

## RESEARCH ARTICLE

# Inhibitory Effects of Syk Transfection on Lung Cancer Cell Invasion

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### Abstract

**Objective:** Spleen tyrosine kinase (Syk) is closely related to tumor invasion and metastasis, and has been shown to have potential inhibitory effects in tumors. In this study, we constructed a eukaryotic expression vector for Syk and analyzed its effects on invasive ability of the A549 non-small cell lung cancer cell line *in vitro*. **Methods:** A fragment of Syk was obtained by RT-PCR from human lung cancer cells and cloned into the expression vector pLNCXSyk. After restriction endonuclease digestion, PCR and DNA sequencing confirmation, the recombinant Syk expression plasmid was transfected into A549 human lung cancer cells using lipofectamine protocols. After selection, the cells stably expressed Syk. Detection of Syk expression of the cells by RT-PCR, and invasive ability were examined. **Results:** The eukaryotic expression plasmid pLNCXSyk was constructed and expressed stably in the A549 human lung cancer cells. The RT-PCR results showed that Syk mRNA expression was upregulated significantly ( $P < 0.05$ ). Lower invasion through a basal membrane were apparent after transfection ( $P < 0.05$ ). **Conclusions:** A eukaryotic expression plasmid to cause Syk expression in lung cancer cells can obviously inhibit their invasive ability *in vitro*.

**Keywords:** Spleen tyrosine kinase (Syk) - non - small cell lung cancer - invasive ability - *in vitro*

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### Introduction

The protein tyrosine kinase (PTKs) is a group of enzyme protein which can catalyze substrate protein tyrosine residues to phosphorylation, and is involved in many signal transduction pathways. It also plays an important role in the control of cell differentiation, proliferation and spread. Recent studies have found that Syk, as a tumor suppressor of PTKs, is closely related to tumor invasion and metastasis (Tatsuya et al., 2003; Yang et al., 2013), and studies showed potential inhibitory effect of the gene Syk in breast, gastric and pancreatic cancer. A few literatures have been reported about Syk expression in lung cancer (Peng et al., 2013). In this study, we analyzed the effect of Syk on invasive ability of Non-small cell lung cancer cell line A549 *in vitro*.

### Materials and Methods

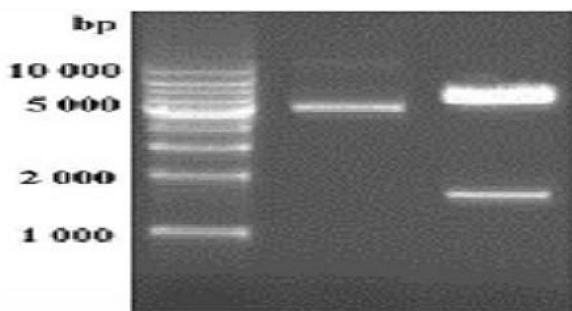
Human lung cancer cell line A-549 cells were purchased from Shanghai Cell Institute of Chinese Academy of Sciences, RPMI-1640 culture medium was from Gibco BRL Company. A-549 cells were maintained in RPMI-1640 (CELLGRO Company) with 10% Fetal bovine serum. TRIZOL and Transfection kit were from Invitrogen Company and the PCR kit from Promega

Company in USA. Primers were synthesized by Jierui Company.

