

# Chimeric antibody targeting SRPK-1 in the treatment of non-small cell lung cancer by inhibiting growth, migration and invasion

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**Abstract.** Non-small cell lung cancer (NSCLC) is one of the most common types of cancer in humans, and is characterized by rapid growth, migration, invasion and reoccurrence. Evidence has indicated that the protein and mRNA levels of serine-arginine protein kinase-1 (SRPK-1) are upregulated in NSCLC tissues. However, the functions of SRPK1 and targeted therapy for SRPK1 in the progression and treatment of NSCLC remain to be fully elucidated. In the present study, the mRNA and protein expression levels of SRPK-1 in NSCLC cells and tissues were analyzed using reverse transcription-quantitative polymerase chain reaction analysis and SDS-PAGE, and the role of SRPK1 in the progression of NSCLC was investigated. In addition, a chimeric antibody target for SRPK-1 (ChanSRPK-1) was constructed, and the therapeutic effects of ChanSRPK-1 were investigated in H358-bearing mice. The curative effects of ChanSRPK-1 on the inhibition of growth, migration and invasion of NSCLC were also examined *in vitro* and *in vivo*. The results revealed that the mRNA and protein levels of SRPK-1 were upregulated in NSCLC cells and tumor tissues. Higher expression of SRPK1 promoted NSCLC cell growth, migration and invasion, whereas lower expression of SRPK-1 suppressed growth, migration and invasion of the NSCLC cells. Animal experiments demonstrated that ChanSRPK-1 inhibited the  $\beta$ -catenin/T-cell factor complex. ChanSRPK-1 treatment also downregulated the phosphorylation levels of glycogen synthase kinase 3- $\beta$  and prolonged the survival of tumor-bearing mice. Taken together, SRPK-1 may offer potential as a therapeutic target oncogenic molecular in NSCLC, and ChanSRPK-1 may be a therapeutic agent with functions as a target and for oncolytic therapy in the treatment of NSCLC.

## Introduction

Non-small cell lung cancer (NSCLC) is one of the most common types of cancer, owing to poor air quality and environmental pollution in the world (1). A previous study reported that tumor growth, migration and invasion in NSCLC are the most important features in tumor metastasis, development and reoccurrence (2). The occurrence of NSCLC has increased due to industrial pollution and the degradation of the ecological environment in the last century (3,4). NSCLC comprises adenocarcinoma, large cell carcinoma and squamous cell carcinoma, and is the most frequent type of lung cancer, which accounts for ~80% of all lung cancer cases (5-7). Despite increasing therapeutic improvements for NSCLC, the survival rates of patients with NSCLC are poor, with overall 5-year survival rates of <15%, indicating it a critical clinical problem (6,8,9). In addition, the migration and invasion of NSCLC are the primary reasons for the poor survival rates in treatment and recurrence for patients with NSCLC (10,11). Therefore, inhibiting the migration and invasion of NSCLC may offer potential as an efficient therapeutic schedule for patients with cancer (12,13).

Li *et al* reported that an increase in a pre-mRNA splicing regulator for serine-arginine protein kinase-1 (SRPK-1) increased cancer development and metastasis (14). Clinical investigations of the expression of SRPK-1 in NSCLC have also been performed, the results of which indicated that SRPK-1 exerted an oncogenic function in NSCLC and suggested that SRPK-1 may serve as a therapeutic target for patients with NSCLC (15). The increased expression of SRPK-1 is correlated with disease grade in several types of tumor, including retinoblastoma, breast cancer, pancreatic cancer and colon cancer (15-18). In the present study, a chimeric antibody target for SRPK-1 (ChanSRPK-1) was constructed and used to examine whether ChanSRPK-1 showed beneficial efficacy on the increased expression of SRPK1 in an NSCLC mouse model. The present study also evaluated the possible long-term survival of NSCLC-bearing mice, and investigated the therapeutic effects on expression of the  $\beta$ -catenin/T-cell factor (TCF) complex and the phosphorylation levels of glycogen synthase kinase 3- $\beta$  (GSK3- $\beta$ ) in cancer-associated processes.

The development of effective drugs to inhibit tumor cell growth, migration and invasion in patients with NSCLC has already focused on public and personalized medication.

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A previous study indicated that SRPK-1 was specifically expressed in epithelial cells in normal breast and colon cells (18). Of note, ChanSRPK-1-mediated neutralization has been demonstrated as a novel and beneficial antibody in the progress and metastasis of NSCLC (19).

