

Network Pharmacology-Based Study on the Mechanism of *Pinellia ternata* in Asthma Treatment

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Abstract

Background: *Pinellia ternata* (PT), a medicinal plant, has had an extensive application in the treatment of asthma in China, whereas its underlying pharmacological mechanisms remain unclear.

Methods: Firstly, the therapeutic effect of PT was verified by an animal experiment. Secondly, a network pharmacology method was adopted to collect activated components of PT from Traditional Chinese Medicine Systems Pharmacology Database and Analysis (TCMSP); binding targets of PT were assessed by exploiting PharmMapper website; asthma-related targets were collected, and Target-Target interaction networks were built. Subsequently, critical nodes exhibiting high-possibility were identified as the hub nodes in the network, which employed to conduct GeneOntology (GO) comment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment analysis. Finally, the tissue expression profiles and single-cell RNA sequencing (scRNA-Seq) data of candidate genes were identified by Gene Expression Omnibus database (GEO) and PanglaoDB databases.

Results: In animal experiments, PT could alleviate the allergic response of mice by inhibiting the activation of *T-helper type 2* (TH2) cells and the secretion of IL-4 and IL-5. Subsequently, 57 achievable targets of PT on asthma were confirmed as hub nodes, included candidate genes matrix metalloproteinase-2 (*MMP2*) and nuclear receptor subfamily 3 group C member 1 (*NR3C1*). Moreover, according to transcriptome RNA sequencing data from lung tissues of allergic mice compared to normal mice, mRNA level of *MMP2* was up-regulated ($P < 0.001$), and mRNA level of *NR3C1* was no significant difference ($P = 0.0749$). Finally, we compared their levels of expression and distributions to those present in the single-cell level.

Conclusions: With network pharmacology, our study provides candidate genes that may be either used for future studies related to diagnosis/prognosis or as targets for asthma management. Besides, more attention should be paid to methods of identifying the origin of these genes and determining their expression at the single-cell level.

1. Background

Asthma refers to an inflammatory disorder of the airways which is induced by common aeroallergens (e.g., house dust mites, fungi, and air pollutants), and characterized by airway hyper-responsiveness, variable airflow limitation, mucous secretion, as well as chronic inflammation (1). In asthma, there exists selective expansion of T lymphocytes (particularly of TH2 cells) that secrete a cluster of cytokines such as interleukins (IL)-2, IL-4, IL-5, IL-9, IL-13, and granulocyte-macrophage colony-stimulating factor (GM-CSF), leading to mast cell differentiation and maturation, eosinophil maturation and survival, basophil recruitment, B cell differentiation and production of IgE, jointly orchestrate the allergic inflammatory cascade (2,3). On the whole, inhaled corticosteroid (ICS) or ICS combined with long-acting β 2-adrenergic agonists has been the common clinical treatment to mitigate asthma. In some cases, however, corticosteroid overload raises a high risk of glucocorticoid-related adverse events and huge difficulty in controlling the onset of asthma (4). For the asthma incidence, a single ingredient medicine cannot satisfy the existing treatment of asthma, and there is an urgent need for practical, multi-target drugs to treat asthma that can remedy the deficiencies exhibited by those that are currently available.

Traditional Chinese medicine (TCM) has been indispensable to the health of China. *Pinellia ternata*, also known as BanXia in Chinese, has been a common and potent medicinal herb in TCM practice. It has been clinically applying for suppression of the cough center to exert antitussive effects, facilitate cell division, decrease the viscosity of the whole blood, and reduce inflammation, in conjunction with other herbs (5-7). Existing studies have reported that PT attenuates ovalbumin (OVA)-induced asthma by the way of reducing the cell numbers of leucocytes, eosinophilic granulocytes and lymphocytes in lung tissues and by the way of decreasing concentration of IL-4, IL-5, IL-13 and TNF- α in bronchoalveolar lavage fluid (BALF) (8,9). However, the detailed mechanisms of their operation at molecular levels are still unknown, making it difficult any rationale in the design of more efficient and less toxic drugs for treatment. Thus, comprehensive and appropriate strategies are urgently required to gain deeper insights into how herbal formulae impact diverse biological processes in the treatment of diseases.

