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Original Article

Biglycan promotes proliferation and metastasis of ovarian cancer

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Abstract: Objective: Ovarian cancer (OC) is a significant threat to the health of women. Biglycan (BGN) plays a crucial role in the oncogenesis and progression of various human cancers. The aim of this study was to clarify the role of BGN in OC. Methods: Immunohistochemical analysis was performed to detect BGN levels in the OC tissues of 68 patients who underwent cytoreductive surgery. Normal ovarian tissues were collected from 21 patients with benign gynecological tumors who underwent oophorectomy. Western blot analysis was conducted to detect BGN levels in human OC and normal ovarian cells. The functions of BGN in OC cells were assessed with the Cell Counting Kit-8, wound healing, and transwell migration assays following upregulation or downregulation of BGN *in vitro*. Results: BGN expression was elevated in OC tissues as compared to normal tissues. The basal level of BGN was also higher in OC cells than in normal cells. Knockdown of BGN reduced the proportion of surviving OC cells, increased wound healing, and decreased cell migration, while overexpression produced the opposite effects. Conclusions: These findings suggest that high BGN expression enhances proliferation and migration of OC cells, indicating that BGN is a potential target for treatment of OC.

Keywords: Biglycan, ovarian cancer, proliferation, metastasis

Introduction

Ovarian cancer (OC) is a highly fatal malignancy affecting women worldwide. Epithelial OC accounts for 85%-95% of OC cases and is often diagnosed at an advanced stage due to the lack of specific clinical symptoms [1, 2]. The current standard treatment strategy for OC includes cytoreductive surgery followed by cisplatin-paclitaxel maintenance chemotherapy. Despite this, OC recurs in 60%-70% of patients after optimal debulking (< 1 cm residual disease) and 80%-85% after suboptimal debulking (> 1 cm residual disease), resulting in a 5-year survival rate of approximately 45% [3, 4]. Therefore, it is crucial to identify new biomarkers and gain deeper insights into the mechanisms of ovarian tumorigenesis and metastasis. Tumor metastasis involves a series of sequential biological cascades, where the extracellular matrix (ECM) is a key modulator of epithelial cell morphology and migration [5].

Biglycan (BGN) is a ubiquitously expressed ECM protein belonging to the family of small leucine-rich repeat proteoglycans. BGN is expressed on the cell surface, within the ECM of various tissues, and is present in almost every organ, although not uniformly distributed [6, 7]. As compared to normal tissues, BGN expression is higher relatively in endometrial, gastric, lung, and colon cancers, and overexpression has been correlated with poor prognosis, indicating a significant role in cancer metastasis and progression [8-11]. The aim of this study was to clarify BGN expression levels in OC tissues and functions in OC cell progression and metastasis.

Material and methods

Study approval and patient consent

The study protocol was approved by the Institutional Review Committee of The First Affiliated Hospital of Nanchang University and

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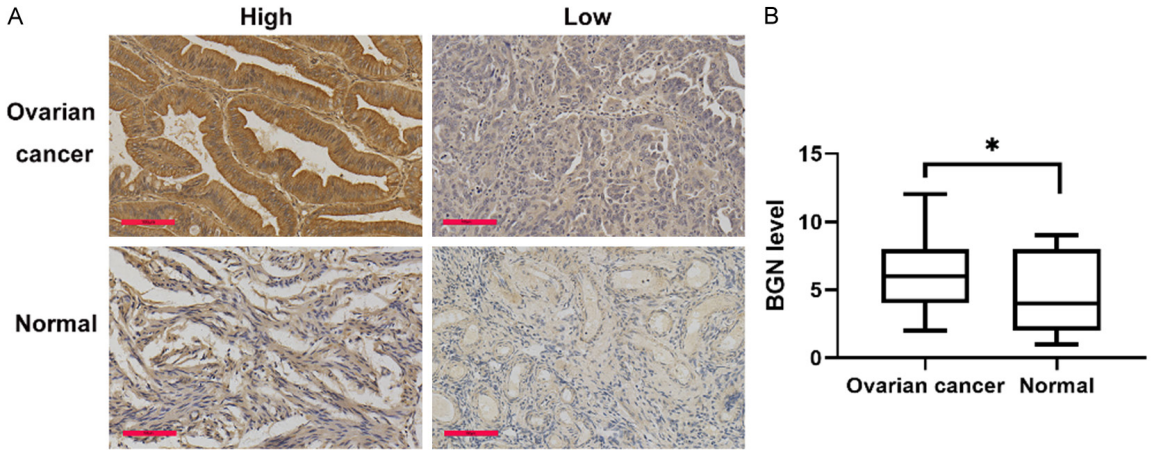