Although suppressing the expression of SRPK-1 in tumor cells has demonstrated beneficial outcomes, the use of anti-SRPK-1 antibody for NSCLC therapy has not been investigated. The present study aimed to investigate the expression of SRPK-1, and the inhibitory effects of ChanSRPK-1 on migration and invasion in an NSCLC mouse model. ChanSRPK-1 inhibited the mRNA expression levels of TCF, matrix metalloproteinase-9 (MMP), collagen type I (CT1) and fibronectin (FBC) in NSCLC-derived vascular endothelial cells (NSCLCDVECs). The present study also investigated the therapeutic outcomes and molecular mechanism via long-term treatment with ChanSRPK-1, which indicated that the migration and invasion of NSCLC were inhibited following treatment with ChanSRPK-1.

## Materials and methods

**Cell culture.** The MRC-5 cells were purchased from American Type Culture Collection (Manassas, VA, USA). The NSCLCDVECs were provided by the University of Toronto (Toronto, Canada). The MRC-5 cells and NSCLCDVECs were cultured in EMEM supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37°C, 5% CO<sub>2</sub> and reasonable humidity.

**MTT assay.** The NSCLCDVECs (5x10<sup>3</sup>) were grown in 12-well plates to ~95% monolayer cells. Subsequently, either ChanSRPK-1 (400 mg/ml) or PBS was added to the plates for 24, 48, 72 and 96 h at 37°C, respectively. MTT, at concentration of 5 mg/ml (50 µl; Amresco, LLC, Solon, OH, USA), was added to the cells and incubated for 4 h. DMSO was added for incubation for 30 min to dissolve the precipitate following removal of the supernatant. The results were determined using a spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 570 nm.

**Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis.** Total mRNA was isolated from the MRC-5 cells and NSCLCDVECs pre- and post-treatment with ChanSRPK-1 using an RNAeasy Mini kit (Qiagen Sciences, Inc., Gaithersburg, MD). The total mRNA (1 µg) was reverse transcribed into cDNA using a reverse transcription kit (Qiagen Sciences, Inc.). The cDNA (10 ng) was used for qPCR (Bio-Rad Laboratories, Inc. Hercules, CA, USA) with the SYBR Green Master Mix system (50 ng of genomic DNA, 200 µM dNTP, 2.5 units of Taq DNA polymerase, and 200 µM primers) according to manufacturers' protocols, followed by preliminary denaturation at 94°C for 2 min, 35 cycles at 94°C for 30 sec, annealing temperature reduced to 64°C for 30 sec and 72°C for 10 min. All forward and reverse primers (Table I) were synthesized by Invitrogen (Thermo Fisher Scientific, Inc.). Relative mRNA expression changes were calculated using the 2<sup>-ΔΔC<sub>q</sub></sup> method (20). The results are expressed as the fold change, compared with the control.

**Cell viability assay.** The MRC-5 cells and NSCLCDVECs were grown in a 24-well plate at a concentration of 2.5x10<sup>5</sup>/ml (total column of 1,000 µl). The MRC-5 cells and NSCLCDVECs were treated with ChanSRPK-1 (400 mg/ml) or PBS (control) for 48 h at 37°C. The viabilities of the MRC-5 cells and NSCLCDVECs were examined using a Cell Counting kit (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) in eight view windows.

**Cell invasion and migration assays.** The MRC-5 cells and NSCLCDVECs were treated with ChanSRPK-1 or PBS (control). For the invasion assay, the cells were suspended as a density of 1x10<sup>5</sup> in 500 µl serum-free EMEM. Following treatment of the MRC-5 cells and NSCLCDVECs with ChanSRPK-1 or PBS, the cells were added to the upper chamber of BD BioCoat Matrigel Invasion chambers (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's protocol. For the migration assay, the cells were inoculated with ChanSRPK-1 or PBS for 48 h using a control insert (BD Biosciences) rather than a Matrigel invasion chamber. The tumor cells invasion and migration were counted in at least three randomly selected stained MRC-5 cells or NSCLCDVECs fields from every membrane under a light microscope (N-STORM, Nikon Corporation, Tokyo, Japan).

**ELISA.** The levels of SRPK-1 were determined using a commercial ELISA kit (CSB-EL022682MO, Cusabio Biotech Co., Ltd, Wuhan, China) for SRPK-1, and the procedure was performed according to the manufacturer's protocol. Briefly, ChanSRPK-1 (20-420 µg/ml, 50 µl) was added to ELISA plates wells for 30 min at 37°C. These were then washed three times with PBS and followed by blocking for 2 h with 300 µl 5% non-fat dried milk in PBS. Then, 100 µl horseradish peroxidase (HRP)-conjugated SRPK-1 (0.2 µg/ml) was added to the wells and incubated for 1 h at 37°C. The plates were washed three times with PBS and absorbance was measured at 450 nm in an ELISA reader.