Network pharmacology refers to an efficient and robust method having been employed extensively for several decades to treat a range of diseases, as coupled with high-throughput group analysis, virtual computing as well as network database retrieval (10,11). Using network pharmacology in search of TCM is conducive to identifying the relationships between drug and disease, discovering active ingredients, elucidating the mechanism of action, and assessing drug safety (12). Furthermore, the recent development of scRNA-Seq technology has combined the single-cell isolation and RNA-sequencing technologies, which enabled the global transcriptome profiling on a single-cell level, offered high-resolution cell-specific gene expression for potentially unraveling of the mechanism of individual cells and greatly advanced our understanding of cellular states (13). Combination with tissue and single-cell transcription data, make it clear to identified the molecular signatures. In this study, a network pharmacological research was conducted to delve into practical components and action objectives of PT, as an attempt to explore the underlying mechanisms of action of disease treatment (Fig. 1).

2. Materials And Methods

2.1 Animal experiments

2.1.1 Mice model

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Shandong Academy of Medical Science. Female C57BL/6 mice, aged 7–8 weeks, were provided by Shandong Laboratory Animal Facility (Shandong Academy of Medical Sciences, Jinan, China). All animals were housed and bred in a temperature and light-controlled environment under a 12h light-dark cycle and they were maintained under specific pathogen-free (SPF) conditions. Subsequently, mice were randomly split into three groups (the control group, the model group, and the herb group) covering four mice respectively. The model group and herb group were injected intraperitoneally (i.p.) with 50 μ g OVA (Sigma-Aldrich, MO, USA) and 2.25 mg adjuvant aluminum hydroxide (Thermo Scientific, Pittsburgh, PA, USA) in a total volume of 100 μ L on day 0, 7, and 14. On day 21, the mice were intranasally (i.n.) administrated with 100 μ g OVA in 50 μ L. The herb group was administrated with PT (1.95 g/kg/d) (provided by affiliated hospital of Shandong University of Traditional Chinese Medicine, China) water decoction for 21 days, whereas the control mice were given the identical volume of phosphate-buffered saline (PBS). All mice were prepared for tissue harvesting.

2.1.2 Flow cytometric staining

Lungs were cut into small pieces and then incubated with 1 mg/ml collagenase D (Sigma-Aldrich, MO, USA) for 30 min and were digested into single-cell suspension. Rat-anti-mouse antibodies cover APC-CD45, PE-Cy7-CD3, PerCP-CD4, and PE-IL-5 (BD Biosciences, CA, USA). Intracellular cytokine staining was performed following the previous description (14). Flow cytometric (BD FACSVerse™, BD Biosciences, USA) data were analyzed with FlowJo software.

2.1.3 Measurement of IL-4/IL-5 in bronchoalveolar lavage fluid (BALF)

The concentration of IL-4 and IL-5 in BALF were measured using a specific mouse ELISA kit (R&D, systems, Minn, USA). ELISA experiments were performed according to the manufacturer's instructions.

2.2 Screening bioactive components of PT

The components data of PT originated from the TCMSP systems pharmacology database (<http://lsp.nwu.edu.cn/tcmsp.php>) (15). To achieve the bioactive components screening of PT in pharmacodynamics studies, two critical indicators have been usually taken as the screening criteria in ADME processes (e.g., absorption, distribution, metabolism, and excretion) and drug design, includes oral bioavailability (OB) and drug similarity (DL), and components were retained only if OB over 30% and DL more than 0.18 to satisfy criteria (16). In this study, based on relevant literature and the PubChem network database (<https://pubchem.ncbi.nlm.nih.gov/>), 11 bioactive components of PT were retained, and their corresponding information (Pubchem ID, OB and DL) was also acquired for subsequent analysis (17).

2.3 Component-related target proteins prediction

To ascertain the relevant targets of the bioactive components in PT, the PharmMapper database (<http://lilab.ecust.edu.cn/pharmmapper/get.php>) was adopted (18). In brief, for a given small molecule, potential candidate targets were identified using the reverse pharmaco-dynamic profiling method. Through the retrieving process, 115 assessed targets were screened out after duplicates were deleted, and make them conform to the correct Uniprot ID. Component-Target network maps were plotted by Cytoscape 3.7.2 for this data (19). Subsequently, GO enrichment and KEGG network pathway analysis were conducted on the targets acquired by using the Database for Annotation Visualization and Integrated Discovery (DAVID, <https://david.nicifcrf.gov/>, updated in Mar. 2017) online (20). Values of $P < 0.05$ were considered exhibiting statistical significance.

2.4 Asthma-related targets prediction

Data on the asthma-related targets were acquired from Online Mendelian Inheritance in Man database (OMIM, Updated December 21, 2019) (21,22), a comprehensive, authoritative compendium of human genes and genetic phenotypes, that is freely available and daily updated. The keyword was 'asthma', and 259 asthma-related targets were harvested jointly.

