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Original Article

Effect of VTCN1 on progression and metastasis of ovarian carcinoma in vitro and vivo

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ABSTRACT

Background and purposes: Through reducing immune response, VTCN1 could promote carcinoma indirectly. However, the direct effect of VTCN1 on carcinoma was not studied clearly, especially on ovarian carcinoma. In this paper, we verified the potential effect and mechanism of VTCN1 on ovarian carcinoma.
Methods: The influence of high or low VTCN1 expression on the viability of ovarian cancer was detected by CKK-8 and annexin V-PI kit. The orthotopicxenograft tumor model was performed to evaluate the effect of VTCN1 on the promotion of tumor in vivo. Western blot was used to verify the signaling pathways predicted by bioinformatics analysis.
Results: Low expression of VTCN1 could inhibit the viability and metastasis of ovarian carcinoma directly in vitro and vivo; Information analysis demonstrated that cell cycle and JAK2/STAT were involved in the regulation of VTCN1. The CDK2/4 and CDC25C expression and phosphorylation of JAK2/STAT had a direct relationship with the reduction of VTCN1.
Conclusions: VTCN1 could affect the viability and metastasis of ovarian carcinoma by reducing the expression of CDK2/4 and CDC25C and phosphorylation of JAK2/STAT. It indicated that VTCN1 was a potential target for treating ovarian carcinoma.

1. Introduction

Ovarian carcinoma which is the fifth most common cause of death from carcinoma and the first most related to gynecologic carcinoma have a poor prognosis at advanced stage and the 5-year survival is less than 30% [1,2]. Worse, the first appearance of this cancer, over 75%, are at a late stage [3]. It enhances the difficulty to cure it. At present, standard therapy is surgery therapy followed by platin and taxan chemotherapy. Although it has good response rates, the disease over, 50% of the cases recur within the following 5 years [4,5]. Hence, it is imperative to find the predicted factors and targets for curing ovarian carcinoma.

V-set domain containing T cell activation inhibitor 1(VTCN1), also known as B7-H4, B7X or B7S1, is a B7 family member and plays critical roles in regulating T-cell activation, cytokine secretion and the development of cytotoxicity [5–7]. In many prior papers, it proved to be a good predictable factor and target for many cancers. Quandt et al. reported that VTCN1 was associated with patients' survival and antitumor immune response in human melanoma [8]. Zang et al. proved that the expression of B7-H4 was high and associated with disease spread and poor outcome in human prostate cancer. Krambeck et al. demonstrated that VTCN1 had a direct relationship with progression and survival of renal cell carcinoma and tumor vasculature [9]. These literatures implied that VTCN1 could influence carcinoma through regulating the immune response or adjusting the progression and survival of carcinoma. However, the mechanism of the direct effect on carcinoma is still not clear, especially in ovarian carcinoma, there is no report about it.

In this study, we explored the biologic functions and underlying mechanisms of VTCN1 in ovarian carcinoma. The results from different experiments showed VTCN1 had a direct relationship wish proliferation, cell circle distribution and metastasis of ovarian cancer in vitro and vivo. Bioinformatics analysis demonstrated that JAK2/STAT3 and CDK2/CDK4/CDC25C signaling pathways linked

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closely with VTCN1 expression. The reports from western blot proved that low VTCN1 expression could reduce the signaling pathways activation of JAK2/STAT3 and CDK2/CDK4/CDC25C.

2. Materials and methods

2.1. Cancer specimens

Tumor tissue samples and normal tissue samples adjacent to tumor (within at least 5 cm of margin) were obtained from 30 patients underwent definitive surgery in Shanghai Tongren Hospital. The independent ethics committee of Shanghai Tongren Hospital provided the ethical approval and patients (or their advisers) offered informed and written consent for the study under the guidelines of ethics committee.

2.2. Bioinformatics analysis

The gene expression data have been deposited in NCBIs Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession number GES12470.

Agilent-012097 Human 1A Microarray for ovarian carcinoma and normal adjacent tissue were downloaded from the GES12470. To obtain more details about the biological pathways involved in ovarian carcinoma pathogenesis through VTCN1 pathway, a gene set enrichment analysis (GSEA) was performed. The *KEGG* gene sets biological process database (c2.KEGG.v4.0) from the Molecular Signatures Database (MsigDB, http://www.broad.mit.edu/gsea/msigdb/index.jsp) were carried out in the analysis of enrichment.

