inhibit proliferation, migration and invasion of ovarian cancer cells.

Despite the initially high response rate to standard front-line debulking surgery followed by platinum-based chemotherapy, high ratio of patients will recur within 6 months of completing platinum-based treatment [14]. Understanding the molecular mechanisms contributes to improving treatment against ovarian cancer. To our knowledge, this is the first report to describe expression and roles of BMP-10 expression in ovarian cancer. Loss of BMP-10 expression might represent a poor prognosis in ovarian cancer patients. Meanwhile, loss of BMP-10 could promote malignant behaviors in ovarian cell lines, suggesting it might be a promising tumor suppressor. However, there were some limitations in this study, including small size of samples and lack of mechanism investigation, which need be explored in near future. Taken together, BMP-10 should be considered as a promising prognostic marker and a crucial regulator for progression of ovarian cancer.

Conflict of interest

All the authors declare that they have no conflict of interest.

Acknowledgements

The study was supported by the Nantong Health and Family Planning Commission, Jiangsu, China (WQ2016074).

References

[1] D.P. Brazil, R.H. Church, S. Surae, C. Godson, F. Martin, BMP signalling: agony and antagony in the family, Trends Cell Biol. 25 (2015) 249–264.
[2] D. Carev, M. Saraga, M. Saraga-Babic, Involvement of FGF and BMP family proteins and VEGF in early human kidney development, Histol. Histopathol. 23 (2008) 853–862.
[3] A. Chatterjee, E.J. Rodger, M.R. Eccles, Epigenetic drivers of tumourigenesis and cancer metastasis, Semin. Cancer Biol. 51 (2018) 149–159.
[4] A.J. Cortez, P. Tudrej, K.A. Kujawa, K.M. Lisowska, Advances in ovarian cancer therapy, Cancer Chemother. Pharmacol. 81 (2018) 17–38.
[5] G. Deng, S. Zeng, Y. Qu, Q. Luo, C. Guo, L. Yin, Y. Han, Y. Li, C. Cai, Y. Fu, H. Shen, BMP4 promotes hepatocellular carcinoma proliferation by autophagy activation through JNK1-mediated Bcl-2 phosphorylation, J. Exp. Clin. Cancer Res. 37 (2018) 156.
[6] A. Gonzalez-Martin, Update on relapsed ovarian cancer treatment: from new consensus to daily clinical practice, Future Oncol. 13 (2017) 3–9.
[7] L. Gou, M. Liu, J. Xia, Q. Wan, Y. Jiang, S. Sun, M. Tang, L. Zhou, T. He, Y. Zhang, BMP9 promotes the proliferation and migration of bladder cancer cells through up-regulating lncRNA UCA1, Int. J. Mol. Sci. 19 (2018).
[8] M. Hu, F. Cui, F. Liu, J. Wang, X. Wei, Y. Li, BMP signaling pathways affect differently migration and invasion of esophageal squamous cancer cells, Int. J. Oncol. 50 (2017) 193–202.
[9] P. Huang, A. Chen, W. He, Z. Li, G. Zhang, Z. Liu, G. Liu, X. Liu, S. He, G. Xiao, F. Huang, J. Stenvang, N. Brunner, A. Hong, J. Wang, BMP-2 induces EMT and breast cancer stemness through Rb and CD44, Cell Death Discov. 3 (2017) 17039.
[10] L. Laatio, P. Myllynen, R. Serpi, J. Rysa, M. Ilves, E. Lappi-Blanco, H. Ruskoaho, K. Vahakangas, U. Puistola, BMP-4 expression has prognostic significance in advanced serous ovarian carcinoma and is affected by cisplatin in OVCAR-3 cells, Tumour Biol. 32 (2011) 985–995.
[11] C. Le Page, M.L. Puiffe, L. Meunier, M. Zietarska, M. de Ladurantaye, P.N. Tonin, D. Provencher, A.M. Mes-Masson, BMP-2 signaling in ovarian cancer and its association with poor prognosis, J. Ovarian Res. 2 (2009) 4.
[12] J.Y. Lee, S. Kim, Y.T. Kim, M.C. Lim, B. Lee, K.W. Jung, J.W. Kim, S.Y. Park, Y.J. Won, Changes in ovarian cancer survival during the 20 years before the era of targeted therapy, BMC Cancer 18 (2018) 601.
[13] H. Lei, J. Wang, P. Lu, X. Si, K. Han, T. Ruan, J. Lu, BMP10 inhibited the growth and migration of gastric cancer cells, Tumour Biol. 37 (2016) 3025–3031.
[14] K. Lindemann, B. Gao, C. Mapagu, S. Fereday, C. Emmanuel, K. Alsop, N. Traficante, Australian Ovarian Cancer Study Group, P.R. Harnett, D.D.L. Bowtell, A. deFazio, Response rates to second-line platinum-based therapy in ovarian cancer patients challenge the clinical definition of platinum resistance, Gynecol. Oncol. 150 (2018) 239–246.
[15] T.M. Peart, R.J. Correa, Y.R. Valdes, G.E. Dimattia, T.G. Shepherd, BMP signalling controls the malignant potential of ascites-derived human epithelial ovarian cancer spheroids via AKT kinase activation, Clin. Exp. Metastasis 29 (2012) 293–313.
[16] P.N. Peters, E.M. Schryver, E. Lengyel, H. Kenny, Modeling the early steps of ovarian cancer dissemination in an organotypic culture of the human peritoneal cavity, J. Vis. Exp. (2015) e53541.
[17] M.H. Vetter, J.L. Hays, Use of targeted therapeutics in epithelial ovarian cancer: a review of current literature and future directions, Clin. Ther. 40 (2018) 361–371.
[18] M. Wu, W. Chen, J. Mi, D. Chen, W. Wang, H. Gao, Expression analysis of BMP2, BMP5, BMP10 in human colon tissues from Hirschsprung disease patients, Int. J. Clin. Exp. Pathol. 7 (2014) 529–536.
[19] L. Ye, S. Bokobza, J. Li, M. Moazzam, J. Chen, R.E. Mansel, W.G. Jiang, Bone morphogenetic protein-10 (BMP-10) inhibits aggressiveness of breast cancer cells and correlates with poor prognosis in breast cancer, Cancer Sci. 101 (2010) 2137–2144.
[20] L. Ye, H. Kynaston, W.G. Jiang, Bone morphogenetic protein-10 suppresses the growth and aggressiveness of prostate cancer cells through a Smad independent pathway, J. Urol. 181 (2009) 2749–2759.
[21] Y. Yokoyama, T. Watanabe, Y. Tamura, Y. Hashizume, K. Miyazono, S. Ehata, Autocrine BMP-4 signaling is a therapeutic target in colorectal cancer, Cancer Res. 77 (2017) 4026–4038.
[22] N. Zhang, L. Ye, L. Wu, X. Deng, Y. Yang, W.G. Jiang, Expression of bone morphogenetic protein-10 (BMP10) in human urothelial cancer of the bladder and its effects on the aggressiveness of bladder cancer cells in vitro, Anticancer Res. 33 (2013) 1917–1925.