2.5 Protein-Protein interaction (PPI) network construction

To delve into the interaction between target proteins from a systematic and holistic perspective, PPI network mapping was conducted with String (<http://string-db.org>) (23). Accordingly, the “component-target-disease” network was established to express the relationships between the targets corresponding to PT and interacting protein; furthermore, such network was visually analyzed with Cytoscape 3.7.2 software. As critical parameters of network analysis, the degree of centrality (DC), betweenness centrality (BC), close to the central (CC), the network centrality (NC), and local edge connectivity topology selection (LAC) were adopted to obtain the significance of respective target. The node exhibiting a DC value higher than twice the median degree of all nodes and achieving the values of the other four indexes over the corresponding median values were defined as a hub (24). To standardly describe these genes, GO enrichment and KEGG pathway analysis were conducted by using DAVID. Values of $P < 0.05$ were considered exhibiting statistical significance.

2.6 Expression Patterns Analysis for MMP2 and NR3C1

Expression patterns in lung tissues may suggest the pathways that the target involved in asthma. GSE6858 was used to analyze expression patterns for genes acquired from the GEO database (<http://www.ncbi.nlm.nih.gov/geo>). In GSE6858, we compared the expression of MMP2 and NR3C1 in OVA stimulated mouse lung tissues compared to the normal group. PanglaoDB database (<https://panglaodb.se/>) was used to find cell types where a certain set of genes are expressed.

3. Results

3.1 PT ameliorated OVA-induced asthma

Asthma is a chronic allergic respiratory disease. Here we validated the potential anti-inflammatory and anti-asthmatic therapeutic properties of PT, using a mouse model of OVA-induced allergic asthma. Unquestionably, OVA-sensitized mice model exhibited an enhanced type 2 immune response, as the percentage of TH2 cells in lung was elevated compared with that of PBS-sensitized control mice ($*P < 0.05$); in the case of drug treatment, the percentage of TH2 cells was noticeably reversed with PT decoction therapy ($\#P < 0.05$) (Fig. 2A). As suggested by ELISA analysis, the concentration of IL-4 and IL-5 were significantly up-regulated by OVA treatment as compared with the control group, whereas PT treatment led to the down-regulation of their levels in BALF of allergic mice (Fig. 2B).

3.2 Screening for the active component of PT

Based on the in vivo anti-asthmatic effects of PT, we further examined the therapeutic mechanism of the drug. Firstly, the 116 reported active ingredients of PT were retrieved from the TCMSP database. Then the values of OB and DL were employed to screen potential active components and 13 of the mentioned bioactive ingredients were preliminarily screened out. Besides, only 11 active ingredients with corresponding targets were screened out after the final screening from PubChem database (Table1), most of which were sterols (beta-sitosterol, stigmasterol, cycloartenol), flavonoids (baicalein, baicalin), unsaturated fatty acid and lipid, exhibiting high oral bioavailability (rang from 30.7% to 44.72%) and high drug similarity (0.2~0.81). Moreover, these ingredients generally displayed the pharmacological activities of antibacterial, anti-inflammatory, and antioxidant.

3.3 Component-target network construction and enrichment analyze

Based on the data acquired above, the component-target network was established with Cytoscape 3.7.2 software (Fig. 3). In the established network, most of the components can act on multiple physiological targets, including baicalin acting on 61 targets, cycloartenol acting on 48 targets, gondoic acid acting on 26 targets, etc. Moreover, the enriched signaling pathways of these targets were analyzed by KEGG and the top 10 KEGG pathways were shown in Table 2, included PPAR signaling pathway, Thyroid hormone signaling pathway, Proteoglycans in cancer, Insulin signaling pathway, Pathways in cancer, Drug metabolism-cytochrome P450, Prolactin signaling pathway, T cell receptor signaling pathway, Metabolism of xenobiotics by cytochrome P450, and Glutathione metabolism.

3.4 Asthma-related targets

By searching the OMIM databases, the target data for the treatment of asthma were collected. A total of 259 targets were screened after false-positive information was checked and removed, including cytokines (e.g. TNF, IL-4, IL-5, IL-33), receptor (e.g. CCR4, IL-6R, IL-9R), chemokines (e.g. CCL2, CCL11, CCL18), and transcription factors (e.g. STAT4, STAT6, GATA3) (Table 3).

