The Mechanism Underlying Hanchuanshu Treatment of Asthma Based on Network Pharmacology and Biological Verification of The Effects in Smooth Muscle Cells

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ABSTRACT

Objective: To construct a molecular regulatory network of "components-core targets of smooth muscle action-pathways" for the treatment of asthma by Hanchuanshu. This model was used to explore the mechanism of its "multiple components-multiple targets-multiple pathways" network. Rat airway smooth muscle cells (ASMCs) were cultured *in vitro*, and the effect of Hanchuanshu on the cytokine expression of contractile and synthetic ASMCs was explored.

Methods: The ADME/T calculation method was used to screen the components of Hanchuanshu for the treatment of asthma. Through network pharmacology methods, the target identification platform based on reverse pharmacophore matching was used to analyze. Predict potential targets, and a biological annotation database (DAVID) was used to analyze the function of target genes and metabolic pathways. Cytoscape software was used to construct a network model of "components-core targets of smooth muscle action-pathways" for the treatment of asthma with Hanchuanshu. Rat ASMCs were cultured in vitro, and ASMCs were exposed to final concentrations of 1, 2, and 4 µg/ml for 48h. The RT-qPCR method was used to determine the mRNA levels of ADRB1, a-SMA, IL-6, TNF-α, IFN-γ, IL-4 and IL-10.

Results: The results of network analysis showed that the 63 active ingredients in Hanchuanshu may regulate the TNF signaling pathway, phosphatidylinositol 3-kinase (PI3K)/AKT (protein kinase B) signaling pathway, HIF-1, and 132 pathways, such as FAK, calcium, and Ras, which play a role in treating asthma. Through *in vitro* cytological simulation of the two phenotypes of asthmatic airway smooth muscle cells, it was found that Hanchuanshu can increase the expression of ADRB1 in contractile ASMCs and has little effect on the expression of a-SMA and IL-4; in contrast, it increased the expression of ADRB1, a-SMA, IL-10 and IFN-γ in synthetic ASMCs and decreased the expression of IL-4, IL-6 and TNF-α.

Conclusion: A network pharmacology analysis found that Hanchuanshu has multiple active components, multiple targets and multiple pathways in smooth muscle cells in the treatment of asthma, providing new ideas and clues for the elaboration of the mechanism of this compound. In vitro experiments verified that Hanchuanshu can inhibit the expression of IL-4, IL-6 and TNF-α and promote the expression of ADRB1, a-SMA, IL-10 and IFN-γ in ASMCs to exert an anti-asthma effect.

KEYWORDS: Network pharmacology; Hanchuanshu; Asthma; Mechanism of action

INTRODUCTION

Asthma is one of the most common respiratory diseases. It is estimated that there are more than 1 million asthma patients worldwide, and the annual cost of treatment is as high as 5.5 billion US dollars [1]. The overall prevalence of asthma in China is 1.24%, and it has substantially increased. The trend indicates that this disease is a serious public health problem. Traditional Chinese medicine is used to treat asthma according to the principle of "treatment of the symptoms if acute and the root cause if slow". Asthma is divided into cold and hot asthma. During the onset of cold asthma, the etiology and pathogenesis are mostly cold phlegm in the lungs, which is triggered by cold, the lung qi is stagnated, the airway is blocked, and cold phlegm is induced. Based on many years of clinical experience, the team of chief physician Zhang Liling has developed Hanchuanshu to warm the lungs, eliminate phlegm and relieve coughs. Nearly 20 years of clinical observation, the effect has been confirmed [2-5]. Details of 200 patients with cold asthma in the 454 Hospital of the People's Liberation Army can be found in the literature [3]. After 3 weeks of treatment, the symptoms of asthma, sputum expectoration, chest tightness and the cold feeling disappeared. Cold asthma is mostly caused by cold phlegm in the lungs; this condition is believed to be caused by cold, phlegm increases the air resistance, and lung qi stagnation causes shortness of breath, phlegm, cough and expectoration. The cause of the disease is cold, yin is abundant in the body, and yang cannot be communicated. Therefore, the limbs are cold, the tongue is pale, the tongue coating is white, and the pulse is rapid. The treatment principle is to warm the lungs and dispel cold and expectoration and relieve asthma. Hanchuanshu is mainly comprised of Ephedra xuanfei, which has anti-asthma effects, combined with dried ginger, asarum to warm the lungs and reduce phlegm, Pinellia ternata, aster to relieve cough, and Xuanfuhua with ochre for lowering qi, reducing phlegm and alleviating asthma. The combination of all medicines can warm the lungs, dispel cold and eliminate phlegm, lowering the qi, relieving asthma and relieving cough. As shown by clinical practice, this drug has obvious effects of relieving asthma and relieving cough, improving the ventilation function of the lungs, restoring Yang Qi, eliminating cold phlegm, smoothing the meridians, and having a satisfactory effect on improving symptoms and signs. However, the components of Hanchuanshu are complex. Through which signaling pathway is the mechanism of treating asthma mediated? At the frontier of Chinese medical research, network pharmacology combines systems biology and multidirectional pharmacology. This field explores the relationship between drugs and diseases from a holistic perspective and emphasizes the integration drugs, targets, and diseases. Starting from a systematic point of view, this strategy reflects and explains the multicomponent-multistage interactions of traditional Chinese medicine, and the holistic and systematic characteristics of this research strategy coincide with the theory of Chinese medicine for the diagnosis and treatment of diseases from the perspective of holistic concepts and syndrome differentiation [6]. In this study, the network pharmacology method was used to study the complex network relationship between the multiple components, multiple targets and smooth muscle effects of Hanchuanshu in asthma, and then, we used in vitro culture of primary rat airway smooth muscle cells (ASMCs) to verify our findings. The effect of this treatment on the expression of biological and inflammatory factors of contractile and synthetic ASMC will provide a reference for revealing the mechanism of Hanchuanshu on smooth muscle.

