

Original Article

Elevated OGN expression correlates with the EMT signature and poor prognosis in ovarian carcinoma

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Abstract: Ovarian carcinoma is the most deadly gynaecological disease, with poor prognosis and limited predictive biomarkers. Recent evidence has indicated controversial roles of OGN in human malignancies, but the pathologic significance of OGN in ovarian carcinoma has not yet been determined. Here, we investigated the expression of OGN in ovarian carcinoma and determined its association with patient prognosis. We found that OGN expression was up-regulated in serous papillary cystadenocarcinoma and endometrioid adenocarcinoma compared to non-tumor tissues, but not in clear-cell ovarian carcinoma. Kaplan-Meier analysis showed that OGN expression was an adverse prognostic factor for both the overall survival and the progression-free survival of ovarian carcinoma patients. Higher OGN levels were positively associated with the activation of EMT-related gene signatures. Histological analysis further confirmed that OGN positive ovarian carcinoma cells expressed vimentin and displayed morphology of mesenchymal identity. Collectively, our preliminary results indicate that elevated expression of OGN is associated with the EMT process and may serve as a potential biomarker for prognosis in ovarian carcinoma.

Keywords: Ovarian carcinoma, OGN, biomarker, epithelial-mesenchymal transition

Introduction

Ovarian carcinoma, the eighth most common cause of cancer-related death, affects 1.2 million women worldwide and leads to over 160,000 deaths per year [1-3]. Since ovarian carcinoma has nonspecific symptoms at early stages, approximately 80% of the cases are diagnosed at advanced stages, with widely metastatic disease within the peritoneal cavity [4]. As a consequence, women with ovarian carcinoma have a relatively poor prognosis, with an overall 5-year survival rate of around 40% [5, 6]. Standard treatment usually includes a combination of surgery, radiation therapy, and chemotherapy, with a high frequency of recurrence within months [7]. Further understanding of the process by which ovarian carcinoma cells disseminate and metastasize is essential for the identification of more reliable biomarkers and the advancement of the treatment strategies.

Accumulating evidence has suggested that epithelial-mesenchymal transition (EMT) plays a

key role in the metastatic spread of cancer cells from primary sites. EMT is a biological process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties to adopt mesenchymal identity [8]. EMT is involved in a wide range of physiologic and pathologic processes including wound healing, organ fibrosis as well as the initiation of metastasis during cancer progression. Additionally, recent studies further revealed roles of EMT in cancer stem cell maintenance and acquired drug resistance, presenting increased danger to cancer patients and predicting poor prognosis [9, 10].

OGN, a subset of small leucine-rich glycoproteins initially found to be highly expressed in bone and blood vessels, plays a key role in extracellular matrix (ECM) remodeling and tissue development [11-13]. As a component of ECM, OGN is implicated in EMT process and the wound healing response. For example, OGN is involved in maintenance of mesenchymal progenitor populations by inhibiting their differentiation into smooth muscle cell lineage during

OGN as a biomarker for ovarian carcinoma

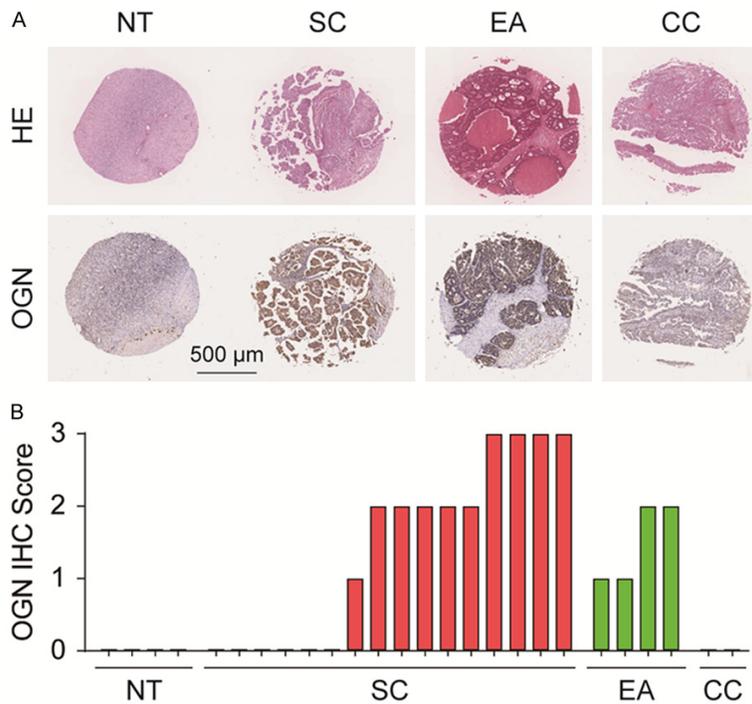


Figure 1. OGN protein expression in ovarian carcinoma. (A) Representative IHC staining of OGN in three pathologic types of ovarian carcinoma tissues and non-tumor controls. Scale bar, 500 μm. (B) Quantification of OGN staining as indicated in (A). Each sample was stained and scored in duplicates.