**Western blot analysis.** The NSCLCDVECs were treated with ChanSRPK-1 (400 ng/ml) or PBS for 48 h. The NSCLCDVECs were then harvested by scraping and were lysed in RIPA buffer, followed by homogenization at 4°C for 10 min. A total of 20 µg protein extracts was electrophoresed on 12.5% polyacrylamide gradient gels and then transferred to nitrocellulose membranes. The membranes were incubated in blocking buffer (5% milk) prior to incubation with primary antibodies at 4°C overnight. The ChanSRPK-1-treated and PBS-treated NSCLCDVECs were inoculated with the correlating mouse anti-human primary antibody: SRPK-1 (ab50927; 1:500), Cytochalasin-D (ab143484; 1:500), G-actin (ab123034; 1:500) and β-actin (ab5694; 1:500; all from Abcam, China) for 12 h at 4°C. All samples were washed with Tris-buffered saline and incubated with HRP-conjugated bovine anti-mouse IgG antibodies (sc-2371; 1:10,000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) for 2 h at 37°C. The results were visualized by using a chemiluminescence detection system (BD Biosciences).

**Animal experiments.** A total of 100 specific pathogen-free male BABL/C (six-week old, body weight: 32-36 g) mice were

Table I. Primer sequences used for reverse transcription-quantitative polymerase chain reaction.

Target gene	Forward primer	Reverse primer
SRPK-1	5'-GGACCGTCTTCGGACATCA-3'	5'-ATCTTTTGGGGTCCGTCAACT-3'
TCF	5'-TGACCCATCTCAGAAGCAG-3'	5'-GCAGAGAGGAGGTTGACTTTC-3'
MMP	5'-TGCCACCGTCCTTTCTTGTT-3'	5'-GCTGACTTGACTCATGGCT-3'
CT1	5'-CACAAAGCAGCCTTGCAGAA-3'	5'-AGAGCAGGCAGCATAGCAGTG-3'
FBC	5'-AACCCGAGGTATGCTTATCT-3'	5'-CCAGTTCTTCATTGCATTGC-3'
$\beta$ -actin	5'-GTGGGCGCCAGGCACCA-3'	5'-CTCCTTAATGTACGCACGATTT-3'

SRPK-1, serine-arginine protein kinase-1; TCF, T-cell factor; MMP, matrix metalloproteinase; CT1, collagen type I; FBC, fibronectin.

purchased from Shanghai Slack Experimental Animals Co., Ltd. (Shanghai, China). All mice were housed in a temperature-controlled facility at  $23\pm 1^\circ\text{C}$  and treated in accordance with the Guide for the Care and Use of Laboratory Animals of Tianjin Chest Hospital (Tianjin, China). Mice were maintained at a 12-h light/dark cycle with free access to food and water. The mice were implanted with NSCLC DVECs and were divided into two groups, which received ChanSRPK-1 or PBS treatment ( $n=50$  in each group). Treatment was started 7 days post-tumor implantation, when the tumor diameter reached 6-8 mm. The tumor-bearing mice were intravenously injected with ChanSRPK-1 (400 ng), with the same volume PBS injected in the control group mice. The treatment was continued for 14 days (one injection per day). The tumor volumes were calculated according to a previous study (21).

**Statistical analysis.** All data are presented as the means  $\pm$  standard error of the mean of triplicate experiments. Unpaired data was analyzed using Student's t-test and comparisons of data between multiple groups were analyzed by one-way analysis of variance (GraphPad version 5.0; GraphPad Software, Inc., La Jolla, CA, USA). The Kaplan-Meier test was used to estimate survival during the 120-day observation period.  $P<0.05$  was considered to indicate a statistically significant difference.