MATERIALS AND METHODS

Database, Processing Software and Reagent Equipment [7]

The selected databases were Traditional Chinese Medicine System Pharmacology Analysis (TCMSP) [8], the BATMAN-TCM online analysis database [9], the UniProt protein database (general) [10], the biological annotation database DAVID V 6.8, and Cytoscape mapping software. Hanchuanshu granules (produced by the Chinese Medicine Preparation Room of 454 Hospital, nonstandard preparation approval number: FP54022, main ingredients: 3g roasted ephedra, 3g dried ginger, 10g asters, 3g asarum, 10g *Piniella*, 10g inula, and 10g ochre) were administered at 10 grams each time, 2 times a day, for 3 weeks [5]. A 100 mesh steel sieve, fetal bovine serum (Gibson), DMEM (HyClone), collagenase-I and trypsin (Beijing Solar bio Technology Co., Ltd.), MTT (Sigma), HiScript II Q RT SuperMix for qPCR and ChamQ SYBR qPCR Master Mix reagent (Nanjing Novazan Biotechnology Co., Ltd.), a 5332 PCR gene amplification instrument (Eppendorf), an HF160F CO_2 incubator (Li Kang Biomedical Technology Holdings Co., Ltd.), an Eclipse Ti inverted fluorescencemicroscope (Nikon), an ELx800 microplate reader (Thermo), and a Millipore water purifier were used.

Collection of the Main Active Ingredients and Target Acquisition

In this study, we searched for the relevant information in the TCMSP and screened all the active ingredients of ginger. The screening condition was oral bioavailability (oral bioavailability, OB) ≥30% and drug-likeness (drug-likeness, DL) ≥0.18 [11]; OB is generally considered to be a reference index for evaluating whether a drug exerts its efficacy. DL indicates the similarity between the identified ingredients and known western medicines, which has important reference significance for determining whether the ingredients of Chinese medicines have an effect in the body. The TCMSP target prediction model was used to predict the target of the relevant active ingredients, and the gene name of the relevant target was extracted from the UniProt database. Cytoscape 3.6.1 was used to construct a network diagram of Hanchuanshu "active ingredients-targets", analyze the network diagram with network topology technology, and study the relationship between the main active components and the targets.

Targets of Action-Construction of Related Disease Networks

The genes of the main targets of the Hanchuanshu compound were imported into the CTD online analysis platform to obtain disease information related to the target and analyze the effects of Hanchuanshu in the treatment of asthma. The role of smooth muscle was combined with the published literature to study the mechanism of its effect. The network topology software Cytoscape 3.5.1 was used to establish a disease information network, which shows the targets of cold asthma and the beneficial effects. In this network, the nodes representing the target protein are nodes, and the interactions of the targets are represented by "edges". The "network analyzer" function of the Cytoscape 3.5.1 software was used to analyze the network topology and calculate its "betweenness centrality" and "node degree distribution", 2 important network topology parameters (the degree value reflects the number of connections between a node "target protein" and other "target proteins", and the betweenness is the path of the target protein

among all the shortest paths in the network). The ratio of the number to the total number of shortest paths was determined. The degree value and betweenness are the main topological parameters to quantify the importance of a target protein (node) in the network. A high degree value and betweenness value allow the subsequent discovery of new drugs and target prediction and finally use the network topology properties to analyze the main active ingredients of Hanchuanshu

Construction of an Interaction Network of Related Target Proteins

To study the interaction between the main active components of Hanchuanshu and related target proteins, we constructed the related target protein-protein interaction (PPI) network with the STRING platform. In the analysis platform, the species was set to human (*Homo sapiens*), the lowest interaction threshold was set to 0.4, that is, medium confidence (medium confidence), and the rest of the parameters had default settings. Cytoscape 3.5.1 was used to study the topological properties of the PPI network and screen and discuss the key target proteins in the PPI network.

Biological Process and Pathway Analysis

Based on the above results, through the biological information annotation database DAVID 6.8, the most enriched biological annotations were screened out. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used for pathway enrichment and Gene Ontology (GO) biological process enrichment for the target genes of the main active components of Hanchuanshu to analyze the main active components of Hanchuanshu. The main metabolic pathways and GO biological processes of the target were identified ($P<0.05$ was defined as significant).