3.5 Hub nodes screening and PPI network construction

To elucidate the molecular mechanism underlying the effects of PT against asthma, the component-related targets and asthma-related targets were uploaded to STRING to acquire the information on PPI. 365 nodes and 3726 correlations are covered in the network. Next, according to the topology of DC, BC, CC, NC and LAC, 57 hub nodes and 808 relationships were ascertained. Among them, 47 hub genes are asthma-related targets, 8 hub genes are component-targets, MMP2 and NR3C1 are two targets which are both component-targets and highly relevant targets for asthma (Fig. 4A). We presumed those targets as the putative targets of PT for the treatment of asthma and constructed a PPI network among these 57 hub nodes (Fig. 4B).

3.6 Hub nodes enrichment analysis

To identify relevant pathways and functions, GO functional analysis and KEGG pathway enrichment analysis pathway enrichment was performed for these 57 putative targets using DAVID. A total of 276 enrichment results were obtained, covering 226 Biological processes (BP), 32 Molecular functions (MF), and 18 Cellular components (CC); the enriched molecular functions of the target proteins, which are mainly associated with responses to stimulus, biological regulation, cellular process (Fig. 5A). As revealed from the KEGG enrichment analysis, the signaling pathways were notably enriched in pathways of T cell receptor signaling pathway, JAK-STAT signaling pathway, and Cytokine-cytokine receptor interaction. After extensive pathways were excluded, the top 15 relevant signaling pathways are presented in Fig. 5B. Values of $P < 0.05$ were considered exhibiting statistical significance; the lower the P -value, the more prominent the relevance will be.

3.7 Expression profiles and scRNA-seq data of MMP2 and NR3C1

Based on the above data, genes MMP2 and NR3C1 are both component-target and highly relevant targets for asthma. In GSE6858, the expression level of MMP2 in OVA stimulated mice was higher than that of the

control group ($P < 0.001$), but between two groups, there was no significant difference in the expression level of NR3C1 (Fig. 6A). From the scRNA-seq data, MMP2 is mainly derived from stromal cells, such as fibroblasts, peritubular cells, and basal cells, while NR3C1 is mainly derived from not only stromal cells but also immune cells (e.g., T cells, B cells, dendritic cells) (Fig. 6B).

4. Discussion

The existing number of patients with bronchial asthma worldwide reaches over 300 million, and considerable patients still face difficulty in mitigating their symptoms after undergoing conventional atomization therapy, thereby developing refractory bronchial asthma (25). The mechanism of asthma pathogenesis remains unclear. Over the past years, numerous researchers have considered that airway inflammation cytokines exhibit abnormally high level on the pathogenesis of asthma and play a critical role in the process; to be specific, IL-4 is capable of stimulating the B lymphocyte to secret large specific IgE, which induces gathered eosinophils, increased airway mucosa, even airway hyperresponsiveness; IL-5 could control the differentiation, maturation, migration, and survival of eosinophils; IL-10 is capable of effectively reducing the secretion activity of mast cells and TH2 cells and exerts a definite effect on the reduction of airway inflammation; both IL-13 and IL-17 are critical pro-inflammatory cytokines in the body, thereby facilitating the synthesis of IgE, elevating the level of vascular adhesion molecules and increasing the degree of eosinophil infiltration in the airway (2,26). All of these genes are included in the asthma-related targets (Table 3). Besides, obesity, metabolic disease, and age can also act as risk factors exacerbating asthma (27). As impacted by heterogeneous in terms of severity, individual difference, and treatment responsiveness, the diverseness of this disease results in multiple therapeutic strategies. Several studies have reported that PT possesses anti-inflammatory and anti-allergic effects both in asthma and in COPD (8,28). However, the physiological and pharmacological actions of PT on asthma and its biological function have not been effectively studied. The incorporation of traditional Chinese medicine into clinical therapy via network pharmacology can provide insights into the possible mechanism and enhance the specificity and effectiveness of the treatment scheme (10).

In our study, flow cytometry analysis revealed that in allergic mice model, PT could attenuate allergic immune response by inhibiting the activation of TH2 cells and the concentration of IL-4 and IL-5. These data suggest that PT have a therapeutic effect on asthma. Then, active ingredients were screened in PT, primarily flavonoids, sterol, glycosides, and unsaturated fatty acid and proteins considered potential targets of ingredients employing to treat asthma were predicted. As further revealed from the KEGG enrichment analysis, the component-related targets primarily participated in PPAR signaling pathway (a nuclear hormone receptor is critical to regulating lipid metabolism, lipid oxidation, and cell proliferation, facilitate adipocyte differentiation and enhance glucose uptake), thyroid hormone signaling pathway (vital regulators of growth, development, and metabolism), proteoglycans in cancer (critical macromolecules contributing to the biology of cancer, covering proliferation, adhesion, angiogenesis, and metastasis) and several metabolic pathways (e.g., cytochrome P450, prolactin signaling pathway, and glutathione metabolism).