Figure 1. BGN expression levels in OC and normal tissues. A. Representative immunohistochemical images of BGN protein in cancer and normal tissues (scale bar = 100 μm). B. BGN expression was higher in OC tissues (n = 68) than normal tissues (n = 21). *P < 0.05.

Table 1. Expression levels of BGN in OC and normal tissues

Groups	High	Low	*p*-value
Normal	13 (61.9%)	8 (38.1%)	0.02
Ovarian cancer	59 (86.8%)	9 (13.2%)	

conducted in accordance with the ethical principles for medical research involving human subjects described in the Declaration of Helsinki. Prior to inclusion in this study, informed consent was obtained from all participants.

Patients and tissue specimens

Paraffin-embedded tumor tissues were collected from 68 epithelial OC patients who underwent cytoreductive surgery at The First Affiliated Hospital of Nanchang University. Normal ovarian tissues were collected from 21 patients with benign gynecological tumors who underwent oophorectomy at the same hospital between 2013 and 2020. None of the patients received radiotherapy or chemotherapy before surgery. Clinicopathological data collected from the medical records included age, pathological type/grade, International Federation of Gynecology and Obstetrics (FIGO) stage, lymphatic metastasis, and ascites. Histological typing was performed by at least two expert pathologists.

Immunohistochemical analysis

Immunohistochemical analysis of paraffin-embedded OC and normal tissue sections was performed to detect BGN expression levels. A streptavidin-peroxidase kit (ZSGB-BIO, Beijing, China) was used for detection and quantification of BGN in accordance with the manufacturer's instructions. An antibody against BGN was obtained from Bioss Biotechnology (Beijing, China). Two independent pathologists scored the staining intensity results of cancer and normal tissues for each case as described in a previous report [12]. The percentage of positive cells was classified as follows: < 10% (0), 10%-25% (1), > 25%-50% (2), > 50%-75% (3), and > 75% (4). Staining intensity was graded as: 0 (no staining), 1 (bright yellow), 2 (orange), or 3 (brown). Total scores of ≤ 2, > 2-5, and ≥ 6 were defined as negative, weakly positive, and strongly positive, respectively.

Cells

Human epithelial OC SKOV3, A2780, and CAOV3 cells, and normal ovarian IOSE80 cells (identified by short tandem repeats; China Center for Typical Culture Collection, Wuhan, China) were cultured in Roswell Park Memorial Institute 1640 medium (Gibco, Beijing, China) supplemented with 10% fetal bovine serum (Biological Industries, Kibbutz Beit Haemek, Israel) at 37°C under an atmosphere of 5% CO_2/95% air.

Western blot analysis

Cells were lysed in radioimmunoprecipitation assay buffer (Beyotime, Chongqing, China) supplemented with phenylmethylsulfonyl fluoride.

