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PTP1B promotes the malignancy of ovarian cancer cells in a JNK-dependent mechanism

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ABSTRACT

Ovarian cancer is the leading cause of death from gynecological malignancies in women. Diagnosis at the early stage remains challenging and efficient treatment is still highly needed. The development and progression of this cancer is associated with many genetic and epigenetic changes, representing the dysregulation of a highly complex signaling network. Previous studies found that protein-tyrosine phosphatase 1 B (PTP1B) was aberrantly expressed in many types of ovarian cancer cells. The exact role of this protein, however, remains controversial. We found that PTP1B was highly expressed in several ovarian carcinoma cell lines. Changing its expression level strongly affected the malignancy phenotypes of the cultured cancer cells and growth of tumors in nude mice. Further analysis at the molecular level found that overexpression of PTP1B activated the JNK (c-Jun N-terminal kinase) signaling pathway and impacted a set of factors involved in cancer metastasis. Overall, our study suggested that overexpression of PTP1B could be a driving factor in the tumorigenesis and progression of ovarian cancer and restoring the normal expression level of this signaling molecule may represent a promising strategy in treating this disease.

1. Introduction

Ovarian cancer is one of the most common and lethal gynecological malignancies in women worldwide [1]. It is a heterogeneous disease and can be divided into at least five different subtypes according to the associated risk factors, cells of origin, clinical features and treatment methods [2]. The disease is often diagnosed at a late stage and the standard treatment has been challenged by a high recurrence rate. The recurrent cancer is highly fatal because of the development of resistant to chemotherapy [2]. Consequently, there is an urgent need to understand the mechanisms underlying the resistance and develop new strategies to manage the disease.

PTP1B (Protein tyrosine phosphatase 1 B), a non-receptor-type oncogenic promoter involved in growth factor signaling, is often upregulated in several types of cancers, leading to enhanced invasion and migration of cancer cells [3–6]. On the other hand, the possibility of its action as a tumor suppressor cannot be ruled out as studies suggest that its phosphatase activity may target and inactivate several kinases, which are often hyperphosphorylated to promote oncogenic signaling in cancers [7,8]. Previous studies indeed found that PTP1B was aberrantly expressed in ovarian cancer cells suggesting its potential role in the development and progression of the disease [6,8]. However, the impact of its action on the signaling network that is ultimately responsible for the tumorigenic phenotypes of the cancer cells still remains to be clarified.

JNK (c-JUN N-terminal kinase) represents another important signaling molecule that has been documented in a variety of human cancer types, including ovarian cancer. Notably, previous studies revealed that aberrant activation of the JNK pathway was associated with shorter progression-free survival of patients with ovarian cancer, suggesting that the activated signaling may be involved in the progression of the disease [9,10]. The JNK pathway can be activated and regulated by a variety of mechanisms, highlighting a complexity of the signaling network and downstream impacts [11]. Consequently, the exact role of the JNK signaling in the biology of

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ovarian cancer remains unclear, although recent study has suggested that it may be required for the maintenance and tumorigenic capacity of ovarian cancer stem cells [12].

Our studies found that PTP1B was upregulated in several ovarian carcinoma derived cell lines. The overexpression of the protein was likely responsible for the enhanced proliferation, invasion and migration of cultured cancer cells, as well as growth of tumors in nude mice. The oncogenic role of PTP1B seemed to be dependent on the activation of the JNK signaling to ultimately regulate a set of factors involved in cancer metastasis. The results strongly supported that PTP1B is an important factor in the development and progression of ovarian cancer, justifying the development of new therapeutic agents targeting this molecule.

2. Materials and methods

2.1. Antibodies and reagents

Antibodies against PTP1B, E-cadherin, vimentin, Twist and β-actin were purchased from Abcam, and antibodies against JNK and phosho-JNK were obtained from Cell Signaling Technology. The JNK specific inhibitor SP600125 was purchased from MCE.

2.2. Cell lines and culture condition

The human ovarian surface epithelial cell line IOSE144 and human ovarian cancer cell lines A2780, HO8910, OVCAR3 and SKOV3 were all purchased from the Cell Bank of the Chinese Academy of Science (Shanghai, China). The IOSE144 cells were maintained in MCDB105 medium. The A2780 and SKOV3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM). The OVCAR3 and HO8910 cells were cultured in RPMI-1640 medium. All cell culture medium were supplemented with 10% fetal bovine serum (FBS) unless otherwise specified. All cell lines were incubated at 37 °C in humidified 5% CO_2.

