Effect of STAT3 siRNA–Induced Inhibition of STAT3 Gene Expression on the Growth and Apoptosis of Lewis Lung Cancer Cells

Chunguang Wang
Mei Sun
Xuejian Zhao
Xingyi Zhang

1 Department of Thoracic Surgery, the Second Hospital of Jilin University, Changchun 130041, China.
2 Department of Pathology, the Second Hospital of Jilin University, Changchun 130041, China.
3 Department of Pathophysiology, School of Basic Medicine, Jilin University, Changchun 130021, China.

This work was supported by the grant to from the Scientific and Technological Office of Jilin Province, China (No. 200505120).

Correspondence to: Xingyi Zhang
Tel: 86-431-8796798
Fax: 86-431-8934741
Email: xyzhang@jlu.edu.cn

OBJECTIVE To determine the effect of short interference RNA (siRNA) against STAT3 induced inhibition of STAT3 gene expression and on the growth and apoptosis of Lewis lung cancer cells.

METHODS pSilencer 2.1–U6 STAT3 siRNA against STAT3–mRNA was synthesized. Lewis lung cancer cells were divided into 3 groups: vehicle, plasmid, and STAT3 siRNA in which the cells were treated with RPMI–1640 culture media, or transfected with pSilencer empty vector, or pSilencer STAT3 siRNA. Semiquantitative RT–PCR and Western blot analysis of STAT3 gene expression in the cells was performed 72 h after transfection. MTT assay for cell proliferation, flow cytometry and DNA laddering electrophoresis were used for determination of cell proliferation and apoptosis.

RESULTS STAT3 was markedly expressed at both the mRNA and protein levels in the cells treated with RPMI–1640 media or transfected with the plasmid vector, whereas STAT3 expression was significantly reduced in cells treated with STAT3 siRNA. These findings suggest that STAT3 siRNA effectively inhibited STAT3 expression. Transfection of the cells with STAT3 siRNA resulted in significant cellular growth inhibition and enhanced apoptosis.

CONCLUSION Transfection of Lewis lung cancer cells with synthetic STAT3 siRNA resulted in effective inhibition of STAT3 gene expression at both protein and mRNA levels, leading to induced apoptosis and growth suppression.

KEYWORDS: STAT3, Lewis lung cancer cells, apoptosis, siRNA.

The morbidity from lung cancer, one of the most common primary malignant carcinomas in the world, has increased annually. Furthermore, to date, the therapeutic efficacy for this malignancy has not improved as manifested by current high mortality and poor long-term survival rates. Proteins, which are signal transducers and activators of transcription (STAT), perform the dual functions of signal transduction and activation of transcription. An increasing number of studies have suggested that STATs have the potential to be novel molecular targets to treat lung cancers. STATs are latent cytoplasmic transcription factors that function as intracellular effectors of cytokine and growth factor signaling pathways. They were originally described in the context of regulating cell signaling, contributing to such diverse process as differentiation, proliferation, and apoptosis. It has been well documented that activation of all of the STAT proteins is caused by phosphorylation of a single tyrosine residue that leads to dimerization via an intermolecular SH2 phosphotyrosine interaction. The dimerized STATs translocate...
to the nucleus where they regulate gene expression by binding directly to high affinity DNA binding sites or by associating with other transcription factors. In normal and/or benign cells, the signaling by STAT3 is under tight regulation so that the signal is transient. However, aberrant signaling by STATs has been reported in many types of malignancies, such as myeloma, head and neck, breast, and prostate cancers. Amongst the Stat family, STAT3 plays a key role in promoting proliferation, differentiation, and anti-apoptosis.

To date, constitutive activation of STAT3 has been detected in a number of tumour-derived cell lines, as well as in a wide variety of human malignancies. We have recently reported that the expression of the STAT3 gene is significantly increased in human laryngeal cancer cell lines (Hep2) and prostate cancer cells at both mRNA and protein levels. Seki et al. have found a significantly increased expression of the STAT3 gene in human lung cancer tissues. Thus, it is plausible that constitutive activation of STAT3 represents an important role in the growth and survival of cancer cells.