Table 2. Association between BGN expression levels and clinicopathological characteristics of OC patients

Clinicopathological	Case no.	BGN expression level		*p*-value
		Low	High	
Age				
< 50	35	3 (8.6%)	32 (91.4%)	0.3
≥ 50	33	6 (18.2%)	27 (81.8%)	
Histological type				
Serous	44	7 (15.9%)	37 (84.1%)	0.48
Others	24	2 (8.3%)	22 (91.7%)	
Pathological grade				
Low	25	3 (12.0%)	22 (88.0%)	0.56
High	43	6 (14.0%)	37 (86.0%)	
FIGO stage				
I/II	37	4 (10.8%)	33 (89.2%)	0.72
III/IV	31	5 (16.1%)	26 (83.9%)	
Lymphatic metastasis				
Positive	19	1 (5.3%)	18 (94.7%)	0.43
Negative	49	8 (16.3%)	41 (83.7%)	
Ascites				
Positive	24	3 (12.5%)	21 (87.5%)	0.57
Negative	44	6 (13.6%)	38 (86.4%)	

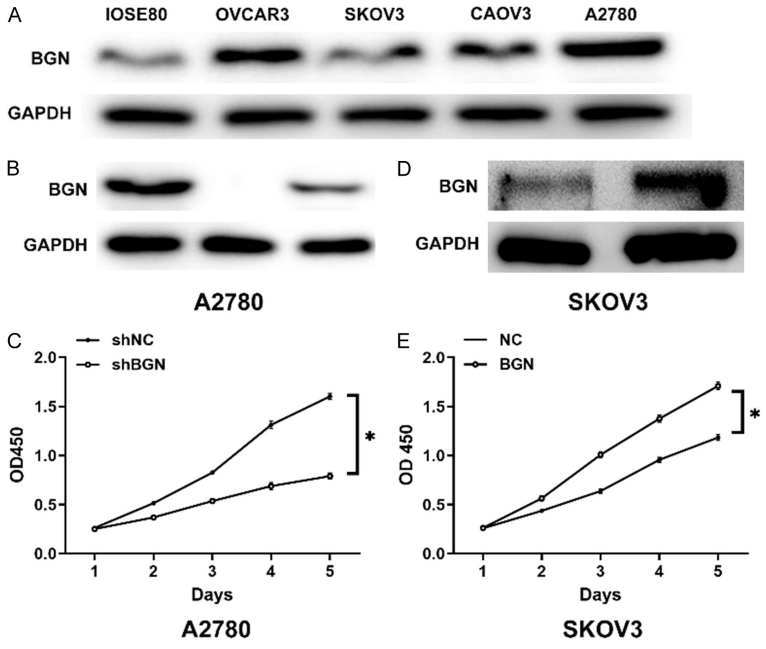

Figure 2. Effects of BGN on survival of OC cells. A. BGN expression was higher in OC cells than normal cells (IOSE80). B, C. BGN levels were decreased in shBGN-transfected A2780 cells and silencing of BGN decreased the proportion of surviving cells. D, E. BGN expression was increased in BGN-transfected SKOV3 cells and overexpression of BGN increased the proportion of surviving cells. *P < 0.05.

Protein concentrations were quantified using a bicinchoninic acid assay kit (Beyotime). Then, the proteins were separated by electrophoresis and electroblotted onto polyvinylidene fluoride membranes, which were probed overnight with primary antibodies against BGN (Abcam, Cambridge, UK) and glyceraldehyde 3-phosphate dehydrogenase (Bioss Biotechnology), followed by a secondary goat anti-rabbit antibody against immunoglobulin G (Cell Signaling Technology, Danvers, MA, USA). The protein bands were visualized using Immobilon Western Chemiluminescent Horseradish Peroxidase Substrate (EMD Millipore Corporation, Billerica, MA, USA).

Cell transfection

Two lentiviral vectors (GenePharma, Shanghai, China) were used to downregulate BGN in A2780 cells, and a third lentiviral vector (GenePharma) was used to upregulate BGN in SKOV3 cells. The cells were transfected with short hairpin RNA targeting BGN (shBGN), a negative control (shNC), BGN, or NC using a Polybrene kit (GenePharma). Puromycin (Solarbio Science and Technology Co., Ltd., Beijing, China) was added to the medium to eliminate uninfected cells and obtain stably transfected cells. The short interfering RNA sequences targeting shBGN1, shBGN2, and shNC were 5'-CCUUUGAGCAGAGAGGCUUTT-3', 5'-CCCUCGUCCUGGUGAACAATT-3', and 5'-TTCTCCGAACGTGTCACGT-3', respectively.