2.3. Modulating the expression level of PTP1B

The human PTP1B full-length cDNA was inserted into the pcDNA3.1 vector (GenePharma Co., Ltd., Shanghai, China) to engineer an overexpression vector pcDNA3.1-PTP1B. The empty vector was used as a control. To knock down the expression of PTP1B in SKOV3 cells, a shRNA targeting PTP1B was designed and obtained from GenePharma Co., Ltd. A scrambled shRNA was used as a control. The reagents were transfected into cells to modulate the expression level of PTP1B.

2.4. Cell transfection

The transfection experiments were performed in 6-well plates. When the cells reached 50% confluency, the PTP1B shRNA or pcDNA3.1-PTP1B vector was transfected into the cells using the Lipofectamine 2000 transfection reagent according to the manufacturer's instructions (Invitrogen). After 6 h of incubation, the transfection medium and reagents were removed and the cells were further incubated in the complete medium.

2.5. Immunoblot analysis

Proteins were detected and quantitated by western blot according to previously described methods [12]. Cells were lysed in a radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors. The supernatants were quantitated by the BCA assay and equal amounts of protein were loaded onto a 10% sodium dodecyl sulfate-polyacrylamide gel for electrophoretic separation (SDS-PAGE). The in-gel proteins were transferred onto PVDF membranes. The membranes were blocked with 5% BSA for 1 h at room temperature and further incubated with specific primary antibodies against PTP1B, E-cadherin, vimentin, Twist, β-actin, JNK or phosho-JNK at 4 °C overnight. β-actin was blotted as an internal reference for each membrane. After the primary antibodies were removed, the membranes were further incubated with horseradish peroxidase-conjugated secondary antibodies (Zhongshan Biotech Co., Ltd, Beijing, China) for 1 h at room temperature. The protein bands were visualized by enhanced chemiluminescence (Applygen Technologies Inc., Beijing, China) and analyzed by the Quantity One software (Bio-Rad, Hercules, CA, USA).

2.6. Cell proliferation assay

A CCK-8 assay kit (Jingmei Biotech, Shanghai, China) was used to determine cell proliferation. In brief, the cells were seeded at 1000 cells/well in a 96-well plate. After incubation with the JNK inhibitor SP600125 or dimethylsulfoxide (DMSO) for the indicated time, 10 μl of CCK-8 was added into each well of the plate and cells were further incubated for 2 h. The optical density of each well at the wavelength of 490 nm (OD_{490}) was measured in a microplate reader (Bio-Rad Model 680) every day for seven days.

2.7. In vitro cell invasion and migration assays

To determine cell invasion, 30 μl of Matrigel (BD Biosciences, USA) was used to cover a 24-well Transwell chamber (Corning

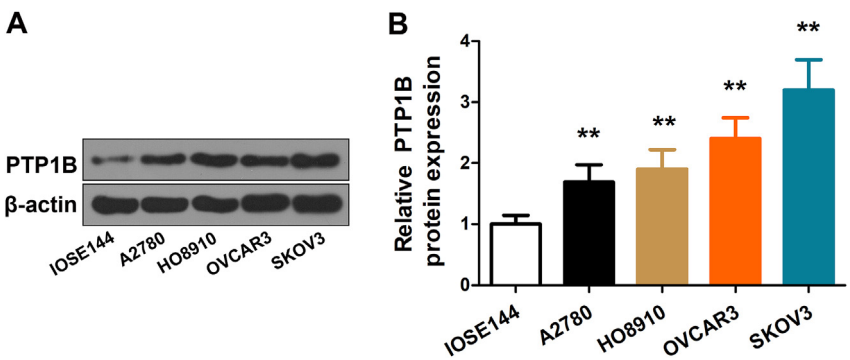

Fig. 1. Quantitation of PTP1B expression in different ovarian cell lines. **(A)** Western blot of PTP1B in human ovarian surface epithelial cells (IOSE144) and ovarian cancer cells (A2780, HO8910, OVCAR3 and SKOV3). β-actin was blotted as an internal reference. **(B)** Bar plot of the PTP1B levels. Data were averaged from three western blot replicates. The error bars are standard deviations. The double asterisks indicate statistical significance ($P < 0.01$).

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