Archives of Medical Research 47 (2016) 411–418

Archives of Medical Research

ORIGINAL ARTICLE

COPB2 Is Upregulated in Prostate Cancer and Regulates PC-3 Cell Proliferation, Cell Cycle, and Apoptosis

Yuanyuan Mi,[a,b,*] Menglei Yu,[c,*] Lifeng Zhang,[d,*] Chuanyu Sun,[a,*] Bingbing Wei,[e,*] Weihong Ding,[a] Yingfeng Zhu,[f] Jianmin Tang,[f] Guowei Xia,[a] and Lijie Zhu[b]

[a]*Department of Urology, Huashan Hospital, Fudan University, Shanghai, PR China*
[b]*Department of Urology, Third Affiliated Hospital of Nantong University, Wuxi, Jiangsu, PR China*
[c]*Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetics and Gene Regulation, Emergency Department, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, PR China*
[d]*Department of Urology, Changzhou No. 2 People's Hospital Affiliated to Nanjing Medical University, Changzhou, PR China*
[e]*Department of Urology, Affiliated Wuxi People's Hospital of Nanjing Medical University, Jiangsu, PR China*
[f]*Department of Pathology, Huashan North Hospital, Fudan University, Shanghai, PR China*

Received for publication April 16, 2016; accepted September 13, 2016 (ARCMED-D-16-00231).

Background and Aims. Transport of membranes and proteins in eukaryotic cells is mediated by vesicular carriers. Coatomer complex I (COPI)-coated vesicles are involved in the transport between endoplasmic reticulum (ER) and Golgi complex. Several studies indicated that some subunits of COPI were correlated with the cell proliferation of malignant tumors. The present study focused on the function of coatomer protein complex subunit β 2 (COPB2), one of seven proteins in COPI, in prostate cancer (PCa).

Methods. COPB2 gene expression was first analyzed by immunohistochemistry (IHC) in 15 paired PCa and carcinoma adjacent normal tissue from patients. To investigate the role of COPB2 in PCa, we used lentivirus-mediated small interfering RNA (siRNA) to knockdown COPB2 expression in human PCa cell line PC-3 and assessed it by RT-qPCR. Cellomics ArrayScan VTI imaging and colony formation were conducted to evaluate cell proliferation. Cell cycle phase arrest and apoptosis were assayed by flow cytometry.

Results. COPB2 gene was upregulated in the PCa tissue. Cell proliferation was significantly inhibited in COPB2-silenced PC-3 cells using both Cellomics ArrayScan VTI imaging and colony formation assays. S-phase cell counts were significantly decreased; G1- and G2-phase cell counts were significantly increased in COPB2-siRNA group than the control group. Apoptosis was significantly increased in COPB2-siRNA cells.

Conclusions. COPB2 significantly promoted PC-3 cell proliferation and colony formation through the cell cycle and apoptosis pathway. Moreover, COPB2 showed a clinical correlation and may serve as a biomarker for the detection for PCa. © 2016 IMSS. Published by Elsevier Inc.

Key Words: COPB2, Lentivirus, Prostate cancer, Proliferation, Immunohistochemistry.

Introduction

Prostate cancer (PCa) is the second most frequently diagnosed cancer and fifth in the cancer-related deaths among men with an estimated 1.1 million new cases diagnosed and 30.7 thousand deaths worldwide in 2012 (1). Although the mortality rate has been decreasing in some parts of the world such as North America, Oceania, Northern and Western Europe, the rate of incidence and mortality has increased in Central and Eastern Europe, Africa, and Asia,

*These authors contributed equally to this work.

Address reprint requests to: Guowei Xia, Professor, Department of Urology, Huashan Hospital, Fudan University, 12 Central Urumqi Rd, Shanghai 200040, PR China; Phone: 021-52889999; FAX: 021-52889999; E-mail: xiaguoweihuashan@sina.com, xiaguowei@fudan.edu.cn; Lijie Zhu, Professor, Department of Urology, Third Affiliated Hospital of Nantong University, 585 North Xingyuan Rd, Wuxi 214041, Jiangsu, PR China; Phone: 0510-82607391; FAX: 0510-82607391; E-mail: zhulijiemeta@hotmail.com.

0188-4409/$ - see front matter.
http://dx.doi.org/10.1016/j.arcmed.2016.09.005

especially in China (2). For example, the total number of PCa cases is 49,000, the rate of incidence is $7.1/10^5$, and the age-standardized incidence/mortality rate was 0.42 in 2011. Moreover, a median period of a 24-year study reported that the incidence in persons from Shanghai and Hong Kong is from $5/10^5$ in the 1980s–$11/10^5$ in 2010 (3,4). The potential causes may be rapid economic development, improvement in quality of life, extended life expectancy, dietary habits (such as fat consumption and obesity), a lifestyle similar to Western countries, and improved diagnosis in hospitals (2,5). Moreover, genetic and environmental factors are also considered and explored.