Preparation of Rat Smooth Muscle Cells (AMSCs); [12]

After the weaned SD rats were sacrificed, the whole body was soaked in 75% alcohol for 5 min and then moved to an ultraclean workbench. The bronchus was removed, cut and spread, and the inner and outer membranes and epithelial and mucosal tissues were scraped off. The inner muscle layer was cut into small pieces less than 1 mm, 1g/L collagenase I was added, and the sample was digested for 1.5-2h. The samples were filtered with steel mesh and centrifuged at 1000r/min for 10min. The cells were resuspended in 20% FBS medium and transferred to cell culture flasks for culture. Based on the different attachment times of tracheal smooth muscle cells and other cells, the cells in the culture flask with a fusion rate of approximately 80% were digested and passaged. After 40min, the upper cell suspension was carefully aspirated and transferred to another culture flask. This procedure was repeated many times until relatively pure tracheal smooth muscle cells were obtained. The a-SMA protein was detected by immunofluorescence.

MTT Cytotoxicity Test

Cells in the logarithmic growth phase were adjusted to 2×10^5/mL, inoculated at 100 µl/well in a 96-well plate, and incubated at 37 ℃ in a 5% CO_2 incubator. Final concentrations of 8 mg/ml, 4 mg/ml, and 2 mg/ml were used for exposure. Three replicate wells were set for each concentration, and the blank group (only containing medium) and the control group (cells and medium) were set for 24h, 48h, and 72 h. Four hours before the end point, MTS reagent with a final concentration of 1 mg/ml was added, and the optical density (OD) value of the microplate reader was measured at 490nm. The OD value of each treatment group can represent the cell activity. The cell survival rate calculation formula is (OD experimental group – OD blank group)/ (OD control group – OD blank group) ×100%.

Cell Transformation and Toxicity

The main function of normally cultured ASMCs is smooth muscle contraction, and thus, these cells are known as contractile cells. ASMCs were inoculated in serum-free basal medium and starved for 6 days. At this time, ASMCs increased the synthesis of inflammatory factors and other cytokines due to artificial damage, that is, transformation from contractile cells to synthetic cells. The contractile and synthetic ASMC cells were digested and suspended, adjusted to a cell concentration of 2×10^5 cells/ml, inoculated into a cell culture flask at 1 ml/bottle, and incubated at 37 ℃ in a 5% CO_2 incubator. The cells were exposed to the final concentrations of 1 mg/ml, 2 mg/ml, and 4 mg/ml or control conditions. Three parallel samples were used for each concentration, and the exposure time was 48h. Cells in each dose group were collected for the experiment.

Fluorescence Quantitative RT-qPCR Analysis of the mRNA Expression of a-SMA, ADRB1, IL-4, IL-6, IL-10, TNF-α, and IFN-γ

After the cells were counted in each dose group, they were adjusted to the same number of cells. After centrifugation, cell lysis buffer was added for lysis, and RNA was extracted according to the kit instructions. A fluorescence quantitative RT-qPCR kit was used, and the protocol was performed according to standard procedures. The primers were synthesized by General Biosystems (Anhui) Co., Ltd., and the reaction conditions are listed in Table 1. a-actin was used as the internal reference, and the Cq values were compared.

Table 1: Primer sequences of genes were described for RT-qPCR.

| Gene	Primer
α-SMA F	CGTGACTACTGCTGAGCGTG
α-SMA R	CAGGCAGTTCGTAGCTCTTC
ADRB1 F	ACTTCGGTAGACGTGCTATGTG
ADRB1 R	AAAGGGCAGCGTGATGG
IL-4 F	CTTACGGCAACAAGGAACACCAC
IL-4 R	GAGCGTGGACTCATTCACGG
IL-10 F	GCAGGACTTTAAGGGTTACTTGG
IL-10 R	TCATTCTTCACCTGCTCCACTG
TNF-αF	CCACCACGCTCTTCTGTCTACTG
TNF-αR	CTTGGTGGTTTGCTACGAACG
IFN-γ F	TCTGGAGGAACTGGCAAAAG
IFN-γR	TGCGATTCGATGACACTTATG
IL-6 F	CATTCTGTCTCGAGCCCACC
IL-6 R	GCTGGAAGTCTCTTGCGGAG
β-actin F	GCCATGTACGTAGCCATCCA
β-actin R	GAACCGCTCATTGCCGATAG

RESULTS

Screening of Active Ingredients of Hanchuanshu in Treating Asthma

We identified 917 asthma-related targets, and 854 targets remained after eliminating duplicates. Based on TCMSP, TCMID, TCM-PTD and other databases and literature mining, 198 main chemical components of Hanchuanshu were selected, and ADME/T calculations were performed on the identified chemical components (OB≥30% and DL≥0.18) [9]. The literature was used to verify whether these components could be used to treat asthma, and we further screened the active ingredients. A total of 63 active ingredients for treating asthma were obtained (Figure1).