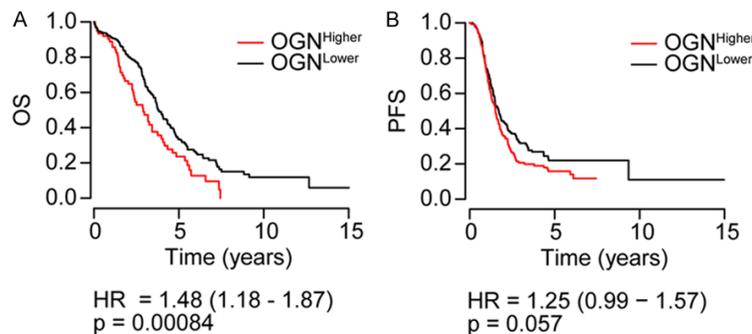


Figure 2. Survival analysis of patients stratified according to levels of OGN expression in ovarian carcinoma. A. Kaplan-Meier survival curve indicating OS of ovarian carcinoma patients with higher or lower OGN expression in the TCGA database by the log-rank test. B. Kaplan-Meier survival curve indicating PFS of ovarian carcinoma patients with higher or lower OGN expression in the TCGA database by the log-rank test.

gut development [14]. Additionally, OGN is also found to be upregulated by human connective tissue growth factor (hCTGF) in primary human tendon fibroblasts (HTFs), suggesting that OGN potentially acts as a modulating factor in wound healing response in human eyes [15]. Recently, emerging evidences have shown controversial roles of OGN in human cancers [16-

21]. However, whether OGN is involved in ovarian carcinoma is largely unknown. Therefore, with an aim of defining novel clinically relevant biomarkers for ovarian carcinoma, we detected expression of OGN and evaluated its association with EMT signature and patient clinical outcomes.

Materials and methods

Tissue specimens

Ovarian carcinoma samples and the adjacent non-tumor tissues included in this study were collected at Danyang People's Hospital Affiliated to Nantong University, Jiangsu, China. Consent forms were signed by all patients. Our present study was approved by the Research Ethics Committee of Nantong University.

Patients in the TCGA database

OGN expression data based on RNA-Seq were extracted from The Cancer Genome Atlas (TCGA) database for ovarian carcinoma patients. Patients were stratified into lower and higher expression groups based on median OGN expression. Prognostic values of OGN for overall survival (OS) and progression-free survival (PFS) were assessed.

Immunohistochemistry assay

Formalin-fixed, paraffin-embedded (FFPE) tissue sections were cut to 4-μm thickness. Antigen retrieval was performed in 0.01 M citrate buffer (pH 6.0) using a microwave oven for 15 min, followed by blocking of endogenous peroxidase with 3% H₂O₂. Primary antibodies were incubated overnight at 4°C, followed by incubation with HRP-conjugated anti-mouse/rabbit secondary antibody (DAKO) for 1 h at room temperature.

