Biomedicine & Pharmacotherapy 100 (2018) 233–239

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

SPC25 upregulation increases cancer stem cell properties in non-small cell lung adenocarcinoma cells and independently predicts poor survival

Jingxia Chen, Hongfen Chen*, Hanbing Yang, Huizhen Dai

Department of Emergency Medicine, The Affiliated Hospital of Weifang Medical University, Weifang, Shandong, 261031, China

ARTICLE INFO

Keywords:
SPC25
Cancer stem cell
NSCLC
Adenocarcinoma
Survival

ABSTRACT

In this study, we investigated the functional role and prognostic value of spindle pole body component 25 (SPC25) in non-small cell lung cancer (NSCLC). *SPC25* expression profile in lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC) and normal lung tissues was examined by using data from the Cancer Genome Atlas (TCGA) and the Human Protein Atlas (HPA). LUAD A549 cells and LUSC H520 cells were used to investigate the influence of SPC25 on cancer stem cell (CSC) properties in terms of the proportion of $CD133^+$ cells, tumorsphere formation and CSC markers, including CD133, ALDH1 and Sox2. Data mining was also performed in the Kaplan-Meier plotter and TCGA-NSCLC to assess the independent prognostic value of *SPC25*. Results showed *SPC25* was significantly upregulated in LUAD and LUSC tissues compared with normal lung tissues. *SPC25* overexpression significantly increased the CSC properties and invasion of A549 cells, but not H520 cells. In comparison, *SPC25* knockdown impaired the CSC properties and invasion of A549 cells, but not H520 cells. Univariate and multivariate analysis confirmed that high *SPC25* expression was an independent prognostic factor for poor overall survival (OS) (HR: 1.622, 95%CI: 1.207–2.178, $p = .001$) and recurrence-free survival (RFS) (HR: 1.726, 95%CI: 1.242–2.399, $p = .001$) in LUAD patients. However, no independent prognostic value of *SPC25* was observed in LUSC patients even under the best cut-off model. Based on these findings, we infer that *SPC25* upregulation can increase CSC properties in LUAD and independently predict poor survival in this histological subtype.

1. Introduction

Spindle pole body component 25 (SPC25) is a component of the nuclear division cycle 80 (Ndc80) complex, together with other three proteins, including Ndc80 (also called Hec1 or KNTC2), Nuf2 and SPC24 [1]. This complex is essential for chromosome segregation by regulating kinetochore assembling and the spindle checkpoint signaling [2]. Functionally, SPC25 can form a heterodimer with SPC24, which governs microtubule-kinetochore attachment, chromosome alignment, and spindle checkpoint activation in mitosis [1,3].

Some recent studies found that dysregulated *SPC25* is associated with the oncogenic process and malignant phenotypes of some cancers. *SPC25* upregulation has been reported in colorectal and gastric cancers [4]. It acts as a Wnt-regulated recurrence-associated gene in hepatocellular carcinoma and is associated with early recurrence after potentially curative liver resection [5]. Its expression is particularly high in basal breast cancer subtype compared with other subtypes and is related to reduced overall patient survival [6]. However, the function and prognostic value of *SPC25* in non-small cell lung cancer (NSCLC) remains unclear.

NSCLC is a heterogeneous group of cancers that includes lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) with similar frequencies, and large cell carcinoma with a lower rate (< 10%) [7]. These subtypes have distinct sites of origin and numerous genetic and epigenetic differences, which are root causes of their unique responses to therapies [8,9]. The hypothesis of cancer stem-like cells (CSCs) indicates that the persistence of CSCs is responsible for tumor initiation, development, metastasis and is the ultimate cause of treatment failure [10]. However, different histological subtypes of NSCLC may acquire self-renewing properties through various genetic or epigenetic mechanisms [10].

In this study, we found that *SPC25* was significantly upregulated in both LUAD and LUSC compared with normal lung tissues. However, its upregulation was associated with enhanced CSC properties and independently predicted poor prognosis in terms of overall survival (OS) and recurrence-free survival (RFS) only in LUAD, but not in LUSC.

* Corresponding author.
E-mail address: hongfenchen@tom.com (H. Chen).

https://doi.org/10.1016/j.biopha.2018.02.015
Received 19 November 2017; Received in revised form 1 February 2018; Accepted 2 February 2018

2. Materials and methods

2.1. Data mining in the cancer genome atlas-NSCLC (TCGA-NSCLC)

The level 3 demographic, clinicopathological and survival data of patients with primary NSCLC in TCGA-NSCLC were obtained by using the UCSC Xena browser (https://xenabrowser.net/). *SPC25* expression data in LUAD and LUSC were extracted separately and were compared with that in normal lung tissues.

2.2. Data mining in the human protein atlas (HPA)

SPC25 expression at the protein level in normal lung, normal bronchus, LUAD and LUSC was examined by using immunohistochemistry (IHC) data in the HPA (http://www.proteinatlas.org/) [11,12].