Target Prediction

After the Chem Bio 3D Ultra12.0 analysis and target prediction results, a total of 21 targets were obtained (Figure 2).
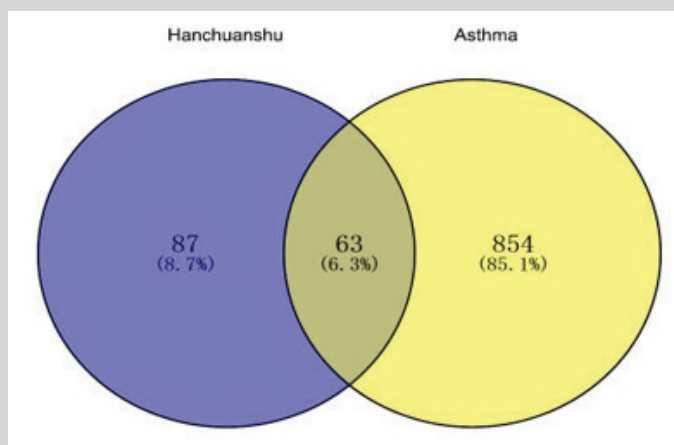
Figure 1: 854 targets remained after eliminating duplicates of 917 asthma-related targets, based on TCMSP, TCMID, TCM-PTD and other databases and literature mining, A total of 63 active ingredients for treating asthma were obtained.
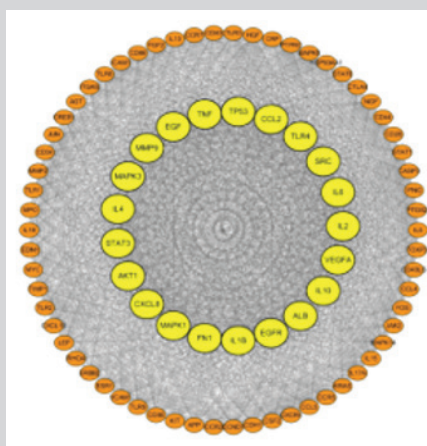
Figure 2: After the Chem Bio 3D Ultra12.0 analysis and target prediction results, a total of 21 targets were obtained.

Target Biological Function Analysis

Figure 3 shows the GO biological process analysis for the enrichment of targets of Hanchuanshu's active ingredients. The analysis results showed that the predicted targets were involved in positive regulation of multicellular organismal process and response to lipids. The top ranked process was multicellular organismal process; the ratio of binding (molecular and protein) was the top category among molecular functions; among cellular components, membrane, cytoplasmic part and cytosol were closely related to the targets. These results reflect that Hanchuanshu may alleviate asthma by improving these biological processes.

Target-Pathway Analysis

DAVID was used to analyze the pathways of cold asthma and the targets. Sixty-three targets were involved in the TNF signaling pathway, phosphatidylinositol 3-kinase (PI3K)/AKT (protein kinase B) signaling pathway, and hypoxia inducible factor 1 (HIF-1), FAK, calcium signaling pathway, Ras signaling pathway, proteoglycans in cancer, cytokine-cytokine receptor interaction MAPK signaling pathway and 132 other asthma-related pathways were identified. In these pathways, TNF signaling participates in systemic inflammation and is one of the cytokines that constitutes the acute phase response. After activating these receptors, various signal transduction pathways (e.g., Ca^{2+}, cAMP, and the

protein kinase cascade) are quickly activated and ultimately affect downstream transcription factors, mainly regulating blood vessel function and participating in the inflammatory response. Hypoxia-inducible factor 1 (HIF-1) is a basic helix-loop-helix-PAS domain transcription factor that is essential in the body's response to low oxygen concentration or hypoxia. FAK plays a very important role in cell signal transduction. This molecule is a key factor for the transduction of intracellular and extracellular signals and mediates multiple signal pathways. FAK can integrate signals from integrins, growth factors and mechanical stimulation, activate intracellular PI3K/Akt, Ras/MAPK and other signal pathways, and regulate cell proliferation and growth. The top 40 related pathways are shown in Figure 4.
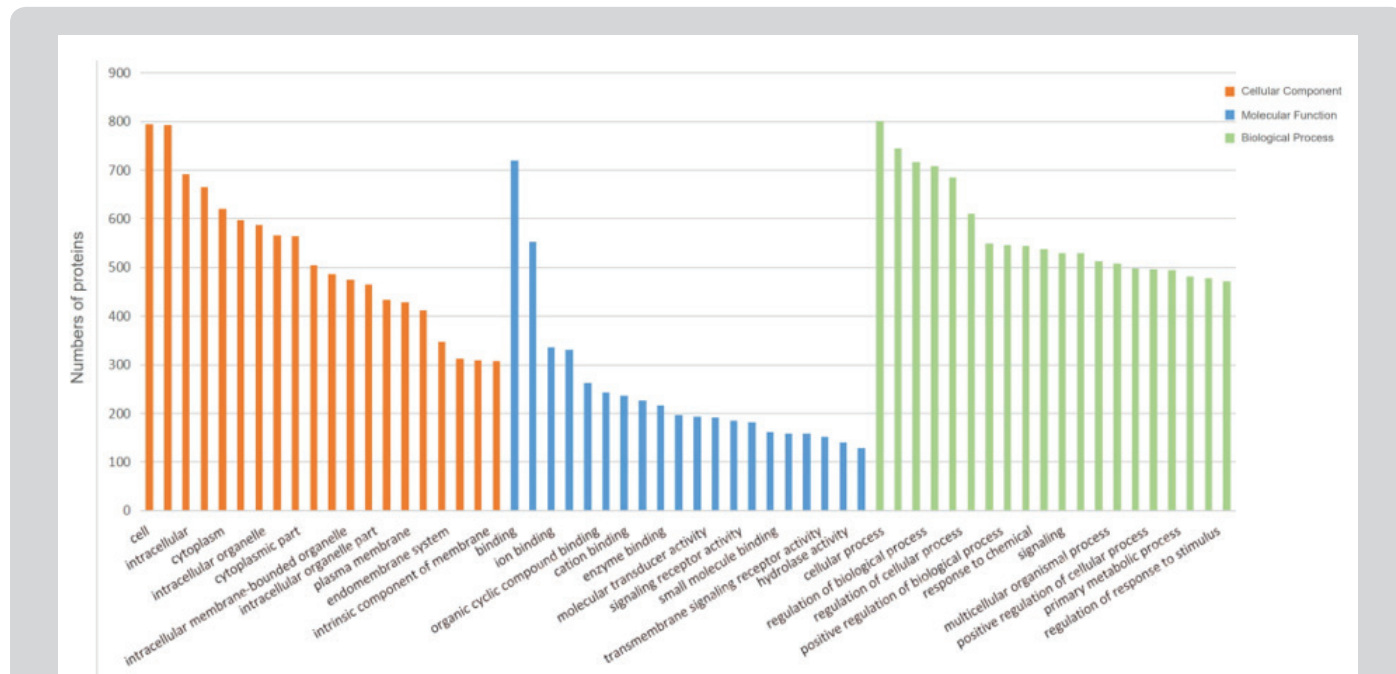

