Research Paper

TGF-β/FAK/AKT Signal Pathway Blocked by Astragaloside, Hinders the Invasion and Metastasis of Non-Small Cell Lung Cancer

QIAN DING, LIJUAN WANG[1], QI ZHU AND XIN CHEN*

Department of Integrated Traditional Chinese and Western Medicine Pulmonary Disease, Zigong First People's Hospital, Zigong 643000, [1]Department of Oncology and Hematology, Sichuan Science City Hospital, Mianyang, Sichuan Province 621000, China

Ding *et al.*: To Examine the Suppressive Impact of Astragaloside

The aim of this study is to examine the suppressive impact of astragaloside on the invasion and spread of non-small cell lung cancer by obstructing the transforming growth factor-beta/focal adhesion kinase/protein kinase B signaling pathway. Sixty mice were categorized into three groups; the normal control group (n=20) (group A), the model control group (n=20) (group B), and the astragaloside IV intervention group (n=20) (group C). To establish the non-small cell lung cancer mouse model for group B and group C, the modulated PC9/ER cell suspension was injected into the left axilla of nude mice, whereas group A received an equivalent amount of normal saline instead. After successful modeling, mice in group C were given astragaloside 30 mg/kg by intragastric administration. The expression levels of transforming growth factor-beta, focal adhesion kinase, and protein kinase B messenger ribonucleic acid were higher in group B compared to group A, whereas the expression levels of transforming growth factor-beta, focal adhesion kinase, and protein kinase B messenger ribonucleic acid were lower in group C compared to group B. The levels of protein expression for transforming growth factor-beta, focal adhesion kinase, and protein kinase B were higher in group B compared to group A, whereas the levels of protein expression for transforming growth factor-beta, focal adhesion kinase, and protein kinase B were lower in group C compared to group B. The non-small cell lung cancer is influenced by astragaloside A, transforming growth factor-beta, focal adhesion kinase, and protein kinase B.

Key words: Astragaloside A, transforming growth factor-beta, focal adhesion kinase, protein kinase B, non-small cell lung cancer

Lung cancer accounts for 11.6 % of the global incidence of widespread malignant tumors and 18.4 % of cancer-related mortality[1]. In all instances of lung cancer, Non-Small Cell Lung Cancer (NSCLC) prevails, accounting for 85 % of cases[2]. Despite advancements in different approaches for treating tumors in individuals diagnosed with lung cancer, such as surgical procedures, chemotherapy, and radiotherapy, the long-term survival rate for patients with NSCLC remains exceedingly poor[3]. Therefore, it is still necessary to explore new therapeutic methods or effective anticancer substances to improve the therapeutic effect of NSCLC patients. The rich and potential anti-cancer properties of natural plants or resources have garnered increasing attention. According to prior research, glycosides have demonstrated potential for reducing inflammation, combating oxidative stress, and inhibiting tumor growth[4]. Astragaloside and is one of the main active components of Radix *Astragalus*, which is obtained by high-tech extraction and separation it has the effect of enhancing immune function and anti-tumor. Clinical studies have demonstrated its efficacy in treating cervical cancer, liver cancer, and other types of tumors[5]. However, the efficacy and mechanism of astragaloside IV in NSCLC are not clear. Transforming Growth Factor-Beta

***Address for correspondence**
E-mail: tuchengzhanfan0540@163.com

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(TGF-β), a significant controller of cellular functions, plays a role in cancer progression by facilitating the movement and growth of tumor cells[6,7]. Increasing amounts of evidence indicate that Focal Adhesion Kinase (FAK) and Protein Kinase B (AKT) have a crucial role in the onset and progression of lung cancer. From a mechanism point of view, the activation of FAK leads to cell migration of various cancers through the phosphorylation regulation signal of AKT. This study aims to investigate the inhibitory impact of astragaloside on the invasion and metastasis of NSCLC by obstructing the TGF-β/FAK/AKT signaling pathway. The findings may offer a fresh perspective for the clinical management of NSCLC.

