Cancer Letters 359 (2015) 218–225

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Original Articles

PTP1B promotes cell proliferation and metastasis through activating src and ERK1/2 in non-small cell lung cancer

Hongbing Liu, Ying Wu, Suhua Zhu, Wenjun Liang, Zhaofeng Wang, Yunfen Wang, Tangfeng Lv, Yanwen Yao, Dongmei Yuan, Yong Song *

Department of Respiratory Medicine, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China

ARTICLE INFO

Article history:
Received 10 September 2014
Received in revised form 14 January 2015
Accepted 14 January 2015

Keywords:
Protein tyrosine phosphatase 1B
Proliferation
Metastasis
Src
Non-small cell lung cancer

ABSTRACT

Previous studies have demonstrated that protein tyrosine phosphatase 1B (PTP1B) can promote tumor progression in breast cancer, colon cancer and prostate cancer. Additionally, PTP1B acts as a tumor suppressor in other cancers, such as esophageal cancer and lymphoma. These findings suggest that PTP1B functions as a double-facet molecule in tumors, and the role of PTP1B in non-small cell lung cancer (NSCLC) is unknown. The present study demonstrates that the expression of PTP1B in NSCLC tissue is significantly higher than its expression in benign lung disease and is associated with the stage and overall survival (OS) of NSCLC patients. *In vitro* studies have demonstrated that PTP1B promotes the proliferation and metastasis of NSCLC cells by reducing the expression of p-src (Tyr527), which activates src and ERK1/2. This study provides the first exploration of the role of PTP1B in the proliferation and metastasis of NSCLC and subsequently elucidates the role of PTP1B in cancer. Our study uncovered that PTP1B can promote NSCLC proliferation and metastasis by activating src and subsequently ERK1/2 and provides a theoretical basis for future applications of PTP1B inhibitors in the treatment of NSCLC.

© 2015 Elsevier Ireland Ltd. All rights reserved.

Introduction

Among all malignancies, lung cancer has one of the highest rates of mortality [1], often due to metastasis of the primary tumor, and non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancers. In recent years, despite the development of new treatments, such as platinum-based chemotherapy and molecular targeted drugs, the five-year survival rate for NSCLC has remained at ~15% because 85% of NSCLCs are at an advanced stage at the time of diagnosis [2,3]. Therefore, researchers continue to explore factors that affect the proliferation and metastasis of lung cancer cells with the aim of finding new ways to control the occurrence and development of lung cancer.

The proliferation and metastasis of lung cancers are regulated by a variety of signaling pathways [4]. Protein tyrosine phosphatases (PTPs) and protein tyrosine kinases (PTKs) maintain a balance of protein activity through the dephosphorylation and phosphorylation of proteins in a signaling pathway [5,6]. An imbalance of PTP and PTK activities can lead to abnormalities in the signaling pathway and then disease, including lung cancer. Many previous studies focusing on PTKs have found that the overexpression or mutation of many PTKs will lead to the development of lung cancer [7–9]; however, less studies have investigated PTPs in lung cancer, therefore, our study aimed to study the roles of PTPs in lung cancer. Protein tyrosine phosphatase 1B (PTP1B) is an important member of the PTP family, and previous studies have shown that PTP1B plays an important role in metabolic diseases, e.g., diabetes and obesity [10,11]. Furthermore, PTP1B may be a double-facet molecule in tumors: it can promote tumor progression in breast, colon, and prostate cancers [12,13] but also functions as a tumor suppressor in other cancers, such as esophageal cancer and lymphoma [14,15]. This dual role of PTP1B suggests that its function is tissue-specific, and no previous study has investigated the roles of PTP1B in NSCLC. Therefore, we conducted this study to explore the roles of PTP1B in NSCLC.

Materials and methods

Materials/antibodies

The agents used in this study and their suppliers are the following: anti-PTP1B (Ab-1) mouse mAb (FG6-1G) (Calbiochem, PH01); GAPDH antibody (SunShineBio, SAP1691); Src (32G6) rabbit mAb (Cell Signaling Technology, 2123); p-src (Tyr527) antibody (Cell Signaling Technology, 2105); ERK1/2 (L352) pAb (bioWorld, BS1112); p-ERK1/2 (T202/Y204) pAb (bioWorld, BS5016); N-cadherin antibody (Cell Signaling Technology, 9782); E-cadherin (24E10) rabbit mAb (Cell Signaling Technology, 9782); β-catenin rabbit mAb (Cell Signaling Technology, 9782); vimentin (D21H3) XP rabbit mAb (Cell Signaling Technology, 9782); p-AKT (ser473) (D9E) XP rabbit

Abbreviations: PTP1B, protein tyrosine phosphatase 1B; NSCLC, non-small cell lung cancer; OS, overall survival; PTPs, protein tyrosine phosphatases; PTKs, protein tyrosine kinases.