Figure 3: GO biological process analysis for the enrichment of targets of Hanchuanshu's active ingredients.
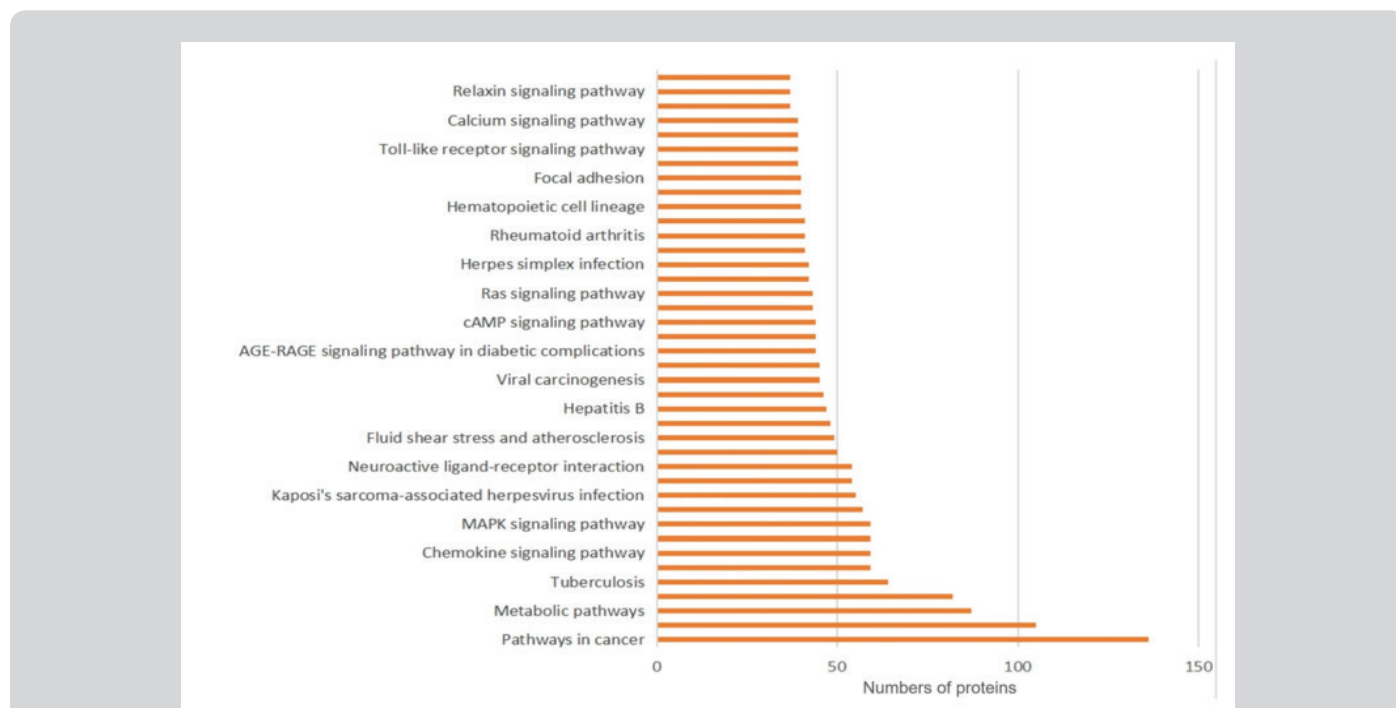

Figure 4: DAVID was used to analyze the pathways of cold asthma and the targets. Sixty-three targets were involved in the TNF signaling pathway, phosphatidylinositol 3-kinase (PI3K)/AKT (protein kinase B) signaling pathway, and hypoxia inducible factor 1 (HIF-1), FAK, calcium signaling pathway, Ras signaling pathway, proteoglycans in cancer, cytokine-cytokine receptor interaction MAPK signaling pathway and 132 other asthma-related pathways were identified.