2.3. Cell culture and infection

Human lung adenocarcinoma A549 and squamous cell carcinoma cell lines NCI-H520 (H520) were purchased from the American Type Culture Collection and were maintained in low passage culture as recommended. In brief, A549 and H520 cells were maintained in RPMI-1640 medium containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in a humidified atmosphere containing 5% CO_2 at 37 °C. *SPC25* Human Lentifect Purified Lentiviral Particles (NM_020675.3) and the corresponding empty control were obtained from GeneCopoeia (Rockville, MD, USA). The ready-to-use *SPC25* shRNA (h) lentiviral particles (sc-76554-V) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The detail of this product can be accessed *via*: https://www.scbt.com/scbt/product/spc25-sirna-h-shrna-and-lentiviral-particle-gene-silencers. A549 and H520 cells were infected with the lentiviral particles or the negative controls in the presence of polybrene. 24 h after infection with the lentiviral particles, the cells were subjected to western blotting analysis of SPC25 expression.

2.4. Western blotting

Cell samples were collected and then were lyzed by using RIPA buffer (Beyotime, Shanghai, China). Denatured samples (30 μg per lane) were separated by using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and were transferred to nitrocellulose membrane (EMD Millipore, Billerica, MA, USA). The membranes were incubated with primary antibodies against SPC25 (1:500, ab20679, Abcam, Cambridge, UK), CD133 (1:1000, ab19898, Abcam), ALDH1 (1:2000, ab52492, Abcam), Sox2 (1:1000, ab97959, Abcam) and GAPDH (1:2500, ab9485, Abcam). After washing, the membranes were further incubated with secondary antibodies coupled to HRP, according to the supplier's recommendations. The blot signals were visualized using the SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, lL, USA).

2.5. qrt-PCR

24 h after infection of lentiviral *SPC25* shRNA particles or the empty control, A549 and H520 cells were lysed to extract total RNA, with the use of Trizol Reagent (Invitrogen, Carlsbad, CA, USA). cDNA was reversely transcribed using the PrimeScript® RT reagent kit (TaKaRa, Dalian, Liaoning, China). Then, qRT-PCR analysis was performed on an ABI 7900HT sequence detector, with the following primers (*SPC25*, forward: 5′-GAGATACCTACAAGGATTCCA-3′; reverse: 5′-GCTGATCTG ATTTTGATATTCC-3′) and TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA). GAPDH was used as the endogenous control gene. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative fold change of gene expression.

2.6. Flow cytometric analysis

Cells after indicating treatment were washed using PBS and then resuspended in FACS buffer (1X PBS, 1% BSA). The cells were labeled with anti-CD133-FITC (Miltenyi, Bergisch Gladbach, Germany) at 10 μl/1 × 10^6 cells at 4 °C in the dark for 30 min followed by washing with PBS. Then, the cells were analyzed using a FACS Calibur (BD Biosciences). Data were analyzed using CellQuest software (BD Biosciences).

2.7. Tumorsphere forming efficiency (TFE)

A549 and H520 cells with indicating treatment were seeded in ultra-low attachment 24-well plate (Corning Inc., Corning, NY, USA) (2 × 10^4 cells/well) and then were cultured in serum-free DMEM/F12 medium (Gibco, Carlsbad, CA, USA), supplemented with commercial hormone mix B27 (Gibco), 20 ng/ml EGF (Sigma-Aldrich, St. Louis, MO, USA), 10 ng/ml bFGF and 1 × B27 (Invitrogen). The plate was incubated at 37 °C with 5% CO_2 for 7 days, without touching the plate. Tumorspheres that were 50 μm or larger in size were counted. Tumorsphere-forming efficiency (TFE) was calculated using the following equation: TFE (%) = (number of spheres)/(number of cells plated) × 100%.

2.8. Transwell assay of cell invasion

Transwell assay was conducted by using a Matrigel invasion chamber (BD Bioscience) in a 24-well cell culture plate following the manufacturer's instruction. Briefly, A549 and H520 cells after transfections were seeded into chamber inserts containing an 8-μm pore size membrane with a thin layer matrigel matrix, with 500 μl serum-free RPMI-1640. The bottom of the well was filled with 700 μl RPMI-1640 medium with 20% FBS. 48 h later, cells invaded to the lower surface of the membrane were fixed, while the non-invading cells on the upper surface were removed. The invaded cells were stained with 0.1% crystal violet. Invasion was then determined for 3 independent fields under a microscope.

2.9. Data mining in the Kaplan-Meier Plotter

The association between the expression of *SPC25* and OS in patients with NSCLC was examined by data mining in Kaplan-Meier plotter, an online database to assess the prognostic value of biomarkers using transcriptomic data in 1715 samples [13]. The patients were divided into two groups by setting the best performing threshold of gene expression as the cut-off. The hazard ratio (HR) with 95% confidence intervals (CI) and log-rank *p*-value were calculated. The number-at-risk was indicated below the survival curves.

2.10. Statistical analysis

Statistical analysis was performed by using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). The association between *SPC25* RNA expression and the clinicopathological features was evaluated using χ^2 tests. Kaplan-Meier curves of OS and RFS after initial therapy were generated by GraphPad Prism v6.0. Log-rank test was performed to assess survival differences. Receiver operating characteristic (ROC) curves for death and recurrence detection were constructed. The optimal cut-off value of *SPC25* expression was determined based on Youden index. Prognostic values were analyzed by univariate and multivariate Cox regression models. Welch's *t*-test was performed to compare *SPC25* RNA expression between LUAD or LUSC and normal tissues groups. $p < .05$ was considered to be statistically significant.