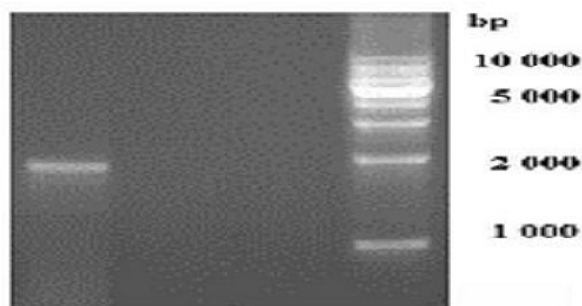
Total RNA was extracted from human lung cancer cells in accordance with the TRIZOL kit instructions, and Syk cDNA was amplified according to Reverse transcription and PCR kit instructions. According to sequence of plasmid pLNCX and Syk cDNA, primers was designed and synthesized by Shanghai Bio-Engineering Company as the following: upstream 5'-CACC ATG GCC AGC AGC GGC ATG GCT G-3', downstream 5'-GTT CAC CAC GTC ATA GTA GTA ATT GCG-3'. The amplification products were analyzed with 5 g/L agarose gel electrophoresis. Fresh PCR product 2  $\mu$ l, salt solution 1  $\mu$ l, sterile water 2  $\mu$ l, and vector 1  $\mu$ l were added to 6  $\mu$ l reaction system, the ligation products were transformed to competent cell DH5 $\alpha$  after water bath for 30 min. Recombinant plasmids were extracted, amplified and double digested with restriction enzyme Cla I and Hind I.

Primers were designed based on the gene sequence in GenBank as follows: Syk (514bp), upstream 5'-CAT GTC AAG GAT AAG AAC ATC ATA GA-3', downstream 5'-AGT TCA CCA CGT CAT AGT AGT AAT T-3'.  $\beta$ -actin (838bp), upstream 5'-ATC TGG CAC CAC ACC TTC TAC AAT GAG CTG CG-3' downstream 5'-CGT CAT ACT CCT GCT TGC TGA TCC ACA TCT GC-3'. Total RNA of the transfected cells was extracted, and reverse

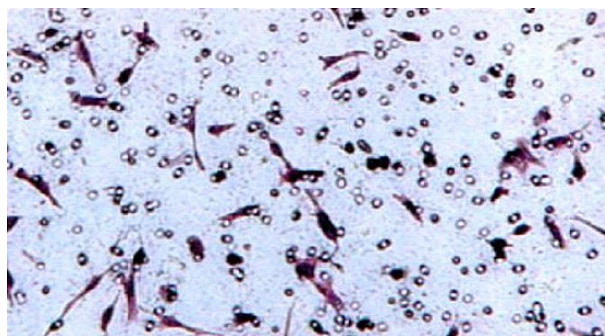
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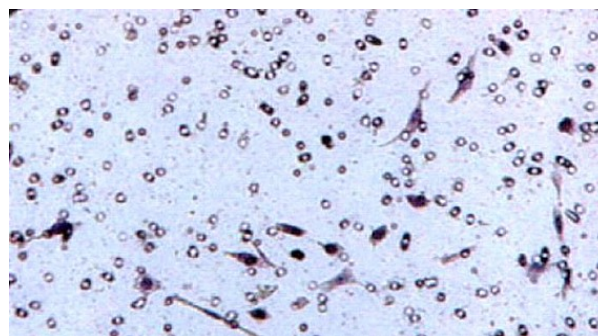
**Figure 1. Identification of the Recombinant Plasmid pLNCX Syk**



**Figure 2. The Syk mRNA was Detected in the Transfected Cells**



**Figure 3. Invasion in the Control Groups (HE×400)**



**Figure 4. Invasion in the Transfected Cells (HE×400)**

transcription and amplification were done refer to kit instructions. The amplification reaction involved 30 cycles of denaturation at 95 °C for 2 minute, annealing at 55 °C for 1 minute, and 72 °C for 1 minute. PCR products were observed under 1.5% agarose gel electrophoresis and photographed. The no transcriptional group is as control. 24-well matrix gel invasion chambers (from BD company) were used. The size of membrane pore between upper and lower chamber is 8um, extracellular matrix gel was covered onto the upper chamber membrane surface, which can simulate the body extracellular matrix and basement membrane environment. The transfected A-549 cells were mixed with serum-free RPMI1640 to the concentration of  $2 \times 10^5/\text{ml}$ , 200 ul of the cell suspension was added to the upper chamber, and 500 ul RPMI1640 with 10% fetal calf serum to the lower chamber. After 48 hours in a incubator filled with 5% CO<sub>2</sub> at 37 °C, the cells into the lower chamber were fixed with formaldehyde (HE staining), and observed under the light microscope (400 times ). We counted the cells in six fields randomly, mean was the number of the invasive cells.