Identification of ASMCs

Morphological observation was conducted: the primary cells were long or polygonal and spindle-shaped, and some cells had multiple foot processes. With continued culture, the cells around the tissue mass grew to confluence, showing the typical "peak-valley" structure of primary tracheal smooth muscle cells. After purification and culture, the cells were mostly long and spindle-shaped, and polypodocytes were occasionally observed. Purified cells were subjected to fluorescence analysis of α-smooth muscle actin (α-SMA) specifically expressed in smooth muscle cells. The results showed that the cells all showed green fluorescence, which confirmed that the purified cells were ASMCs (Figure 5).
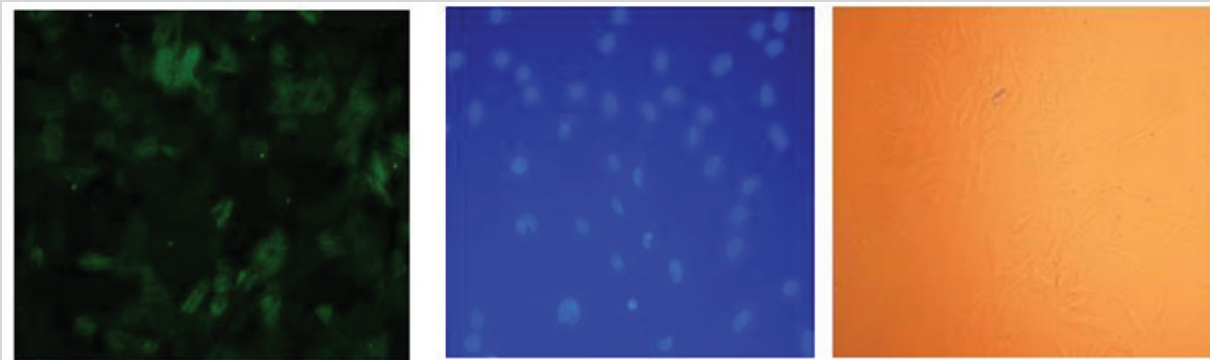

Figure 5: The primary cells were long or polygonal and spindle-shaped, and some cells had multiple foot processes. With continued culture, the cells around the tissue mass grew to confluence, showing the typical "peak-valley" structure of primary tracheal smooth muscle cells. After purification and culture, the cells were mostly long and spindle-shaped, and polypodocytes were occasionally observed. Purified cells were subjected to fluorescence analysis of a-smooth muscle actin (a-SMA) specifically expressed in smooth muscle cells.

MTT Test Results

When the final concentration of Hanchuanshu was less than 8 mg/mL, the cell proliferative activity of each dose group at the 3 time points was slightly higher than that of the control group, and the cell proliferative activity between each dose group was relatively similar. When the final concentration of Hanchuanshu was greater than 8 mg/mL, the cell proliferative activity at both 24h and 48h showed a steep decline. The cell proliferative activity at 72h also began to decrease sharply when the final concentration of Hanchuanshu was greater than 16 mg/ml. Based on Figure 6 data, plus a control group our doses for this experiment were set as 4 mg/ml, 2 mg/ml, and 1 mg/ml; 3 dose groups and a solvent control group were used, and the time was 48h.
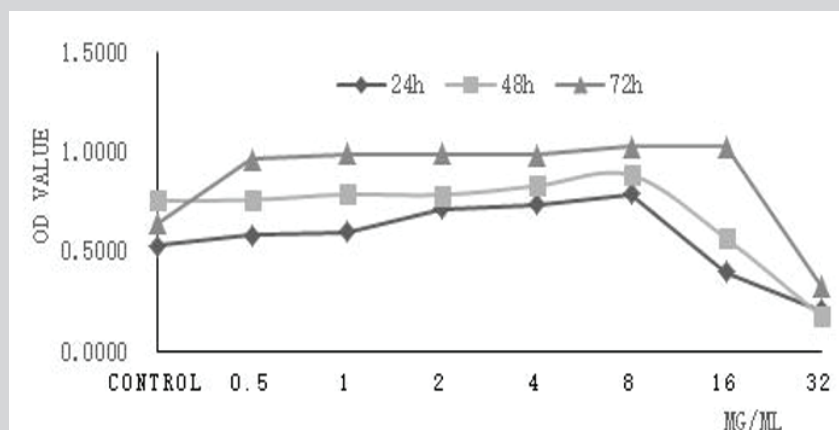
Figure 6: When the final concentration of Hanchuanshu was less than 8 mg/mL, the cell proliferative activity of each dose group at the 3 time points was slightly higher than that of the control group, and the cell proliferative activity between each dose group was relatively similar. When the final concentration of Hanchuanshu was greater than 8 mg/mL, the cell proliferative activity at both 24h and 48h showed a steep decline. The cell proliferative activity at 72 h also began to decrease sharply when the final concentration of Hanchuanshu was greater than 16 mg/ml. Based on this data, plus a control group our doses for this experiment were set as 4 mg/ml, 2 mg/ml, and 1 mg/ml; 3 dose groups and a solvent control group were used, and the time was 48h.