MATERIALS AND METHODS

Substances and chemicals:

Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. provided 60 male C57BL/6 mice. The NSCLC cell line PC9/ER was obtained from Shanghai Chuanqiu Biotechnology Co., Ltd. Astragaloside IV was acquired from Nanjing DOS Biotechnology Co., Ltd. Antibodies for TGF-β, FAK, AKT, B-Cell Lymphoma 2 (BCL-2), caspase-3, Bcl-2-Associated X Protein (BAX), and β-actin were purchased from Abcam Biotechnology Co., Ltd. Messenger Ribonuclic Acid (mRNA) primers for TGF-β, FAK, AKT, and β-actin were obtained from Sigma, United States of America (USA). The quantitative Polymerase Chain Reaction (qPCR) detection kit, Bicinchoninic Acid (BCA) protein concentration determination kit, immunohistochemically sheep anti-rabbit second antibody, and gel electrophoresis preparation kit are products provided by Shanghai Biyuntian Co., Ltd.

Animal model:

A total of sixty mice were divided into three groups; group A (n=20) as the normal control group, group B (n=20), and group C (n=20) as the astragaloside IV intervention group. To establish the NSCLC mouse model for group B and group C, the modulated PC9/ER cell suspension was injected into the left axilla of nude mice, whereas group A received an equivalent amount of normal saline instead. Following a successful modeling procedure, the mice in group C were administered astragaloside at a dosage of 30 mg/kg, whereas group A and group B received an equivalent volume of normal saline for a duration of 2 w.

Determination of survival time:

The survival time of rats in each group was recorded after duration of 2 w of administration for each group.

Sampling and sample preparation:

After the death of each group, part of the tumor samples were fixed overnight with 4 % paraformaldehyde solution, cut, dehydrated, embedded and sliced, followed by immunohistochemically and Hematoxylin and Eosin (H&E) staining, the number of Ki67 positive cells and total cells were counted, and the Ki67 index was calculated.

Western blot:

Frozen tumor tissues were homogenized at a temperature of 4° to create a 10 % homogenate. Subsequently, the supernatant was acquired through centrifugation. The protein concentration was assessed using the BCA method. Gel preparation, electrophoresis for 90 min, gel cutting, film transfer for 90 min, milk sealing, washing, and incubation with primary and secondary antibodies were performed. Following that, development took place, and the obtained results were analyzed using Bio-Rad image laboratory software.

qPCR:

The synovium's total RNA was obtained using an RNA extraction kit. The microRNA (miRNA) was then converted into complimentary Deoxyribonucleic Acid (cDNA) using the one Step Prime Script miRNA cDNA synthesis kit. Subsequently, miRNA fluorescence qPCR detection kit was utilized to conduct q-PCR, following the kit's instructions to complete the cycle. Following the completion of the reaction, the software was used to calculate the mRNA's relative expression.

Statistical method:

Statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS) 20.0, and the measurement data were represented as the mean plus or minus the standard deviation ($\bar{x}\pm s$). The comparison among groups was conducted using

analysis of variance, while pairwise comparison between groups was done using either Least Significant Difference (LSD) test or Tamhane test. Group A showed a significant difference ($^{a}p<0.05$) compared to group B ($^{b}p<0.05$).

RESULTS AND DISCUSSION

The Ki67 index of tumor cell proliferation was higher in group B compared to group A, and the Ki67 index in group C showed further increase compared to group B (Table 1). Table 2 shows that the mice in group B had a shorter survival time compared to group A, while group C had a longer survival time than group B.

In group B, the level of Bcl-2 expression was lower compared to group A, whereas the levels of BAX and caspase-3 expression were higher in group B than in group A. In Table 3, the group C exhibited an increased expression level of Bcl-2 compared to the group B, whereas the group C showed decreased expression levels of BAX and caspase-3 in comparison to the group B.

In group B, the expression levels of TGF-β, FAK, and AKT mRNA were higher compared to group A, whereas in group C, the expression levels of TGF-β, FAK, and AKT mRNA were lower compared to group B (Table 4).

In Table 5, the group B exhibited higher protein expression levels of TGF-β, FAK, and AKT compared to the group A, whereas the group C showed decreased expression levels of TGF-β, FAK, and AKT compared to the group B.