OGN as a biomarker for ovarian carcinoma

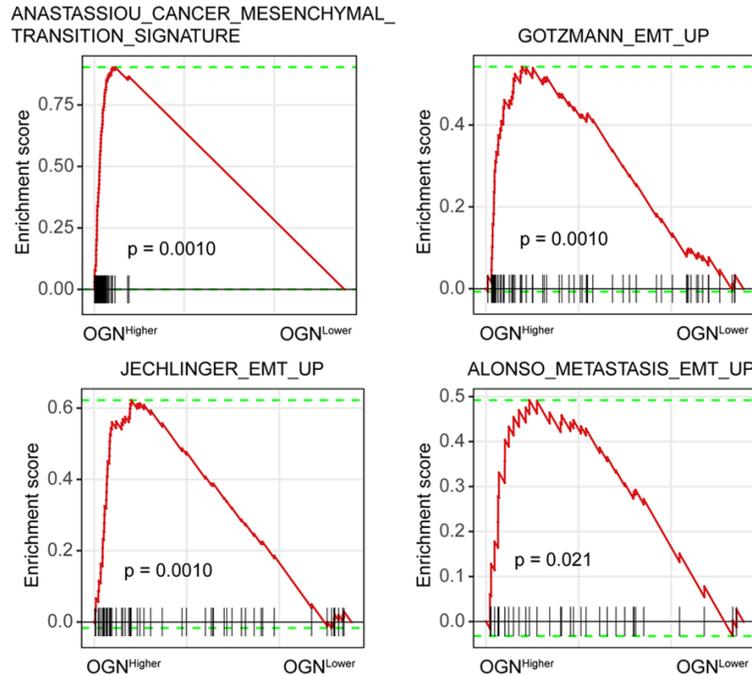


Figure 3. GSEA analysis showing robust enrichment of EMT signatures in ovarian carcinoma patients with higher OGN expression relative to those with lower OGN expression.

Antibody binding was detected by 3,3'-diaminobenzidine (DAB). The sections were counterstained with hematoxylin, dehydrated and mounted. Duplicates of tissue samples were scored separately, with the intensity of staining scoring from 0 to 3. The following primary antibodies were used: OGN (1:200, ab211456, Abcam) and vimentin (1:500, ab8069, Abcam).

Statistical analysis

Statistical analyses were performed with SPSS statistical software. Gene Set Enrichment Analysis (GSEA) was performed to assess the enrichment of the curated gene sets from the Molecular Signature Database in the patient subgroups [22]. Statistical significance of the OGN expression difference in survival was computed using Kaplan-Meier analysis with the *P* value determined by log-rank test.

Results

OGN is upregulated in ovarian carcinoma

To evaluate the expression of OGN in ovarian carcinoma tissues, we performed immunohistochemistry (IHC) in ovarian carcinoma specimens and non-tumor control tissues. Three

common pathologic subtypes of ovarian carcinoma, as confirmed by hematoxylin and eosin (H&E) staining, were included in our analysis (**Figure 1A**). As compared to non-tumor tissue control, OGN IHC showed intensive, diffuse staining in serous papillary cystadenocarcinoma (SC) and endometrioid adenocarcinoma (EA) (**Figure 1A, 1B**). Interestingly, OGN expression was hardly detected in clear-cell ovarian carcinoma (CC) (**Figure 1A, 1B**), indicating that expression of OGN is unregulated in ovarian carcinoma in a subtype-specific manner.

OGN expression is associated with poor prognosis in ovarian carcinoma

To assess the prognostic significance of OGN, we analyzed the association between OGN

expression levels and the survival outcomes of ovarian carcinoma patients utilizing datasets from the TCGA database, including overall survival (OS) and progression-free survival (PFS). Kaplan-Meier analysis and log-rank test showed that higher OGN expression in ovarian carcinoma predicted poor overall survival (HR = 1.48, *P* = 0.00084) (**Figure 2A**). Similarly, patients expressing higher OGN had trends for shorter PFS than those with lower OGN expression (HR = 1.25, *P* = 0.057) (**Figure 2B**). These results suggest a prognostic value for OGN expression in ovarian carcinoma patients.

OGN expression correlates with EMT signature in ovarian cancer

Tumorigenesis and adverse progression are frequently accompanied by EMT. Given the previous observations that OGN is implicated in EMT [14, 15], we asked whether ovarian carcinoma patients with higher OGN expression possess EMT properties using GSEA analysis on the gene sets in the Molecular Signature Database [22]. Four EMT-related gene sets, namely Anastassiou cancer mesenchymal transition signature, Gotzmann epithelial to mesenchymal transition, Jechlinger epithelial to mesenchymal transition and Alonso metastasis