It is known that dysregulation of apoptosis contributes to cancers and many diseases. Studies have shown that over active STAT3 promotes uncontrolled growth and survival through aberrant expression of downstream targeted genes, such as cyclin D1, cyclin D2, c-Myc, p53, Bcl-xL, Bcl-2, Mcl-1, and survivin; these genes influence cell cycle progression or inhibit apoptosis. The up-regulation of these genes in cancers has been well-documented, and it has been demonstrated that STAT3 can directly regulate expression of several survival genes. The anti-apoptotic genes encoding Bcl-xL, Mcl-1, and survivin proteins are STAT3 target genes. It has been reported in studies with breast cancer cells that activation of STAT3 signaling induces enhanced expression of Mcl-1 and survivin genes with anti-apoptotic activity, whereas disruption of STAT3 signaling results in a dramatic reduction in the expression of Mcl-1 and survivin and induction of apoptosis.

Our recent studies along with others have demonstrated simultaneously increased STAT3 gene expression and decreased expression of Bcl-2 or Bcl-xL genes in human laryngeal cancer cell lines (Hep2), prostate cancer cells, and astrocytoma cells at both mRNA and protein levels. Likewise, the knockdown of STAT3 expression by RNA interference (RNAi), significantly increased expression of Bcl-2 or Bcl-xL genes, suggesting induction of apoptosis in human Hep2 cells, prostate cancer cells, and astrocytoma cells.

There is evidence to suggest that malignant cells having constant activation of STAT3 become STAT3-dependent for survival, and that deactivation or down-regulation of STAT3 results in apoptosis. Studies have shown that using a variety of approaches, such as tyrosine kinase inhibitors, antisense oligonucleotides, decoy oligonucleotides, and dominant-negative STAT3 proteins can inhibit STAT3 expression in cancer cells to suppress proliferation and induce apoptosis. In human head and neck squamous carcinoma cells, blocking of STAT3 signaling by decoy oligonucleotides or antisense oligonucleotides inhibits transforming growth factor effects and suppresses oncogenic growth of these cells. STAT3β is a naturally occurring dominant negative STAT3 variant that is identical to STAT3 except for the absence of the trans-activation domain. Blockade of STAT3 signaling by STAT3β in human myeloma cells and breast cancer cells in vivo down-regulates IL-6-induced expression of the Bcl-xL antiapoptotic gene and induces apoptosis, thereby suggesting that targeting STAT3 by STAT3β may enhance in vivo antitumor responses.

The use of RNA interference (RNAi) represents a novel alternative to gene inhibition that may be capable of inhibiting STAT3 expression in cancer cells leading to suppression of proliferation and induction of apoptosis. RNAi is triggered by introducing long double-stranded RNA (dsRNA) molecules into cells where the dsRNAs are cleaved by an endonuclease named dicer into 21- to 23-nt RNAs referred to as short interference RNAs (siRNAs). The siRNA molecules that serve as a guide for sequence-specific degradation of homologous mRNAs have been used for functional analysis of genes in many species. siRNA targeting STAT3 has been successfully used for treating numerous cancers. However, to our knowledge, no reports have been published to date concerning the effect of siRNA against the STAT3 gene in lung cancers cells. In our studies we have used Lewis lung cancer cells, which have shown to be of value for the study of lung cancer.

The objectives of the present study were (1) to determine if STAT3 siRNA can inhibit the expression of STAT3 gene in Lewis lung cancer cells and (2) to investigate the effect of STAT3 siRNA on the growth and apoptosis in these cells.

**MATERIALS AND METHODS**

**Experimental protocols**

pSilencer 2.1-U6 STAT3 siRNA against STAT3-mR-