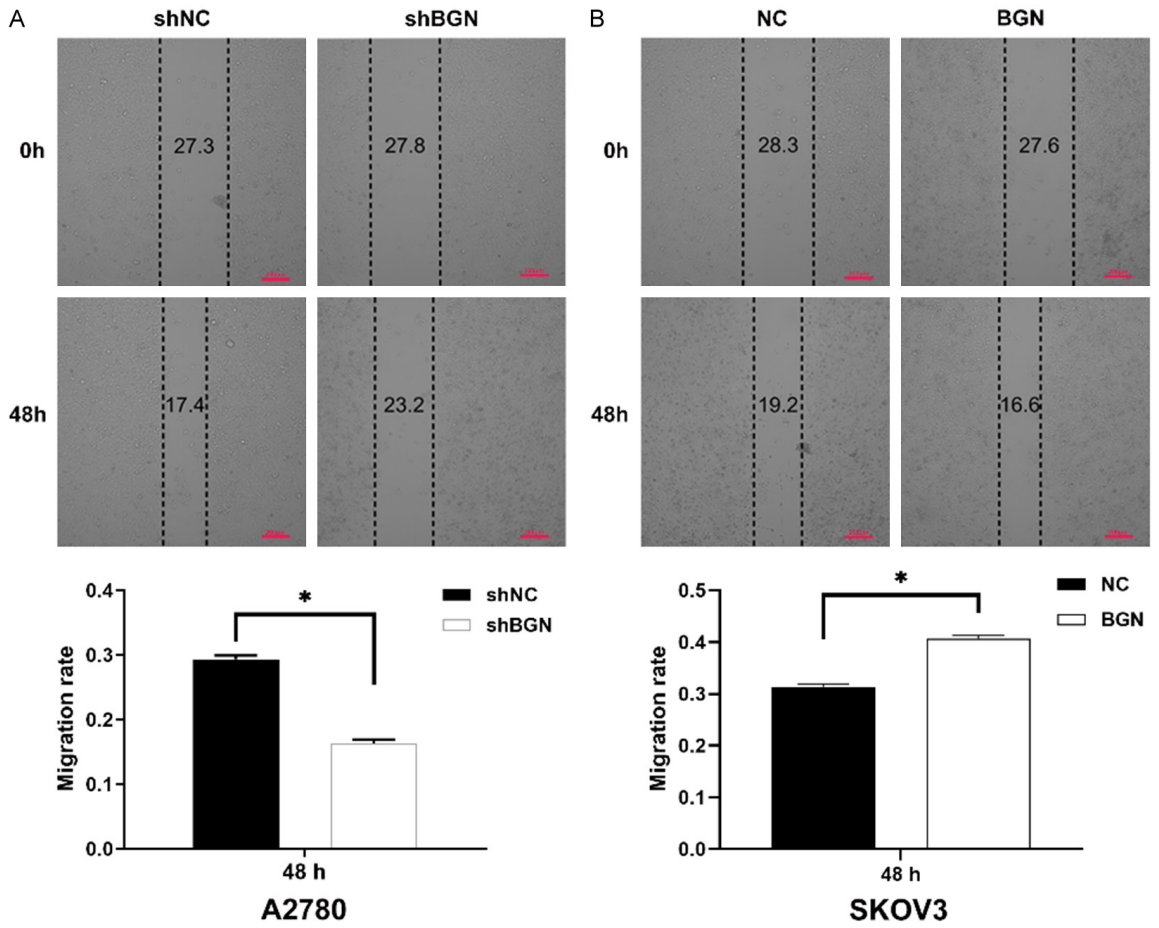

Figure 3. Effects of BGN on wound healing of OC cells, as determined with the wound healing assay (200×). A. Microscopic observations and the distances between the wound edges of cells were recorded at 0 and 48 h after scratching the cell surface. A longer wound was observed in A2780 cells following BGN silencing. B. A shorter wound was observed in SKOV3 cells following BGN overexpression. *P < 0.05.