* Corresponding author. Tel.: +86 25 80860148; fax: +86 25 8086 3591.
E-mail address: yong_song6310@yahoo.com (Y. Song).

http://dx.doi.org/10.1016/j.canlet.2015.01.020
0304-3835/© 2015 Elsevier Ireland Ltd. All rights reserved.

Table 1
NSCLC patient demographics and clinicopathology.

Characteristic	Value (%)
Number of patients	63
Gender	
Male	45 (71.43)
Female	18 (28.53)
Age	
<60	30 (47.62)
≥60	33 (52.38)
Smoking habits (case-years)	
<20	36 (57.14)
≥20	27 (42.86)
Tumor size	
<4 cm	18 (28.57)
≥4 cm	45 (61.43)
Histology	
Adenocarcinoma	28 (44.44)
Squamous	30 (47.62)
Adeno-squamous	5 (7.94)
Lymph node metastasis	
No	27 (42.86)
Yes	36 (57.14)
pTNM stage	
I	20 (31.75)
II	19 (30.16)
III	24 (38.09)

mAb (Cell Signaling Technology, 4060); Akt (pan) (11E7) rabbit mAb (Cell Signaling Technology, 4685); β-actin pAb (bioWorld, AP0060); and adenovirus Ad-PTP1B (Hanbio, China); and Ad-GFP (Hanbio, China).

Patients

Sixty-three patients with NSCLC (Table 1) and thirty patients with benign lung disease (pneumonia) who were diagnosed at Jingling Hospital (Nanjing, Jiangsu Province, China) between 2000 and 2006 were enrolled in this study. All of the patients underwent lung surgery, and all of the final diagnoses were confirmed by pathological examination. None of the enrolled NSCLC patients had received any prior chemotherapy or radiotherapy. Patients with NSCLC were administered two to four cycles of adjuvant chemotherapy based on the stage of their tumor. Patient follow-up visits were continued until June 01 2012, and the overall survival (OS) of the NSCLC patients was measured from date of NSCLC diagnosis until date of death or last follow-up.

Cell culture

A549, SPCA1 and PC9 cells were grown in DMEM (Gibco, C11995) supplemented with 10% fetal bovine serum (Hyclone) and maintained at 37 °C in a humidified atmosphere with 5% CO_2.

siRNA transfections

A549, SPCA1 and PC9 cells were seeded in six-well plates (3×10^5 cells/well) and cultured in DMEM (Gibco, C11995) supplemented with 10% fetal bovine serum (Hyclone) without antibiotics for 24 h prior to transient transfection. The Lipofectamine® 2000 reagent (Invitrogen, 1070962) was used for siRNA transfection following the manufacturer's instructions, and the media were replaced 6 h post-transfection. The siRNA of PTP1B, src and ERK1/2 was from Shanghai GenePharma Co., Ltd., Shanghai, China.

MTT cell growth assay

After incubation for 24 h, the cells were seeded in six wells of a 96-well plate at a density of 1×10^3 cells/well. The total cell numbers were assessed every 24 h after incubation with 20 µL of MTT (5 mg/mL) for 4 h. Following incubation, the cell supernatants were carefully aspirated from each well and discarded, and 100 µL of DMSO was added to each well. The plates were then oscillated in the dark for 10 min until the crystals were fully dissolved. The optical density (OD) of each well was then measured with a microplate reader at 490 nm, and the OD values are reported as the means ± SD.

Colony formation assays

The cells were transfected in six-well plates for 24 h with siRNA or adenovirus, placed in six-well plates (500 cells per well) and maintained in media containing 10% FBS. The medium was replaced every four days; after 14 days, the cells were fixed with methanol and stained with 0.1% crystal violet. The visible colonies were then counted. Each group was assessed using triplicate wells.