#### Statistical Methods

The results were expressed as mean ± standard deviation. Analysis of variance and q test were made with SPSS version 12.0 software.  $p < 0.05$  was considered statistically significant.

### Results

After the recombinant plasmid DNA was cut with the restriction enzyme, 1.5% agarose gel electrophoresis showed that strip size was consistent with the expected design. The sequencing results was the same as that in the Gene bank (AF015950), and no base mutation

occurred (Figure 1). Total RNA of transfected cells was extracted, and PT-PCR examination showed the gene Syk transcription in the mRNA level, while there was no transcription in control group (Figure 2). In the invasion assay, the number of the transmembrane cells was significantly reduced compared with control group ( $20.143 \pm 1.51, 50.29 \pm 7.56, p < 0.05$ ), cell structure was not clear under light microscope, and invasion on the reconstituted basement membrane weakened (Figure 3 and 4).

### Discussion

The PTKs is a group of protease which can catalyze objects protein tyrosine residue phosphorylation, and involved in many signal transduction pathways and plays an important role in control of cell differentiation, proliferation and spread. PTKs can be divided into receptor and non-receptor types according to different topology. The combination of ligand and the PTKs receptor can activate intracellular tyrosine kinase domain, and trigger a specific signal transduction pathways. Non-receptor type PTKs in intracellular, as s signal transduction members, play an important role in many aspects. Syk is a non-receptor tyrosine kinase, which composes of 629 amino acids and locates on human chromosome 9q22, its protein relative molecular mass is  $72 \times 10^9$  (Li et al., 2001).

The spleen tyrosine kinase Syk was long thought to be a hematopoietic cell-specific signaling molecule. Recent evidence demonstrated that it is also expressed by many non-hematopoietic cell types (Hoeler et al., 2005; Ulanova et al., 2005) and that it plays a negative role in cancer (Hoellenriegel et al., 2012). A significant drop in its expression was first observed during breast cancer progression, but an anomalous Syk expression has now also been evidenced in many other tumor types. Sung et

al. (2009) found that in the mouse mammary gland, loss of one Syk allele profoundly increases proliferation and ductal branching and invasion of epithelial cells through the mammary fat pad during puberty, and mammary carcinomas develop by one year. Mechanistic studies using Syk re-expression demonstrated its suppressive function in tumorigenesis and metastasis formation, which is surprising for a tyrosine kinase. The molecular mechanism of its tumor-suppressive function remains largely unknown; An increasing number of clinical studies reveal a correlation between reduced Syk expression and an increased risk for metastasis formation, and assign Syk as a potential new prognostic marker in different tumor types (Coopman and Mueller, 2006).

In this study, fragment of Syk was obtained from human lung cancer cells and cloned into the expression vector pLNCXSyk. The recombinant Syk expression plasmid was transfected into human lung cancer cells A549 by using lipofectamine protocols. We found that Syk mRNA expression were higher than that of control group ( $P < 0.05$ ). The number of invasive cells and invasive ability decreased obviously ( $P < 0.05$ ). It suggested that high expression of Syk can reduce invasive ability of lung cancer cell. These results are similar to previous reports about Syk expression in other types of malignant tumors (Nakashima et al., 2006; Layton et al., 2009).

In conclusion, Syk involved in lung cancer cell invasion and played an important role. These observations led us to hypothesize that upregulation of Syk expression could inhibit tumor cell invasion, and these may serve as part of the basis for gene therapy. However, the following issues should be addressed to reveal the molecular mechanism of Syk's tumor suppressive function: (1) relationship between transduction network and tumor; (2) regulation mechanisms in the tumor; (3) Syk specific positioning and functional differences in tumor; (4) Syk subtypes detection and functional studies.

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