## Results

**Construction of a chimeric antibody targeting SRPK-1 and determination of *in vitro* activity.** The antibody targeting of SRPK1 was screened using a conventional approach and the constant region of antibody was replaced with a constant region from human antibody. To enhance the stability and half-life of the antibody targeting SRPK-1, the crystalline fragment was linked to the antibody targeting SRPK-1, and ChanSRPK-1 was analyzed. The structure of ChanSRPK-1, containing the crystalline fragment and cell-penetrating peptide to enhance stability and transmembrane ability, is shown in Fig. 1A. ChanSRPK-1 was expressed by pET-27b in *Escherichia coli*. As shown in Fig. 2B, the molecular weight of ChanSRPK-1 was  $\sim 67.5$  kDa, determined using SDS-PAGE gel electrophoresis. In addition, the affinity of ChanSRPK-1 for SRPK-1 was determined using ELISA and western blot analysis. The results (Fig. 1C) demonstrated that ChanSRPK-1 maintained a high affinity with SRPK-1 in the ELISA assay. A band at  $\sim 67.5$  kDa was confirmed by SDS-PAGE gel

electrophoresis, and the specific binding to SRPK-1 was confirmed using western blot analysis (Fig. 1D). These results indicated that ChanSRPK-1 was expressed at a high level and had the capacity to bind with SRPK-1.

***In vitro* effects of ChanSRPK-1 on the expression of SRPK-1 in NSCLC cells.** In order to confirm the *in vitro* effects of ChanSRPK-1 (400 mg/ml) on the expression of SRPK-1 in NSCLC cells, NSCLC DVECs and MRC-5 cells were analyzed using RT-qPCR analysis and ELISA. The results (Fig. 2A and B) showed that the expression of SRPK-1 was upregulated in the NSCLC DVECs, compared with that in the MRC-5 human lung cells. In addition, the data (Fig. 2C) showed that ChanSRPK-1 treatment neutralized the expression of SRPK-1 in a dose-dependent manner (200-2,000 ng/ml). ChanSRPK-1 treatment also neutralized the expression of SRPK-1 in a time-dependent manner (24, 48, 72 and 96 h; Fig. 2C). The *in vitro* effect of ChanSRPK-1 on NSCLC DVEC growth was also examined. The results (Fig. 2D) showed that ChanSRPK-1 (400 mg/ml) suppressed NSCLC DVEC growth from 48 h. These data suggested that the expression of SRPK-1 was increased in NSCLC DVECs, and that ChanSRPK-1 decreased the expression of SRPK-1 in NSCLC DVECs and inhibited growth.

***In vitro* inhibitory effects of ChanSRPK-1 on migration and invasion in NSCLC.** The expression of SRPK-1 has been associated with the prognosis of human cancer, as TCF has been shown to promote the migration and invasion of NSCLC cells (15). Although the role of SRPK-1 in NSCLC cells has been investigated, the role of ChanSRPK-1 in the inhibition of migration and invasion in NSCLC cells has not been reported previously (21). To investigate the inhibitory effects of ChanSRPK-1 on NSCLC DVECs, cell viability, migration and invasion were analyzed. The results (Fig. 3A) showed that the viability of NSCLC DVECs was significantly decreased in the ChanSRPK-1-treated groups (200, 400 and 1,000 mg/ml for 48 h), compared with that of the MRC-5 cells. In addition, the results (Fig. 3B and C) indicated that the presence of SRPK-1 markedly promoted NSCLC DVEC migration and invasion at doses of 200, 400 and 1,000 mg/ml for 24 h, compared with the untreated group. By contrast, it was demonstrated that 200-1,000 ng/ml of ChanSRPK-1 markedly inhibited the migration, invasion and apoptosis of NSCLC DVECs (Fig. 3D-F). Of note, 400 mg/ml of