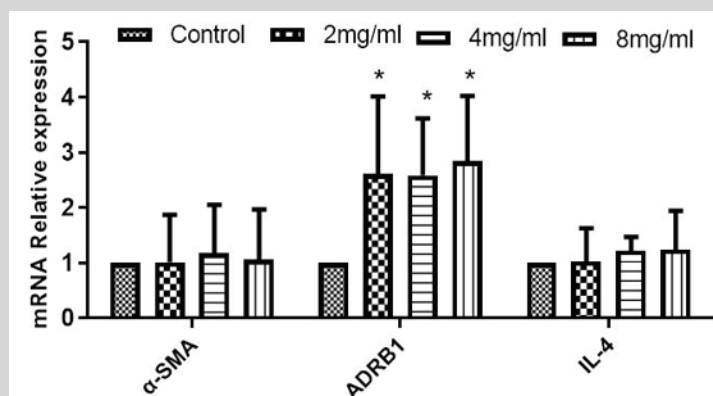## mRNA Expression of a-SMA, ADRB1, IL-4, IL-6, IL-10, TNF-α, and IFN-γ

The expression of contractile ASMCs cells is shown (Figure 7):

The expression of ADRB1 tended to increase with increasing dose, especially in the high-dose group (8 mg/ml group) compared with the control group ($P<0.05$). The expression levels of IL-4 and ADRB1 were similar, and each dose group and the control group showed a
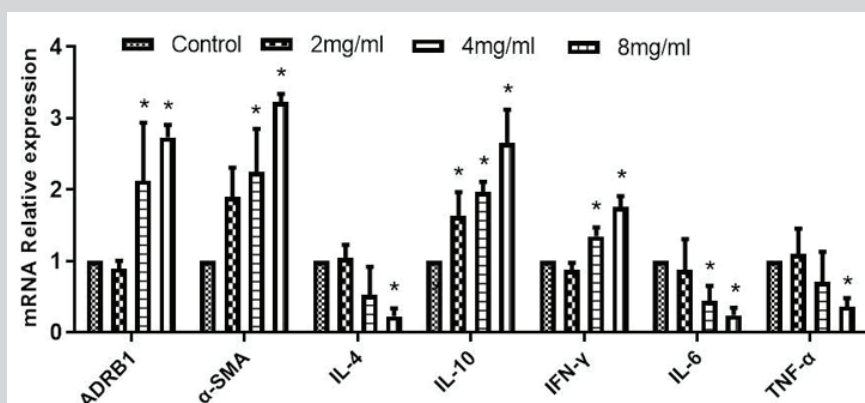
large increase, but the difference was not significant (*P*>0.05). The mRNA expression of a-SMA increased with increasing dose, and the mRNA expression of a-SMA was not significantly different from that of the control group (*P*>0.05).

Synthetic ASMC expression was also examined (Figure 8): In contrast to contractile cells, synthetic cells treated with Hanchuanshu

showed increased expression of ADRB1 and a-SMA with increasing doses. The expression of ADRB1 and a-SMA increased at medium and high doses compared with that of the control group (*P*<0.05), the expression levels of IL-4, IL-6 and TNF-α were all reduced, and the high-dose group was significantly different from the control group (*P*<0.05). The expression of IL-10 and IFN-γ increased with increasing dose, with significant differences (*P*<0.05).

Figure 7: The expression of ADRB1 tended to increase with increasing dose, especially in the high-dose group (8 mg/ml group) compared with the control group (P<0.05). The expression levels of IL-4 and ADRB1 were similar, and each dose group and the control group showed a large increase, but the difference was not significant (P>0.05). The mRNA expression of a-SMA increased with increasing dose, and the mRNA expression of a-SMA was not significantly different from that of the control group (P>0.05).

Figure 8: In contrast to contractile cells, synthetic cells treated with Hanchuanshu showed increased expression of ADRB1 and a-SMA with increasing doses. The expression of ADRB1 and a-SMA increased at medium and high doses compared with that of the control group (P<0.05), the expression levels of IL-4, IL-6 and TNF-a were all reduced, and the high-dose group was significantly different from the control group (P<0.05). The expression of IL-10 and IFN-γ increased with increasing dose, with significant differences (P<0.05).

DISCUSSION

Hanchuanshu is composed of seven compatible medicinal materials, including ephedra and dried ginger. There are a total of 21 different smooth muscle-related gene targets that act on different pathways by regulating different smooth muscle-related gene targets. This study found that Hanchuanshu may play a therapeutic effect through three mechanism: 1. This treatment participates in the PPAR signaling pathway, calcium ion signaling pathway, and cGMP PKG signaling pathway, and it can eliminate oxygen free radicals by regulating various inflammatory factors such as TNF-α and IL. 2. Hanchuanshu can alleviate asthma through

various metabolic pathways in the body; 3. Hanchuanshu regulates steroid biosynthesis, oxidoreductase activity, transcription factor binding process, homeostatic process and transmembrane transport protein activity pathway, inhibiting lung and bronchus epithelial cell proliferation and activation.

