Supplementary Materials and Methods

RT-PCR and primers

Total RNA was isolated using TRIzol reagent (Catalog No. 51-0700, Invitrogen, Camarillo, CA, USA). RNA was then reverse transcribed using Moloney murine leukemia virus reverse transcriptase and random primers (Catalog No. 51-0700, Invitrogen). Specific primers for each gene are shown below.

Gene Name	Sense (5'---3')	Anti-sense (5'---3')
BPIFA1 (SPLUCN1)	GCCTCATTGTCTTCTACGGGCTGTT	CTGAGGGCATTTGTCAAGCTTCCT
BPIFA2 (SPLUCN2)	ATGCTTCAGCTTTGGAA ACTTGTTCTCCTG	GATGAGGGTTTGCAGC TGGGTTTTGTGCTG
BPIFA3 (SPLUCN3)	TTGGGGACAGACTGAATGCC	GTTCATGAGCAGCCTCCTGT
BPIFA4p (BASE)	CAAGCCCTTAATGATTTGACTC	AGGTTTCTCTCTATGTTTGCCAC
BPIFB1 (LPLUNC1)	CCCTGCCCAATCTAGTGAAA	TCACCTTTCCCTGTGAGTCG
BPIFB2 (LPLUNC2)	TGTCCACCTGGGCACCTTAA	GGAAT AGCGGATCTGGGACTC
BPIFB3 (LPLUNC3)	CAGAATGTGCTGGGATCGGT	GACAACGCCAAAAACCCCTC
BPIFB4 (LPLUNC4)	GGTGACAACACCAAGTCCCA	CAGCATCACAGATGGTGGGT
β-actin	TTGCCGACAGGATGCAGAA	GCCGATCCACACGGAGTACTT

Antibodies

Antibodies against the following proteins and tags were purchased from the indicated sources: 14-3-3 (Catalog No. 51-0700, Invitrogen); p-14-3-3 (phosphor-Ser58, Catalog No. PPS042, R&D Systems, Minneapolis, MN, USA); 14-3-3 θ, 14-3-3 ζ

(Santa Cruz Biotechnology, Dallas, TX, USA); 251512 (LunX antibody, Catalog No. MAB1897, R&D Systems); Ki-67, (Abcam, HKSP, N.T. Hong Kong, China); anti-Flag (Sigma, St. Louis, MO, USA); Erk, phosphor-Erk, p38, phosphor-p38, AKT, phosphor-AKT, JNK, phosphor-JNK, c-Jun, phosphor-c-Jun, ATF-2, phosphor-ATF-2, (Cell Signaling Technology, Beverly, MA, USA); mIgG (Meridian Life Science, Memphis, TN, USA).

Determination of the toxic effects of the LunX antibody

Male C57BL/6 mice (5 weeks old, Shanghai Experimental Animal Center, China) were used to analyze the LunX antibody toxic effects *in vivo*. LunX antibody (30 mg/kg body weight) or PBS vehicle was injected i.v. twice weekly, and mouse behavior and body weight were analyzed 4 weeks later. Lung sections were stained with eosin and hematoxylin to determine histological changes.

Plasmid construction and oligonucleotides

The following expression plasmids were constructed in our lab: pCDNA3.0/LunX; pCDNA3.1/luciferase; p3XFLAG-myc-CMV-24/LunX; and pET-22b/LunX (His tagged). Small interfering RNAs against LunX were designed using the online tools on the Clontech web site. Short hairpin RNAs against LunX were constructed with the pRNAT-H1.1 vector and used for the establishment of stable cell lines. The small interfering RNAs and short hairpin RNAs targeted sequences were as follows: 5'-GGAAAAGUGACGUCAGUGA-3' ($LunX^1$); 5'-UCGGCAUAAAGCU CCAA

GU-3' ($LunX^2$); and 5'-UUCUCCGAACGUGUCACGUTT-3' (mock). 14-3-3 θ si-RNA and 14-3-3 ζ si-RNA were purchased from Santa Cruz Biotechnology.

Patient information

All tumor samples were collected between October 7th, 2004 and April 15th, 2010. Patients with pathologically proven NSCLC identified by routine imaging and cytologic assessments were selected. In addition, these specimens were selected based on the presence of primary NSCLC, mainly primary adenocarcinomas and squamous cell carcinomas, and no treatment prior to surgical resection. All cancer samples were classified according to TNM stage (American Joint Committee on Cancer, AJCC, TNM system). Informed consent was obtained from each subject, and the research was performed in compliance with the principles enunciated in the Helsinki Declaration with the approval of the Ethics Committee of the University of Science and Technology of China.

Analyses of human clinical samples

For immunohistochemistry, the grading of LunX expression was mainly based on the cell staining intensity and not the number of positive cells. LunX expression in each lung cancer tissue section was recorded using the following 4-point scale: 3 for intense staining (brown); 2 for moderate expression (yellow brown); 1 for faint or equivocal expression (light yellow); and 0 for no expression (no staining) (35). Two

board-certified pathologists scored each section independently without prior knowledge of the clinical characteristics of cancer patients and reached an agreement.

Establishment of the ELISA method

We established a double-antibody sandwich ELISA assay for LunX protein using two strains of hybridoma cell lines stably secreting anti-LunX monoclonal antibodies, S-20-15 and S-35-8, which were purified by protein-A affinity chromatography (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The S-20-15 was diluted in carbonate buffer (coating buffer) to 10 μg/ml and was packaged on 96-well polystyrene microtiter plates at 4°C overnight. Next, 1% BSA was used for sealing, and the samples of pleural fluid of NSCLC patients were diluted 1:1 in PBS. Purified His-tagged LunX protein and PBS were used as positive and blank controls, respectively. The horseradish peroxidase-marked S-35-8 (dilution at 100 ng/ml) was added for testing, and TMB was added for coloration. OD450<0.1, negative; OD450>0.1, positive.

Cytometry, wound-healing and transwell assays

Cell proliferation was determined by cytometry. In brief, $6x10^5$ cells were seeded into plates and then counted and scanned by microscopy after incubation for 24, 48 or 72 h. For the antibody blockade, the cells were treated with PBS (vehicle control), 160 μg/ml IgG, 80 μg/ml S-35-8 antibody or 160 μg/ml S-35-8 antibody for 48 h. Cell migration and invasion were analyzed using wound-healing and transwell permeable

support systems (Catalog No. CBA-100-C, Cell Biolabs, San Diego, CA, USA). In the wound-healing assay, the cells were serum-starved for 12 h in six-well plates. A linear wound was created using a pipette tip. Wound healing was observed at 12 and 24 h. The transwell system was used for migration and invasion studies according to the manufacturer's protocol. The cells were counted, placed on the inserts ($1 x 10^5$ cells) in serum-free medium and incubated for 4 h (migration assay) or 24 h (invasion assay) in a cell culture incubator. The cells were removed from the insert tops, and the cells that migrated/invaded through the polycarbonate basement membrane were stained and counted (20 random 400x fields per well).

Reference

1. Sun Y, Campisi J, Higano C, Beer TM, Porter P, Coleman I, et al. Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy resistance through WNT16B. Nature medicine 2012;18(9):1359-68.