In the PPI network of component-targets and asthma-related targets, hub nodes were screened, and the GO enrichment analysis and the KEGG pathway enrichment analysis were performed to clarify the multiple

mechanisms systematically. Based on the GO terms, the activity of hub nodes was associated with numerous biologic processes (e.g., inflammatory response, chemotaxis and immune response), variety molecular functions (e.g., cytokine activity, growth factor activity, and protein tyrosine kinase activity) and diversified cellular components (e.g., extracellular space, extracellular region, and external side of plasma membrane). Thus, the results demonstrated that PT primarily exerted various therapeutic effects on asthma by participating in these processes and functions. In the cytokine-cytokine receptor interaction pathway, 17 of 57 hub nodes were included and critical to the department of asthma; among them, TSLP, IL-2, and IL-4 were strong inducers capable of directly activating TH2 cells and ILC2s even on the macrophage, respectively (25,29-31). IL-5 could activate and recruit eosinophils, IL-13 activates goblet cells, CCL2, CCL11 and CXCL16 recruit and chemotactic immune cell, all of which led to an allergic reaction. Furthermore, the JAK-STAT signaling pathway is one of a handful of pleiotropic cascades and plays essential roles during development and cellular processes (e.g., segmentation, proliferation, and organogenesis) (32). JAK kinases could selectively phosphorylate STATs, leading to their activation. To be specific, STAT6 acted as one of the primary members in JAK-STAT pathway, capable of specifically inducing TH2 cell differentiation and promoting the differentiation of TH2 cytokines (e.g., IL-4, IL-5 and IL-13), and facilitating the dominant expression of TH2 cells in asthma (33). T cells are critical to defending the body against pathogens. Different T cell lineages express different types of T cell antigen receptors (TCRs). Stimulation of T cells with antigens or cytokines requires the translation of antigen binding to the TCR, so T-cell proliferation, cytokine production, cell motility, and proliferation are to be induced (34).

Undoubtedly, matrix metalloproteinase-2 (MMP2) and nuclear receptor subfamily 3 group C member 1(NR3C1) are two hub nodes, members of both component-targets and asthma-targets, making them crucial accounting for PT in asthma treatment. MMPs are a family of enzymes characterized by a common zinc ion at their active site. In general, MMPs are thought to be involved in the normal maintenance of the extracellular matrix and inflammation and cell-cell signaling (35). Studies reported that the main function of MMP2 protease is to degrade extracellular matrix proteins and participate in airway remodeling in asthma (36). Using MMP-2-cleavable activatable cell-penetrating peptides (ACPPs) with the cleavage sequence PLGC(Me)AG, show that MMP-2 is up-regulated in the lungs of allergen OVA-challenged mice (37), this result is consistent with findings in Fig. 6A. Furthermore, the highest MMP-2 activity was detected in the areas of inflammation surrounding airways, a major site of fibroblasts, epithelial cells, basal cells, this finding is also consistent with data in scRNA-Seq. Besides, NR3C1 is vital for regulating inflammatory responses and capable of impacting the number of the glucocorticoid receptor (GR) and the affinity between the glucocorticoid receptor and glucocorticoid (38). The stress hormone cortisol binds to glucocorticoid receptors produced by NR3C1, which triggers transcriptional processes resulting in reduced inflammation (39). Reduced expression of NR3C1 may lead to fewer receptors for cortisol to bind to and carry out its functions and lead to glucocorticoid resistance, which gives rise to the inflammatory response that can become chronically over-activated and increase susceptibility to inflammatory disease over time (40). Actually, many individuals with asthma rely on corticosteroid medications to control asthma symptoms (41). While in animal experiments, NR3C1 is apparently revealed a large gap in lung tissues of normal mice, ubiquitously expressed lower in most allergic mice lung tissues (Fig. 6B). scRNA-Seq revealed that NR3C1 is mainly derived from germ cells, stromal cells (e.g., basal cells, fibroblasts, epithelial cells) and immune cells (e.g., T cells, B cells, dendritic cells), these data indicate that the expression level of NR3C1 is related to gender,

genetic background, and immune microenvironment individuals. Thus, identifying factors affecting NR3C1 expression is important for improving health, particularly for those with asthma.