The overall prevalence rate of asthma in China is 1.24%, and it has shown an obvious upward trend. This disease has become a serious public health problem in China [13]. Many studies have shown that asthma is caused by various cells (such as eosinophils, mast cells, T lymphocytes, neutrophils, airway epithelial cells, etc.) and cellular components that participate in chronic inflammatory diseases of the airway [14,15]. Airway

inflammation, airway remodeling and airway hyper responsiveness (AHR) are the three main characteristics of asthma. Airway smooth muscle cells (airway smooth muscle cells, ASMCs) are the main effectors in the pathogenesis of asthma. Their proliferation and thickening are involved in asthma airway remodeling and airway hyperresponsiveness. After their transformation from a contractile to synthetic phenotype, these cells will produce a variety of inflammatory mediators and active factors, resulting in increased airway resistance, and other symptomatic contractile ASMCs develop a synthetic phenotype. Synthetic ASMCs can produce various cytokines and chemokines, causing multiple inflammatory cells to infiltrate the airway. The signal transduction pathway mediated by this aggregation and inflammatory cells themselves can be further amplified under the stimulation of chemokines, eventually generating more chemokines, driving the inflammatory response, and participating in chronic inflammation in the airway of individuals with asthma. In addition, synthetic ASMCs synthesize and release a large number of inflammatory mediators and cytokines, leading to airway remodeling and AHR and further causing the recruitment and activation of inflammatory cells, expanding the inflammatory cascade. Therefore, phenotypic transformation of ASMCs can change physiological activities, such as intracellular signal transduction pathways, inflammatory mediator release, cell proliferation and apoptosis, and directly affect the development and outcome of asthma. It is extremely important to reduce chronic airway inflammation, improve airway remodeling, and reduce AHR, which can effectively intervene in the phenotypic transformation of ASMCs. Airway smooth muscle cells cultured in vitro conform to the animal welfare trend of animal substitution and have the advantages of a short cell cycle, low cost, and easy control of varying factors. As primary cultured cells, ASMCs have more relevant biological activity than immortal cells such as P59. Therefore, ASMCs can be used as an economic and convenient system for the screening of anti-asthma drugs.

α-SMA is a characteristic protein in smooth muscle cells, an important immunological detection index for ASMCs, and one of the specific marker proteins of ASMCs [15], which can be used to distinguish these cells from epithelial cells. We performed a fluorescence immunoassay of the cultured primary cells, and the cytoplasm showed green fluorescence under the laser microscope, confirming that the cultured cells are the required ASMCs. Starving ASMCs for 1 week promotes the secretory function of some epithelial cells, which can synthesize high levels of active substances, such as IL-4 and ADRβ, and partially simulate the physiological changes of smooth muscle cells in asthma. ASMCs can transform from normal contractile cells to synthetic cells [16]. Our experiments used Hanchuanshu to act on contractile and synthetic ASMCs to evaluate the effect of Hanchuanshu on normal and asthmatic airway smooth muscles.

The test results showed that after 48 h of treatment, when the final concentration of Hanchuanshu was >4 mg/mL, the mRNA expression of α-SMA in contractile ASMCs decreased, and the expression of ADRβ1 increased significantly. The amount of ADRβ on the cell surface is related to smooth muscle relaxation. As expression increases, the smooth muscle is more easily regulated, muscle tension is relieved, and airway remodeling is decreased to antagonize the occurrence of asthma and improve the symptoms of asthma [17]. IL-4 can induce the synthesis of IgE, and its expression increases, which can promote the secretion of inflammatory signal factors and aggravate the occurrence of respiratory diseases [18]. The secretion of IL-4 in patients with asthma is also higher than

that in healthy individuals [19], which plays an important role in the development of asthma. Hanchuanshu can significantly reduce the IL-4 expression and secretion of synthetic ASMCs and relieve allergic symptoms and inflammatory reactions. In addition, the expression of IL-6 and TNF-α decreased, and the expression of IL-10 and IFN-γ increased with the increase of dose, indicating that the inflammatory response was alleviated. These changes indicate that Hanchuanshu can promote the transformation of ASMCs into normal smooth muscle cells. This change can soothes smooth muscle cells, opens airways, relieves inflammatory reactions, and helps relieve asthma symptoms.

CONCLUSION

The above studies show that network pharmacology analysis of the mechanism of action of Hanchuanshu in the treatment of asthma provided accurate results, and it also revealed the characteristics of Hanchuanshu's multiple components and multiple targets, providing a reference for future research.

DECLARATION

Ethics approval and consent to participate: All experiments were carried out in adherence with the guidelines of the Institutional Animal Care and Use Committee of China and were approved by the Animal Care and Research Committee of Jiangxi University of Traditional Chinese Medicine.

Consent for publication: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All participants agreed to publish.

Availability of data and materials: The data used to support the findings of this study are available from the corresponding author upon request.

Competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

FUNDING

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