Advanced and metastatic PCa patients have been managed with androgen-deprivation therapy (ADT). Virtually, most patients show disease progression at a median of 18–24 months with recurrent castration-resistant PCa (CRPC) (6). After ADT, docetaxel/prednisone chemotherapy has been regarded as the standard for metastatic CRPC (mCRPC) (7). However, none of these therapies is curative, and patients ultimately demonstrate metastatic progression. Over the last decade, agents in gene therapy or immunotherapy such as aflibercept, sipuleucel-T, CTLA-4, and PD-1 have shown a survival benefit to men with mCRPC (8,9). Despite these initial successes, additional gene therapy targets for PCa are essential (10).

Coatomer protein complex subunit β (COPB) is one of the seven nonclathrin-coated vesicular coat subunits that form the coatomer complex I (COPI), which plays a role in membrane transport between endoplasmic reticulum (ER) and Golgi apparatus (11). COPB2, the main member of COPB family, is comprised of 906 amino acids, and its chromosomal localization is 3q2.3 (12). Two recent studies reported that some subunits (including COPB2) in COPI were correlated with the cell proliferation of malignant tumors (13,14), suggesting that COPI may play a vital role in cancer development and progression. In the current study we examined the expression of COPB2 in human PCa tissue and cell lines such as PC-3, using lentivirus-mediated small interfering RNA (siRNA) targeting COPB2 in order to ascertain its function in cell proliferation, cell cycle, and apoptosis.

Materials and Methods

Tissue Specimens and Collection

PCa and paired carcinoma adjacent normal specimens were obtained from 15 patients who underwent radical prostatectomy (RP) between January 2014 and November 2015 in the Huashan Hospital of Fudan University and the Third Affiliated Hospital of Nantong University. The diagnosis was based on the pathological examination at the Department of Pathology in the same hospital. None of the patients recruited in our study was subjected to chemotherapy or radiotherapy before surgery. The specimens were fixed with 10% formaldehyde and embedded in paraffin for histological sectioning. Clinical information including age, body height, weight, pathology, and TNM stage was assimilated in a database. Approval for the experiments was obtained from the ethics committees at the Huashan Hospital of Fudan University and the Third Affiliated Hospital of Nantong University. The samples were collected after obtaining informed consent from patients.

Immunohistochemical Analysis

For IHC analysis, 4-μm sections from formalin-fixed-paraffin-embedded tissue were transferred to polylysine-coated slides. The sections were deparaffinized in xylene and dehydrated with ethanol as alcohol gradient followed by blocking endogenous peroxidase activity with 0.5% H_2O_2 in methanol for 10 min. Nonspecific binding was blocked by incubating the sections with 10% normal goat serum in PBS for 1 h at room temperature. Subsequently, without washing, the sections were incubated with rabbit anti-COPB2 antibody (1:100, Abcam, USA) in PBS at 4°C overnight in a moist chamber. The slides were then probed for 10 min with an HRP-labeled polymer conjugated to an appropriate secondary antibody. The percent of positive cells was scored as '0' (0%), '1' (1–25%), '2' (26–50%), '3' (51–75%), and '4' (>76%). The staining intensity was scored as '0' (no staining), '1' (weakly stained), '2' (moderately stained), and '3' (strongly stained). The final COPB2 integrated haplotype score (IHS) was calculated using the percent of positive cell score × staining intensity score (0–2). A high COPB2 expression level was defined as a total score ≥6 and a low COPB2 expression level was defined as a total score <6.

Cell Culture

Four PCa cell lines PC-3, DU-145, CWR22RV1, and LNCaP, were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China) and cultured in RPMI-1640 medium (Gibco, USA) containing 10% fetal calf serum (Gibco), 100 μg/mL streptomycin (Sangon Co. Ltd., Shanghai, China), and 100 U/mL penicillin (Sangon Co. Ltd.) at 37°C in a 5% CO_2 incubator. The media of all four cell lines was replaced every 3 days and the cells were harvested upon reaching about 80% confluence.

Real-time Quantitative PCR (RT-qPCR) for Endogenous COPB2 Expression Detection in Prostate Cancer Cell Lines

RNA extraction from the 4 PCa cell lines was performed with a TRIzol kit (Pufei Corp., Shanghai, China) and reverse transcribed into cDNA with random primers by M-MLV reverse transcriptase (Promega, Madison, WI) according to the manufacturer's instructions. Two sets of primers used for PCR were designed by Beacon Designer 2 software (Premier Biosoft International, Palo Alto, CA) as follows:

GAPDH-F, 5′-TGACTTCAACAGCGACACCCA-3′, GAPDH-R, 5′-CACCCTGTTGCTGTAGCCAAA-3'; COPB2-F, 5′-GTGGGGACAAGCCATACCTC-3′, COPB2-R, 5′-GTGCTCTCAAGCCGGTAGG-3'.