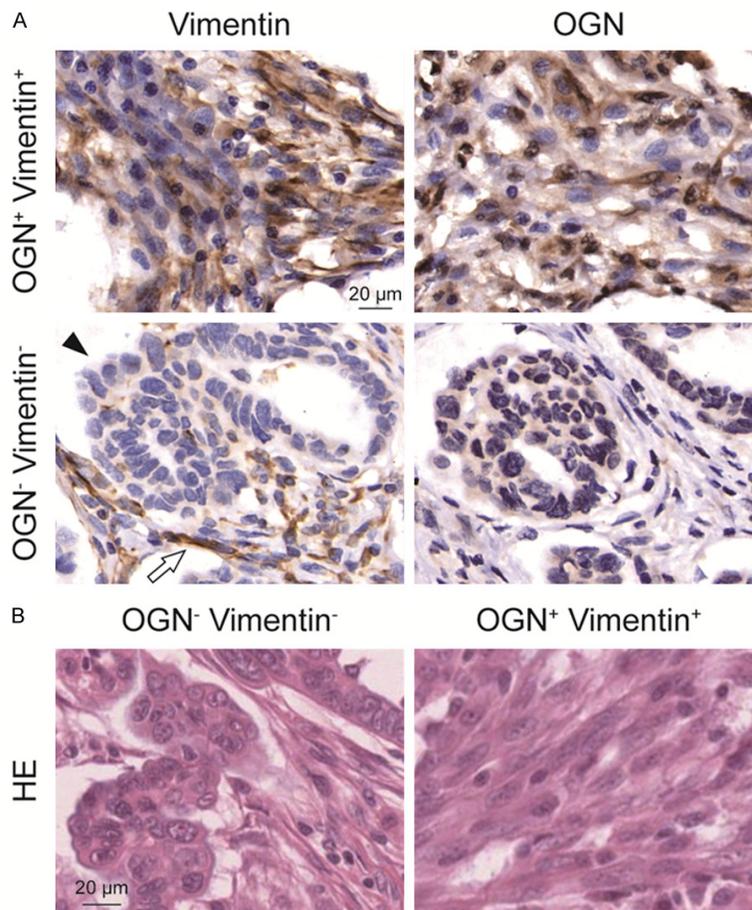


Figure 4. OGN expression correlates with the invasive phenotype in ovarian carcinoma. (A) Representative IHC staining demonstrating correlation of OGN and vimentin protein expression in ovarian carcinomas. Black arrowhead: negative staining of vimentin in ovarian cancer cells; White arrow: positive staining of vimentin in the surrounding cells. Scale bar, 20 μ m. (B) Representative H&E staining corresponding to specimens in (A).

epithelial to mesenchymal transition, were significantly enriched in patients expressing higher OGN levels (Figure 3). These data suggest that OGN upregulation might correlate with the EMT program in ovarian carcinoma.

Expression of OGN correlates with vimentin expression in ovarian carcinoma

To further address the pathological relevance between OGN expression and EMT activation, we performed IHC staining of vimentin, an important marker and regulator of EMT process [23], on ovarian carcinoma specimens. In agreement with the GSEA data, cancer samples with strong OGN expression also stained heavily for vimentin (Figure 4A). Additionally, the vimentin⁺ OGN⁺ cancer cells displayed a spin-

dle-shaped morphology, further indicative of their mesenchymal phenotype (Figure 4B).

Discussion

In the present study, we provide the first analysis of OGN protein expression in human ovarian cancer tissues and demonstrate that OGN is significantly increased in a substantial proportion of ovarian cancer cases compared with unaffected tissues. High OGN expression is associated with stronger enrichment of EMT-related transcriptional program and a significant trend toward worse clinical outcomes.

Several recent studies have suggested a broader function of OGN in tumorigenesis. However, the role of OGN in cancer biology remains controversial and seems to be cancer-type specific. For example, OGN has been shown to be dramatically upregulated in meningioma compared to normal brain as well as a spectrum of other brain tumors [20]. Further mechanistic assays revealed that over-

expression of OGN in meningioma cells enhances cell proliferation, cell cycle progression, and colony formation by activating AKT/mTOR pathway [20]. Similar role was observed in neuroblastoma, where OGN significantly increases neuroblastoma cell growth induced by insulin-like growth factor 2 [18]. On the other hand, OGN has also been reported as tumor suppressor, with its downregulation observed in some cancer types including gastric cancer, colorectal adenoma and laryngeal carcinoma [19, 21, 24]. Most recently, Hu et al. further demonstrated a restrictive role of OGN in colorectal cancer progression by reduced activation of EGFR/AKT pathway and enhanced T lymphocyte tumor infiltration [16, 17]. Indeed, we also found that OGN can function as tumor suppressor and predict better clinical outcome in certain other

cancer types (data not shown), strongly suggesting that the functions of OGN are largely tissue-context dependent. Therefore, the molecular basis underpinning the distinct biological roles of OGN is worth further investigation.

In conclusion, our study suggests that expression of OGN is elevated in ovarian carcinoma tissues compared with the benign counterparts. Higher OGN mRNA levels correlate with shorter overall survival and progression-free survival. The GSEA analysis further highlights significant association between OGN expression and previously described EMT signatures in ovarian carcinoma patients. Further work will be needed to determine the mechanisms by which increased expression of OGN associates with EMT process in ovarian carcinoma.

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Disclosure of conflict of interest

None.

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