TABLE 1: KI67 INDEX OF TUMOR CELL PROLIFERATION

Group	n	Ki67 index
A	20	0.19±0.06
B	20	2.93±0.38[a]
C	20	1.53±0.26[b]
F		522.412
p		0.000

Note: [a]p<0.05 compared to group B, and [b]p<0.05 compared to group C

TABLE 2: DURATION OF SURVIVAL FOR MICE

Group	n	Survival time/d
A	20	57.71±18.22
B	20	27.66±9.82[a]
C	20	37.38±11.20[b]
F		157.823
p		0.000

Note: [a]p<0.05 compared to group B, and [b]p<0.05 compared to group C

TABLE 3: APOPTOTIC PROTEIN EXPRESSION

Group	n	BAX	Caspase-3	Bcl-2
A	20	0.37±0.09	0.41±0.14	0.54±0.03
B	20	0.86±0.16[a]	0.78±0.12[a]	0.31±0.03[a]
C	20	0.43±0.14[b]	0.44±0.14[b]	0.49±0.04[b]
F		80.413	47.276	258.235
p		0.000	0.000	0.000

Note: [a]p<0.05 compared to group B, and [b]p<0.05 compared to group C

TABLE 4: IMPACT OF TGF-β, FAK AND AKT mRNA IN THE LUNG TISSUE OF MICE

Group	n	TGF-β mRNA	FAK mRNA	AKT mRNA
A	20	1.24±0.32	0.83±0.35	0.77±0.09
B	20	2.47±0.54[a]	2.59±0.67[a]	2.05±0.46[a]
C	20	1.65±0.40[b]	1.76±0.43[b]	1.32±0.35[b]
F		212.744	318.451	321.687
p		0.000	0.000	0.000

Note: [a]p<0.05 compared to group B, and [b]p<0.05 compared to group C

TABLE 5: TGF-β, FAK AND AKT PROTEIN EXPRESSION

Group	n	TGF-β	FAK	AKT
A	20	0.28±0.04	0.30±0.03	0.33±0.14
B	20	0.86±0.28[a]	0.76±0.11[a]	0.78±0.12
C	20	0.57±0.08[b]	0.52±0.09[b]	0.44±0.14[b]
F		115.521	245.156	218.481
p		0.000	0.000	0.000

Note: [a]p<0.05 compared to group B, and [b]p<0.05 compared to group C

Bronchial mucosa or gland-originating malignant tumor is known as lung cancer. Histopathological classification categorizes lung cancer into two types; NSCLC and small cell lung cancer. NSCLC is the prevailing form, constituting approximately 80 % of all reported cases of lung cancer[8]. Throughout every phase of tumor progression, metastasis is an intricate pathological phenomenon regulated by various genes and proteins. Hence, it is highly important to investigate the metastatic process of NSCLC and the correlation between invasive genes and proteins for the purpose of developing targeted treatments. Chinese herbal medicine, along with other Asian countries, has a rich history spanning thousands of years, with each type possessing distinct medicinal properties and therapeutic benefits. Chinese herbal medicine, as a novel approach in cancer treatment, has garnered increasing interest from both domestic and international scholars. Chinese herbal medicine, when combined with conventional cancer treatments like chemotherapy, has demonstrated the ability to enhance the responsiveness of tumors to chemotherapy. This helps to reduce the drug resistance of tumors to chemotherapy, thus improving the effectiveness of treatment[9]. By reducing toxicities and side effects, improving the efficacy of chemotherapeutic drugs, and providing safer and more comprehensive treatments, Chinese herbal medicine can achieve this goal. In addition, the active components in Chinese herbal medicine usually show multi-target effect. This means that they can affect a variety of biological pathways and molecules associated with cancer at the same time[10]. This multi-target effect makes Chinese herbal medicine have the ability to comprehensively interfere with tumor development and inhibit the growth and spread of tumor cells, so as to enhance the diversity and comprehensiveness of treatment.

Astragalus membranaceus (*A. membranaceus*) is extracted from the roots of plants and belongs to Leguminosae. Herbal medicines highly regard it as a safe and effective ingredient[11], as it has gained extensive recognition in the field of traditional Chinese medicine. Astragaloside IV, derived from the root of *A. membranaceus*, is a naturally occurring herbal compound. Astragaloside IV, belonging to triterpenoid saponins, possesses a chemical structure that closely resembles saponins. *A. membranaceus* is widely recognized as a crucial element in its pharmacological effects, exhibiting a diverse range of pharmacological activities. The effects of antioxidants, anti-inflammatory agents, antivirals, and immunomodulatory are included[12]. Furthermore, numerous *in vitro* and *in vivo* investigations have demonstrated that astragaloside possesses the ability to impede the proliferation of various cancer cell types and facilitate apoptosis[13]. Research has indicated that astragaloside IV has the potential to enhance liver cancer by means of Nuclear Factor Erythroid 2–Related Factor 2 (Nrf2)-mediated Mothers against Decapapentaplegic (SMAD) 3C/3L conversion. This study aims to investigate the suppressive impact of astragaloside on the invasion and spread of NSCLC by obstructing the TGF-β/FAK/AKT signaling pathway. The findings may offer a fresh perspective for the clinical management of