Transwell migration and invasion assay

After transfection with siRNA or adenovirus for 24 h, the cells were digested with 0.25% trypsin–EDTA and then centrifuged (1000 rpm for 5 min). The cells were then washed once or twice with PBS and resuspended in DMEM containing 0.2% BSA. The cells were counted using a hemocytometer and adjusted to a cell density to 2×10^5/mL. Then, 200-µL aliquots of the diluted cells were added to the upper portion of a Transwell® migration chamber (invasion with coated with Matrigel), and 500 µL of DMEM with 10% FBS was added to the lower chamber. The chambers were incubated with 5% CO_2 at 37 °C for 24 h, the supernatants were discarded, and the membranes were fixed with 4% formaldehyde for 20 min. The cells from the upper well were swabbed using cotton buds, rinsed with PBS, and air-dried on a table. Then, 800 µL of freshly prepared Giemsa working solution was added to each well, and the upper chamber was immersed in the Giemsa working solution for 15–20 min and then rinsed with distilled water. Six fields of each Transwell® chamber were photographed using an inverted wide-field microscope at 200× magnification. Each condition in the experiment was performed at least in duplicate. The number of cells in each image was counted using the ImageJ software, and the investigators were blinded to which image was being counted to avoid bias.

Western blotting

The cell proteins were extracted using an extraction kit purchased from KeyGEN Biotech (Nanjing, China) according to their recommended protocol. The protein concentrations were determined using the Bradford method with a Protein Assay kit (KeyGEN Biotech). The protein samples (50 µg) were resolved by 10% SDS-PAGE and transferred to PVDF membranes. The membranes were blocked with PBST buffer (PBS plus 0.05% Tween-20) containing 5% w/v nonfat milk, incubated overnight with several antibodies at 4 °C, and then incubated with a specific secondary antibody for 2 h at room temperature. Protein bands were visualized using an ECL detection system (Alpha Innotech, Johannesburg, South Africa).

Apoptosis by flow cytometry

Apoptosis was investigated by flow cytometry using an Apoptosis Kit (Invitrogen, V13241). Forty-eight hours after transfection with siRNA or adenovirus, the cells were harvested and washed in cold PBS. The cells were then re-centrifuged, the supernatant factions were discarded, and the washed cells were resuspended in 1× annexin-binding buffer. The cell density in the resuspended dilution was adjusted to 1×10^6 cells/mL with 1× annexin-binding buffer, and a 100-µL aliquot of the suspension was used for each assay. Then, 5 µL of Annexin V and 1 µL of PI working solution (100 µg/mL) were added to each 100-µL aliquot of the cell suspension, and the suspension was incubated at RT for 15 min. After the incubation period, 400 µL of 1× annexin-binding buffer was added to the suspension and mixed gently, and the stained cells were analyzed by flow cytometry (BD FACSCalibur) by measuring the fluorescence emissions at 530 nm and 575 nm (or equivalent) following excitation at 488 nm.

Immunohistochemistry

Immunohistochemistry (IHC) was performed as described by Wang et al. [16]. The stained slides were independently scored by two observers blinded to the clinicopathological information of the patients. In cases of different opinions, agreement was reached through careful discussion between the evaluators. The percentage of positive cells was classified according to four grades (percentage scores): <10% (grade 0), 10%–20% (grade 1), 21%–50% (grade 2), and >50% (grade 3). The staining intensity was classified according to four grades (intensity scores): no staining (grade 0), light-brown staining (grade 1), brown staining (grade 2), and dark-brown staining (grade 3). PTP1B staining positivity was determined using the following formula: overall score = percentage score × intensity score. Overall scores of <2 and ≥2 were defined as negative and positive staining, respectively.

Tumorigenicity assay in mice

PC9 cells were transfected with either PTP1B siRNA or control siRNA. Twenty-four hours after transfection, the trypsinized cells were harvested by low-speed centrifugation (4 °C, 1000 rpm, 5 min), washed twice with PBS and resuspended to a concentration of 5×10^7 cells/mL in PBS. The cells in 0.1 mL of PBS were inoculated s.c. into the front-right armpit of seven-week-old male severe combined immunodeficient (SCID) mice (five mice in each group). Measurements of the tumor diameter (a) and short axis (b) were taken every two to three days with a veneer caliper. The data were recorded as the average diameter of measurements a and b. The tumor volume was calculated using the following formula: $ab^2/2$. The mice were sacrificed 26 days after inoculation, and the tumors that formed at the site of injection were excised, weighed, and photographed.

Download English Version:

https://daneshyari.com/en/article/2112515

Download Persian Version:

https://daneshyari.com/article/2112515

Daneshyari.com