Cell viability assay

Cells (2×10^3) were seeded in the wells of a 96-well plate and cultured for 1, 2, 3, 4, or 5 days. Cell viability was determined using a Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan).

Wound healing assay

Cells were seeded in the wells of a 6-well plate and cultured until confluent. A 20-μL pipette tip was used to scratch the cell monolayer, and plates were washed with phosphate-buffered saline to remove non-adherent cells. Cells were photographed at 0 and 48 h.

Transwell migration assay

Cells in serum-free Roswell Park Memorial Institute 1640 medium were plated in the chambers of a transwell apparatus (CostarTM; Corning, Inc., Corning, NY, USA) without Matrigel. Medium with 10% serum was added to the lower chamber as a chemoattractant. After 24 h, cells in the lower chamber were stained with 0.5% crystal violet solution and quantified under an inverted microscope.

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics for Windows (version 26; IBM Corporation, Armonk, NY, USA). The two-sided Student's t-test and one-way analysis of variance were used to compare differences between and among groups, respectively. The correlation between BGN levels and clinicopathological factors was analyzed with the chi-square test. A probability (p) value < 0.05 was considered statistically significant.

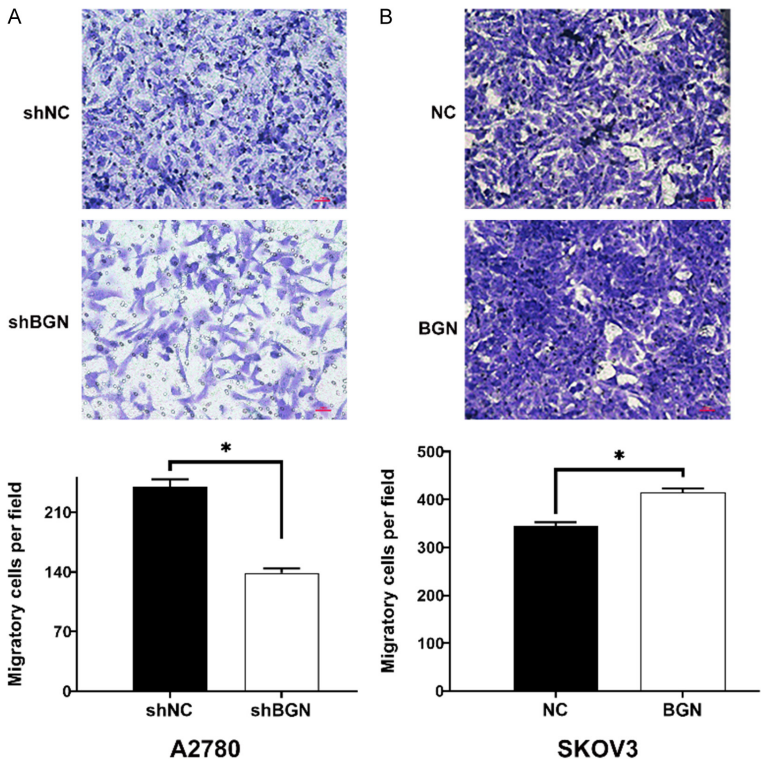

Figure 4. Effects of BGN on migration ability of OC cells, as determined with the transwell assay. A. Photographs of cells that traveled through the micropore membrane without Matrigel. The cell migration rate was reduced in BGN-silenced A2780 cells as compared to control cells. B. Photographs of cells that traveled through the micropore membrane without Matrigel. The cell migration rate was increased in BGN-overexpressing SKOV3 cells as compared to control cells. *P < 0.05.