NSCLC[14]. The findings indicated that the tumor cell proliferation Ki67 index was higher in group B compared to group A, whereas it was lower in group C compared to group B. Mice in group B had a shorter survival time compared to group A, while mice in group C had a longer survival time than group B. In group B, the level of Bcl-2 expression was lower compared to group A, whereas the level of BAX and caspase-3 expression was higher in group B than in group A. Group C exhibited higher expression levels of Bcl-2 compared to group B, whereas the expression levels of BAX and caspase-3 were lower in group C than in group B. Astragaloside IV is recommended for its potential to hinder the invasion and spread of NSCLC, enhance apoptosis, and extend the duration of survival.

The metastasis of NSCLC cells initiates with the process of Epithelial-Mesenchymal Transition (EMT). EMT-generated cells have the ability to undergo specific changes in their structure, migrate and invade, as well as prevent apoptosis and break down the extracellular matrix[15]. EMT occurs mainly in various physiological and pathological processes, including tissue and embryonic development, organ injury and repair, organ fibrosis, metastasis of cancerous tumors, and other events. The pathway controls various cellular processes, such as cell specialization, programmed cell death, and cell growth, and has been demonstrated to hinder or facilitate the advancement of tumors through various mechanisms[16]. Dysfunction or atypical stimulation of TGF-β may result in various pathological disorders, such as cancerous growths, fibrotic ailments, aberrant immune reactions, and more. Reports indicate that TGF-β acts as a typical initiator for EMT and has a vital function in maintaining EMT in various cancer cells derived from epithelial or epithelioid sources[17]. It acts antitumor by causing the death of normal and precancerous cells in the early stages of cancer. On the other hand, the promotion of cancer is facilitated by TGF-β through its enhancement of the EMT and metastasis of cancer cells[18]. Furthermore, TGF-β has the ability to impact various intracellular signaling pathways, such as the VEGF and PI3K/AKT pathways, which play a crucial role in the proliferation, growth, and metastasis of lung cancer cells. They are effective therapeutic targets for lung cancer[19]. The findings indicated that the group B exhibited an increase in the relative expression of TGF-β, FAK, and AKT mRNA compared to group A, whereas the group C demonstrated a decrease in the relative expression of TGF-β, FAK, and AKT mRNA compared to group B. The levels of protein expression for TGF-β, FAK, and AKT were higher in group B compared to group A, whereas they were lower in group C compared to group B. Blocking the TGF-β/FAK/AKT signal pathway with astragaloside is believed to have inhibitory effects on the invasion and metastasis of NSCLC.

Several research studies have indicated that the potential of TGF-β to trigger caspase 3 and reduce Vascular Endothelial Growth Factor (VEGF) levels, as well as its ability to hinder the PI3K-mediated AKT signal pathway, may contribute to its anti-tumor properties[20]. The PI3K/AKT pathway is not just a significant signaling factor in the development of cancer, but also a crucial intracellular pathway involved in cell cycle regulation, including cell inactivation, proliferation, cancer development, and longevity[21]. To accomplish various biological functions, such as activating camp response element binding protein, mammalian rapamycin target protein, and PI3K[22,23], PI3K hinders phosphorylation and subsequently triggers AKT activation. Furthermore, certain biomolecules like insulin-like growth factor-1, EGF, calmodulin, and FAK have the ability to trigger this pathway. Furthermore, the FAK-triggered PI3K/AKT pathway has been discovered to have links to numerous types of cancers, and controlling this pathway offers a fresh outlook on treating diseases. As a cytoplasmic kinase, FAK participates in the regulation of extracellular matrix integrin pathway and participates in metastasis and invasion by regulating tumor cells.

In conclusion, astragaloside IV has the ability to hinder the invasion and spread of NSCLC by obstructing the TGF-β/FAK/AKT signaling pathway.

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Conflict of interests:

The authors declared no conflict of interests.

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