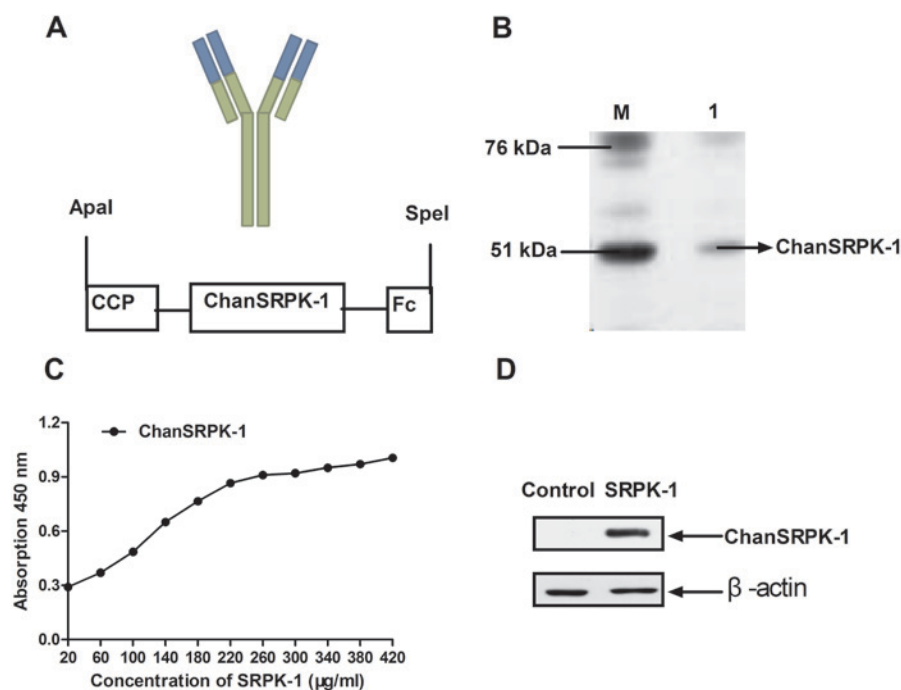


Figure 1. Characteristics of ChanSRPK-1. (A) Structure of the chimeric antibody of ChanSRPK-1. ChanSRPK-1 was aggrandized with CCP and Fc by genetic engineering. (B) SDS-PAGE analysis of purified ChanSRPK-1 under reducing conditions. Lane 1, molecular weight protein markers; lane 2, ChanSRPK-1. (C) Specific interaction of ChanSRPK-1 with SRPK-1. (D) Specificity of ChanSRPK-1 was analyzed using western blot analysis. SRPK-1, serine-arginine protein kinase-1; ChanSRPK-1, chimeric antibody target for SRPK-1; Fc, crystalline fragment; CCP, cell-penetrating peptide.

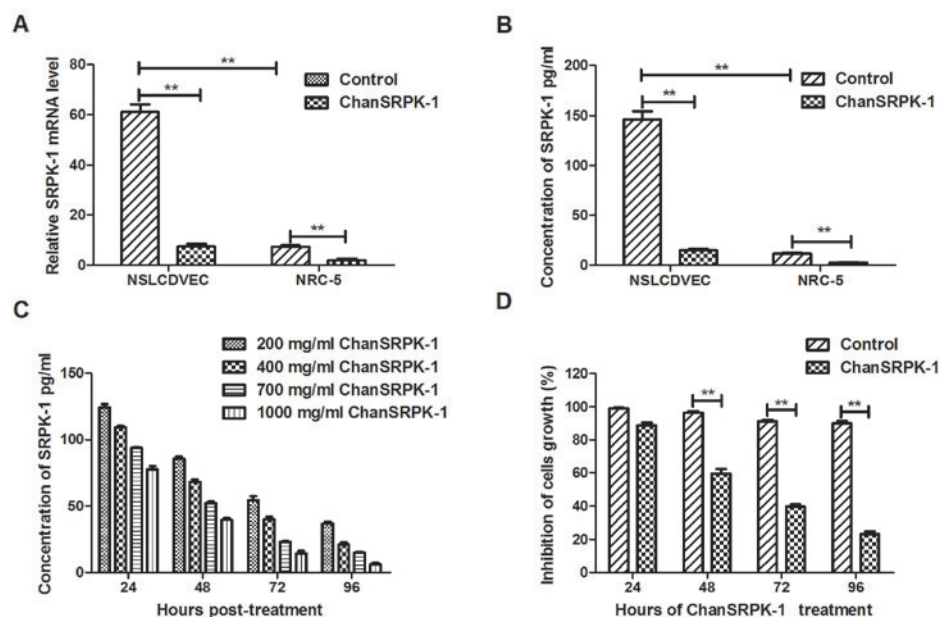


Figure 2. Effects of ChanSRPK-1 on the expression of SRPK-1 and growth of NSCLC-VECs. (A) Analysis of mRNA expression of SRPK-1 in NSCLC-VECs and MRC-5 cells pre- and post-treatment with ChanSRPK-1. (B) Detection of SRPK-1 levels in NSCLC-VECs and MRC-5 cells pre- and post-treatment with ChanSRPK-1. (C) Effects of time and dose in treatment with ChanSRPK-1 on levels of SRPK-1 in NSCLC-VECs. (D) Inhibitory effects of ChanSRPK-1 on NSCLC-VECs. SRPK-1, serine-arginine protein kinase-1; ChanSRPK-1, chimeric antibody target for SRPK-1; NSCLC-VECs, non-small cell lung cancer-derived vascular endothelial cells.

ChanSRPK-1 was sufficient to inhibit the migration and invasion of NSCLC-VECs. These results suggested that ChanSRPK-1 not only affected cell viability, but also inhibited the migration and invasion of NSCLC-VECs.

