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Original article

Importin-11 overexpression promotes the migration, invasion, and progression of bladder cancer associated with the deregulation of CDKN1A and THBS1

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Abstract

Objectives: We recently determined that a novel oncogene, IPO11 from 5q12, participates in bladder cancer (BCa) progression. However, the biological function of IPO11 and the molecular mechanisms through which it contributes to BCa progression remain unclear. The aim of this study was to investigate the role of IPO11 in BCa aggressiveness and elucidate the molecular mechanisms underlying its effects in BCa.

Materials and methods: The mRNA expression levels of IPO11 in BIU-87, RT4, UMUC3, EJ, 5637, T24, J82, and HT-1376 cell lines were determined using quantitative real-time polymerase chain reaction. Expression of importin-11 was detected in 134 formalin-fixed and paraffin-embedded (FFPE) BCa tissues and 10 paired nonneoplastic bladder tissue specimens by immunohistochemistry. The copy number of IPO11 was examined in 25 FFPE BCa specimens using fluorescent in situ hybridization. The effects of IPO11 on migration, invasion, and cell proliferation were investigated in EJ and 5637 cell lines using RNA interference. Potential molecular mechanisms were investigated using whole transcriptome sequencing and bioinformatic approaches in EJ cells and IPO11-silenced EJ cells and verified using quantitative real-time polymerase chain reaction.

Results: Endogenous IPO11 mRNA was highly expressed in 6 invasive BCa cell lines (EJ, HT-1376, UMUC3, 5637, J82, and T24) but had a low expression in the noninvasive BCa cell line BIU-87 and the papillary BCa cell line RT4. Immunohistochemical staining revealed that 87 (64.9%) of 134 FFPE BCa tissues displayed importin-11 overexpression. Moreover, importin-11 overexpression was positively associated with increased tumor stages and tumor grades, lymphatic invasion, and lymph node metastasis. Furthermore, importin-11 overexpression was detected in 100% (14/14) of BCa tissues with IPO11 amplification, and IPO11 amplification was not observed in 2 additional BCa tissues with importin-11 overexpression. Small interfering RNA-mediated knockdown of IPO11 is sufficient to inhibit the motility and invasiveness of EJ and 5637 cells. IPO11 knockdown also inhibited cell proliferation in EJ cells, whereas this was not observed in 5637 cells or the in vivo experiments. Using whole transcriptome sequencing, we found that 22 genes (including IPO11) were differentially expressed in IPO11-silenced EJ cells compared with wild-type EJ cells, 4 of which were upregulated, and 18 of which were downregulated. KEGG pathway enrichment analysis of the significantly differentially expressed genes showed that the proteoglycans in cancer pathway (pathway Id: hsa05205) was most significantly enriched among 10 genetically altered pathways and referred to 6 significantly altered genes (CDKN1A, HBEGF, PTK2, THBS1, CCNG2, and EGR1). The next 3 most significantly enriched pathways in order were the p53, ErbB, and BCa pathways. CDKN1A and THBS1 were the most 2 frequently covered genes and were involved in 9 and

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6 pathways, respectively. They were also 2 key proteins in the BCa pathway (pathway Id: hsa05219) that were downregulated in IPO11-knockdown EJ cells compared with wild-type EJ cells.

Conclusions: Importin-11 overexpression can promote BCa cell invasiveness, probably associated with the deregulation of CDKN1A and THBS1 primarily through the activation of the proteoglycans in cancer pathway and the classical BCa pathway. Importin-11 may be a useful target through which the progression of noninvasive BCa to invasive BCa can be blocked.

Keywords: Bladder cancer; Tumor progression; IPO11; CDKN1A; THBS1

1. Introduction

Bladder cancer (BCa) is the most common urinary malignancy in both China [1] and the United States [2]. BCa can be divided into 2 disease subgroups: non–muscle-invasive BCa (NMIBC) subgroup and muscle-invasive BCa (MIBC) subgroup. These subgroups have distinctive molecular and clinical properties [3]. The progression of NMIBC, noninvasive papillary cancer or carcinoma in situ, to MIBC is the key step leading to a poor prognosis in patients with the disease. However, the factors that trigger this progression remain unclear.

We previously found that IPO11 copy number amplification and importin-11 overexpression were the early molecular events in BCa progression by performing intratumoral spatial heterogeneous studies using exon sequencing, fluorescent in situ hybridization (FISH) and immunohistochemical analysis. Importin-11 overexpression was first reported to be correlated with poor survival in patients with BCa [4]. Importin-11 is encoded by IPO11 and is a member of the karyopherin family, which mediates the nucleocytoplasmic transport of macromolecules through nuclear pore complexes [5]. In recent years, abnormal subcellular distributions of tumor-related proteins have been found to be responsible for carcinogenesis and tumor progression [6–8]. Karyopherins have gradually been identified as critical nodes in the regulation of nuclear transport processes and tumorigenesis. Karyopherin family members, such as karyopherin-α2 and karyopherin-β1, have been reported to be overexpressed in various cancers, and their overexpression caused worse oncological outcomes [9–21]. However, the biological function of IPO11 and the molecular mechanisms by which it contributes to BCa progression remain unknown.

In this study, we investigated the function of IPO11 using in vivo and in vitro experiments and explored the molecular mechanisms through which BCa progression is regulated by importin-11 overexpression via mRNA sequencing and bioinformatic analysis.

2. Materials and methods

2.1. Cell lines and cell cultures

Eight bladder cell lines (BIU-87, RT4, UMUC3, EJ, 5637, T24, J82, and HT-1376) were selected for this study. The human BCa cell lines RT4, UMUC3, EJ, 5637, T24, J82, and HT-1376 were purchased from the American Type Culture Collection (ATCC, Manassas, VA), and the BCa cell line BIU-87 was purchased from Shanghai Obio Technology Corp., Ltd. (Obio Technology Co., Ltd., Shanghai, China). All cell lines were cultured in RPMI-1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (GE Healthcare—HyClone Laboratories Inc., South Logan, UT). All cells were grown in a humidified incubator at 37°C with 5% CO_2.

2.2. Patients and specimen characteristics

We obtained formalin-fixed and paraffin-embedded (FFPE) tissues from 134 patients with BCa who underwent radical cystectomy between January 2009 and December 2013 from the archives of the Department of Pathology of Changhai Hospital (Shanghai, China). Ten adjacent normal bladder mucosa specimens were also collected from these patients in 2013. All tissue samples were reviewed by an attending genitourinary oncology pathologist to confirm the diagnoses of BCa and normal mucosa. All sample collection procedures were approved by the Institutional Review Board of Changhai Hospital.

2.3. Immunohistochemistry

A polyclonal rabbit antibody (14403–1-AP; Proteintech, Chicago, IL) was used to assess importin-11 expression. Optimal staining was achieved at a 1:50 antibody dilution. Immunohistochemical staining was analyzed independently by 2 pathologists. Importin-11 staining was evaluated according to the staining intensity (0, 1+, 2+, and 3+) and determination of the percentage of positive tumor cells for each slide. A final immunohistochemistry (IHC) score was calculated according to these parameters as described previously [22], indicating negative, weak, moderate, or strong staining. Moderate and strong importin-11 staining were considered as importin-11 overexpression.

2.4. Fluorescent in situ hybridization

Two-color FISH probes were applied to the FFPE BCa tissue sections using an IPO11/AHRR FISH Probe Kit (Cytotest Inc., Rockville, MD). The kit includes a locus-specific orange probe for the IPO11 gene, which is located