Results

BGN expression is upregulated in OC tissues

BGN expression levels in OC and normal tissues were detected using an immunohistochemical assay. The results showed that BGN expression levels were significantly higher in OC tissues than normal tissues (P = 0.02, **Figure 1**; **Table 1**). BGN was upregulated in 86.8% (59/68) of OC patients. The correlation between BGN protein expression in cancer tissues and clinicopathological variables in 68 epithelial OC cases was explored (**Table 2**). Notably, the results indicated that BGN expression was not related to age, pathological type/grade, FIGO stage, lymphatic metastasis, or ascites. BGN expression in OC and normal cells was also detected by western blot analysis. The results indicated that BGN expression was higher in OC cells than normal cells (**Figure 2A**).

BGN promotes the proliferation of OC cells

A2780 cells were used to silence BGN expression, and SKOV3 cells were used to upregulate BGN expression due to the high BGN levels in A2780 cells and low levels in SKOV3 cells (**Figure 2A-C**). Silencing of BGN decreased the proportion of surviving A2780 cells (P < 0.001, **Figure 2D**), while overexpression of BGN increased the proportion of surviving SKOV3 cells (P < 0.001, **Figure 2E**). These findings demonstrate that BGN plays a role in proliferation of OC cells.

BGN accelerates wound healing of OC cells

The wound healing assay was performed to investigate the effect of BGN on the migration ability of OC cells. Wound healing was prolonged in A2780 cells following BGN silencing (P < 0.001, **Figure 3A**), as compared to SKOV3 cells following BGN overexpression (P < 0.001, **Figure 3B**).

BGN promotes the migration of OC cells

The cell migration rate was reduced in BGN-silenced cells as compared to control cells (P < 0.001, **Figure 4A**). Conversely, the migration rate was increased in SKOV3 cells overexpressing BGN (P < 0.001, **Figure 4B**). These results suggest that BGN plays a critical role in metastasis of OC.

Discussion

OC is among the most common malignant tumors of the female reproductive system and poses a serious threat to the health of women [13]. Hence, it is essential to explore the mechanisms underlying progression of OC.

BGN, an ECM protein, plays a key role in maintaining cell stability and organization in tissues

by interacting with other ECM proteins [14]. BGN is also known to promote cancer cell proliferation, invasion, and metastasis [15]. The Wnt signaling pathway, which regulates tumor progression, is closely related to carcinogenesis. BGN can enhance the canonical Wnt signaling pathway by interacting with the Wnt co-receptor, thus promoting cancer cell proliferation, invasion, and metastasis [16, 17]. Additionally, BGN can promote cancer cell migration and invasion, while inhibiting apoptosis, through a p38- and MAPK-dependent manner [18].

Analysis of the clinical data indicated that OC patients had higher BGN levels in cancer tissues as compared to normal tissues. The OC cells also exhibited higher BGN levels than normal cells. These findings suggest abnormal BGN expression in OC. However, the correlation between BGN levels and clinicopathological variables was inconsistent with other studies that linked BGN levels to lymph node metastasis and tumor invasion depth [19]. This discrepancy may be due to the limited number of OC patients included in this study. Hence, future research with more cases is warranted to explore the correlation between BGN and clinicopathological variables.

Further functional studies demonstrated that downregulation of BGN increased cell death, while upregulation decreased cell death, indicating that BGN is involved in proliferation of OC cells. The wound healing assay showed that BGN overexpression accelerated the wound healing process of OC cells. In the transwell assay, more OC cells migrated to the lower chamber in the BGN-overexpression group as compared to the control group. These results suggest that BGN enhances migration of OC cells, consistent with other studies demonstrating that BGN promotes cancer cell migration [20, 21]. However, the mechanisms employed by BGN to promote proliferation and migration of OC cells were not explored in this study. Thus, further investigations are needed.

Conclusions

BGN levels were higher in OC tissues than in normal tissues. Moreover, BGN overexpression promoted the proliferation and migration of OC cells. Thus, BGN may serve as a potential target for OC treatment.

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Disclosure of conflict of interest

None.

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