*Effects of ChanSRPK-1 on NSCLC-VECs by targeting TCF and in vivo efficacy in NSCLC-bearing mice.* To determine the efficacy of ChanSRPK-1 for NSCLC treatment, the present study analyzed the expression of TCF, MMP, CT1



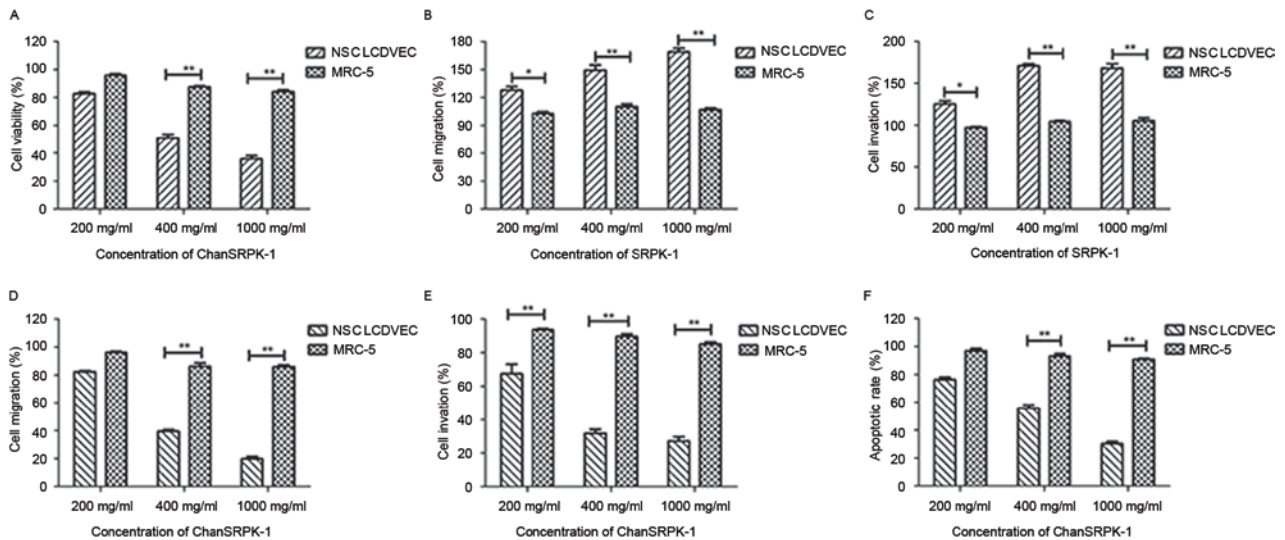


Figure 3. Effects of ChanSRPK-1 on the viability, migration, invasion and apoptosis of NSCLCDVECs. (A) Cell viabilities were significantly altered in ChanSRPK-1-treated NSCLCDVECs at different doses, compared with MRC-5 cells. (B) SRPK-1 promoted the migration of NSCLCDVECs following exposed to 200 mg/ml, compared with control cells. (C) SRPK-1 promoted the invasion of NSCLCDVECs exposed to 200 mg/ml, compared with control cells. (D) ChanSRPK-1 inhibited migration of NSCLCDVEC after exposure 200 mg/ml, compared with control cells. (E) ChanSRPK-1 inhibited the invasion of NSCLCDVECs following exposure to 200 mg/ml, compared with control cells. (F) ChanSRPK-1 induced the apoptosis of NSCLCDVECs following exposure to 400 mg/ml, compared with control cells. \* $P < 0.05$  and \*\* $P < 0.01$ . SEP-1, serine-arginine protein kinase-1; ChanSRPK-1, chimeric antibody target for SRPK-1; NSCLCDVES, non-small cell lung cancer-derived vascular endothelial cells.