2.3. Cell culture

The ovarian carcinoma cell lines HO-8910, SKOV3 and OVCAR3 were purchased from the Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China), and maintained in DMEM (10% fetal bovine serum, 1% penicillin and streptomycin) at 37 °C in a water-saturated carbon dioxide incubator (5%).

2.4. Establishment of VTCN1 knockdown OVCAR3 cells

RNA interference sequence, GAAGGACCTTTCACCTTGTCT(1786), was designed and synthesised by JRDUN Biotechnology(Shanghai) co. Ltd. Under the guidelines of manufacturer, shRNA (or negative control) was transfected into cells through lentiviral vector (purchased from Addgene). Stable shRNA expression cells were harvested and processed for the follow analysis after transfection.

2.5. Cell proliferation assay

96-well plates containing 1×10^5 cell in each well were incubated in the water-saturated carbon dioxide incubator (5%). Cell counting kit (Dojindo, Japan) was used to detect the cell proliferation at 12, 24 and 48 hours, respectively. The operation was carried out under the guidance of manual.

2.6. Cell apoptosis and cycle assays

Cells in 24-well plate were digested by trypsin and collected through centrifugation at 48 h after being seeded. The collected cells were resuspended by PBS and counted. Annexin V-Propidium Iodine(PI) staining kit (Becton Dickinson, for apotosis) or PI staining kit (for cycle, Beyotime) were used to detect the apoptosis and cycle of cells in a FACSCalibur cytometer (FACSCalibur, BD Biosciences), respectively.

2.7. Cell migration and invasion assays

Boyden chamber containing 24-well transwell plates (Corning Inc.) with 8-μm pores membrane was used in the experiment of migration and invasion assays. The processes of migration and invasion were similar as described in Dong's paper [10]. The differences of migration and invasion were as follows: in invasion assay, the inserts were coated with Matrigel (BD Bioscience, Franklin Lakes, NJ, USA) and cells were incubated for 24 hours before detection; while in migration assay, the inserts were not coated and cells were incubated for 1 hour before detection.

2.8. Quantitative real-time PCR

The details for qRT-PCR was as described in Sadun et al. [11] with some modifications: total RNA was prepared with Trizol Reagent (Invitrogen) and cDNA was synthesized with AMV reverse transcriptase (Fermentas, USA) according to the instructions of manufacturer, respectively. The primer pairs were shown in Table 1.

2.9. Western blot

Cells cultured in 6-well plate were harvested and washed with PBS twice before lysis. Cell lysis solution was centrifuged to get supernatant for electrophoresis. The process was similar with that described in Tulunay [12]. The primary antibodies against VTCN1 (Abcam), GAPDH (Fermentas), CDK2 (Abcam), CDK4 (Abcam), CDC25C (Abcam), p-JAK 2 and JAK 2 (CST), and p-STAT 3 and STAT 3 (CST) were used in this study.

2.10. In vivo orthotopicxenograft tumor model

Twelve 6-week-old male BALB/c nude mice (purchased from Charles River Japan Inc.) were divided into two groups. The method was described in Zhang et al. [13] with some modifications. OVCAR3 cells with stable VTCN1-shRNA or the respective empty vectors (both 4×10^6 cells) were injected into oxter. The size of tumors was determined at intervals of 3 or 4 days after the appearance of tumor about 2 weeks later. The tumors were harvested and weighed at 46 days after the injection.

2.11. Statistical analysis

The results were showed as mean ± error. The statistical difference was analyzed by SPSS Version 16.0. The criterion of statistical significance was $P < 0.05$.

Table 1
Primers used in RT-PCR analysis.

Gene	Primer sequence	Species	Amplicon size (bp)
VTCN1	Forward: 5′-AGGGAGTGGAGGAGGATACAG -3′ Reverse: 5′-GCAGCAGCCAAAGAGACAG -3′	Human	137
GAPDH	Forward: 5′-CACCCACTCCTCCACCTTTG -3′ Reverse: 5′-CCACCACCCTGTTGCTGTAG -3′	Human	110