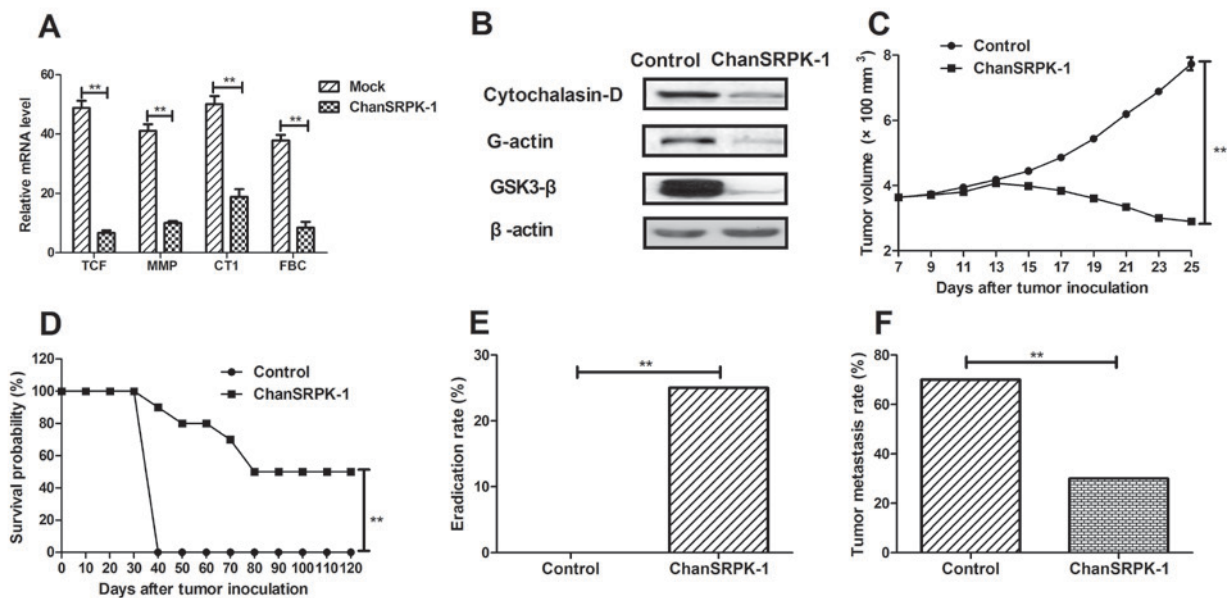


Figure 4. Beneficial therapeutic effects and inhibitory migration effects of ChanSRPK-1 in NSCLC-bearing mice. (A) ChanSRPK-1 had a beneficial effect on protein expression of tumor metastasis-associated TCF, MMP, CT1 and FBC in tumor-bearing mice, compared with control treatment. (B) ChanSRPK-1 decreased expression levels of Cytochalasin-D and G-actin, and the phosphorylation of GSK3- $\beta$  in tumors, compared with the untreated group. (C) ChanSRPK-1 treatment significantly inhibited tumor cell growth in NSCLC-bearing mice. (D) Survival was prolonged following treatment with ChanSRPK-1 over a 120-day observation period, compared with survival in the control group. (E) Eradication rate of NSCLC was enhanced following treatment with ChanSRPK-1. (F) Metastatic rate of NSCLC was decreased following treatment with ChanSRPK-1. The Kaplan-Meier test was used to estimate survival during the 120-day treatment period. \* $P < 0.05$  and \*\* $P < 0.01$ . SEP-1, serine-arginine protein kinase-1; ChanSRPK-1, chimeric antibody target for SRPK-1; NSCLCDVES, non-small cell lung cancer-derived vascular endothelial cells; TCF,  $\beta$ -catenin/T-cell factor; MMP, matrix metalloproteinase; CT1, collagen type I; FBC, fibronectin; GSK3- $\beta$ , glycogen synthase kinase 3- $\beta$ .

and FBC. As shown in Fig. 4A, the mRNA expression levels of TCF, MMP, CT1 and FBC in the NSCLCDVECs were significantly higher, compared with those in the ChanSRPK-1-treated cells. The difference in the expression

of migration-promoting proteins between the ChanSRPK-1 and control groups was also determined to be statistically significant using a paired t-test ( $P < 0.01$ ). A previous study showed that upregulation in the phosphorylation level of

GSK3- $\beta$  by SRPK-1 was correlated with tumor cell migration (15). In the present study (Fig. 4B), it was found that the downregulation in the expression of Cytochalasin-D and G-actin, and the phosphorylation of GSK3- $\beta$  were significantly different in the ChanSRPK-1-treated tumor cells, compared with those in the control. In addition, NSCLC-bearing mice were used to determine the *in vivo* antitumor efficacy of ChanSRPK-1. As shown in Fig. 4C, tumor size was significantly suppressed in xenograph mice in the ChanSRPK-1-treated group, compared with those in the control group. In addition, long-term (120 day) survival was preceded following treatment with ChanSRPK-1. The data (Fig. 4D) revealed that treatment with ChanSRPK-1 (n=10 in each group) prolonged the survival of NSCLC-bearing mice, compared with control mice. Furthermore, it was demonstrated (Fig. 4E) that treatment with ChanSRPK-1 significantly protected tumor-bearing mice via eradicating tumors. As demonstrated in Fig. 4F, it was found that ChanSRPK-1 inhibited NSCLC tumor metastasis compared with PBS-treated mice. The data showed that the inhibitory effect of ChanSRPK-1 on tumor metastasis in NSCLC-bearing mice was enhanced, compared with that in the control. These results suggested that ChanSRPK-1 suppressed the migration-promoting protein and contributed to beneficial efficacy in NSCLC-bearing mice.

## Discussion

Lung cancer is a respiratory disease leading to the majority of cancer-associated mortality in the world owing to air contamination (22). At present, NSCLC accounts for ~80% of lung cancer cases and its incidence is increasing. It includes adenocarcinoma, which comprises large cell carcinoma and squamous cell carcinoma (5-7). The high mortality rates of NSCLC present a challenge, and a rapidly increasing trend has been observed, which has gradually become a focus of public opinion and a significant burden on human health (23). The difficulty in the early detection of NSCLC is the primary reason for lower survival rates in advanced NSCLC (24). The majority of patients with NSCLC are at an advanced stage of lung cancer at diagnosis (23). In addition, conventional therapeutic techniques show poor efficacy for NSCLC, and recurrence and metastasis are frequently observed clinically. Statistical data have also shown that the survival rates of patients with NSCLC, in terms of the overall 5-year survival rate, are poor (25). Therefore, the identification of more efficient anti-NSCLC agents is required for patients with NSCLC in preclinical and clinical trials.

Growth, migration and invasion in NSCLC are the most important drivers for tumor metastasis and poor survival rates in treatment and recurrence for patients with NSCLC (10,11). Previous studies have reported that SRPK-1-encoding transcripts are ubiquitously expressed in several human tissues, and higher expression levels of SRPK-1 in the precise cellular localization of NSCLC, pancreatic carcinoma, colon cancer and breast cancer have been reported previously (13,15). It has been identified that the majority of SRPK-1 protein is expressed exclusively within NSCLCDVECs and that SRPK-1 is positively correlated with the grade of cancer progression. Furthermore, the downregulation of SRPK-1

increases the apoptosis of tumor cells (17). These results suggest that therapeutic agents, through suppressing the activity of SRPK-1, may be effective as a potential single anticancer agent or an adjuvant agent in combination with conventional therapeutic regimens.

In the present study, the design involved construction of a Chan-SRPK-1 for NSCLC therapy via inhibition of growth, migration and invasion. The expression of SRPK-1 and inhibitory effects on NSCLCDVECs were also examined *in vitro* and *in vivo*. Lehman *et al* (12) reported that two major kinases responsible for the protein phosphorylation of mitogen-activated protein kinase (MAPK)3 and MAPK1 were reduced through inhibited phosphorylation levels of GSK3- $\beta$ . In the present study, the mRNA expression levels of the TCF, MMP, CT1 and FBC tumor cell metastasis-associated proteins were significantly decreased in NSCLCDVECs treated with ChanSRPK-1, compared with levels in untreated cells.

The ability of SRPK-1 to enhance proliferation and survival via the regulation of multiple signaling pathways in tumor cells has been exploited and suggested as an opportunity to develop novel anticancer therapeutics targeting SRPK-1 (26). A previous study reported that SRPK-1 regulates migration and growth in various cancer cells (27). In the present study, it was shown that the expression of SRPK-1 was superfluous in NSCLCDVECs, compared with normal MRC-5 lung cells. It was found that ChanSRPK-1 interrupted the signaling pathway involved in tumor cell growth, migration and invasion regulated by SRPK-1. In addition, ChanSRPK-1 treatment at a concentration of 400 ng/ml for 24 h markedly suppressed NSCLCDVEC migration, compared with that in the untreated cells *in vitro*, and inhibited tumor metastasis in tumor-bearing mice *in vivo*. Primary data analysis of tumor diameter and long-term survival showed that injection with ChanSRPK-1 once a day led to tumor regression and resulted in a survival rate of 50% in a 120-day period in tumor-bearing mice. Data also indicated that treatment with ChanSRPK-1 against NSCLC was sufficient to partially protect the animals via the eradication of tumors in experimental mice, which translated into long-term survival and tumor-free living in the mice of the lung cancer model.

In conclusion, the present study introduced ChanSRPK-1 as an anticancer candidate via once daily administration through intravenous injection. In the NSCLC mouse model, the results demonstrated that, ChanSRPK-1 downregulated the expression of SRPK-1, which led to the reversal of TCF-induced cell migration. Taken together, the data obtained in the present study suggested the beneficial effects of cellular targeted therapy, which can assist in further elucidating the clinical value of applying such a regimen.

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