5. Conclusions

TCM has been commonly used to treat various diseases and exhibits favorable efficacy, whereas the molecular mechanism of TCM has not been elucidated. In the present study, the network pharmacology method was used to study the scientific basis of PT in the treatment of asthma. 57 hub nodes were screened and signaling pathways associated with asthma were enriched, these data lay a scientific basis for the clinical treatment of asthma and also provide insights into the development and utilization of drugs. The recent development of scRNA-Seq technology has combined the single-cell isolation and RNA-sequencing technologies which enabled the global transcriptome profiling on a single-cell level, greatly advanced our understanding of cellular states, and also provides an accurate direction for TCM treatment of diseases. Furthermore, systematic and rigorous experiments are required to verify our findings.

Abbreviations

TH2 cells, T helper type 2 cells; IL-2, interleukin-2; GM-CSF, granulocyte macrophage colony–stimulating factor; ICS, inhaled corticosteroid; TCM, Traditional Chinese medicine; PT, *Pinellia ternata*; OB, oral bioavailability; DL, drug similarity; GO, GeneOntology; KEGG, Kyoto Encyclopedia of Genes and Genomes; OMIM, Online Mendelian Inheritance in Man database; DC, degree of centrality; BC, betweenness centrality; CC, close to the central; NC, the network centrality; LAC, local edge connectivity topology selection; SPF, specific pathogen-free; OVA, ovalbumin; BALF, bronchoalveolar lavage fluid; BP, biological processes; MF, molecular functions; CC, cellular components; MMP2, matrix metalloproteinase-2; NR3C1, nuclear receptor subfamily 3 group C member 1; ACPPs, activatable cell-penetrating peptides; GR, glucocorticoid receptor;

Declarations

Acknowledgment

none

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

Author contributions

YML carried out the major experiments and drafted the manuscript. XJC, QX, and SSZ supported in the study design, reviewed the protocol, and participated to interpret the primary outcome. CFY led the overall project and designed experiments.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Competing interests

The authors have no competing interests.

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Tables

Table 1 Active components and ADME parameters of *Pinellia Ternate*

MOL ID	Molecule Name	OB	DL
MOL001755	24-Ethylcholest-4-en-3-one	36.08	0.76
MOL002670	Cavidine	35.64	0.81
MOL002714	Baicalein	33.52	0.21
MOL002776	Baicalin	40.12	0.75
MOL000358	beta-sitosterol	36.91	0.75
MOL000449	Stigmasterol	43.83	0.76
MOL005030	Gondoic acid	30.7	0.2
MOL000519	Coniferin	31.11	0.32
MOL006936	10,13-eicosadienoic	39.99	0.2
MOL003578	Cycloartenol	38.69	0.78
MOL006967	beta-D-Ribofuranoside, xanthine-9	44.72	0.21

ADME, (absorption, distribution, metabolism, and excretion); OB, oral bioavailability; DL, drug similarity.

Table 2 The Top 10 KEGG pathways of component-related targets.

Term	Count	P Value	Gene
PPAR signaling pathway	9	2.79E-06	FABP6, FABP7, PCK1, PDPK1, PPARA, PPARD, PPARG, RXRA, RXRB
Thyroid hormone signaling pathway	10	2.21E-05	ESR1, GSK3B, MDM2, PDPK1, RAF1, RXRA, RXRB, SRC, THRA, THRB
Proteoglycans in cancer	12	7.21E-05	ESR1, FGFR1, GRB2, KDR, MAPK14, MDM2, MET, MMP2, PDPK1, PPP1CC, RAF1, SRC
Insulin signaling pathway	10	9.34E-05	CALM1, EIF4E, GRB2, GSK3B, INSR, PCK1, PDPK1, PPP1CC, PYGL, RAF1
Pathways in cancer	16	2.23E-04	DAPK1, FGFR1, GRB2, GSK3B, GSTP1, MDM2, MET, MMP2, PPARD, PPARG, RAF1, RARA, RARB, RXRA, RXRB, XIAP
Drug metabolism - cytochrome P450	7	2.90E-04	ADH5, GSTA1, GSTA3, GSTM1, GSTM2, GSTP1, GSTT2
Prolactin signaling pathway	7	3.67E-04	ESR1, ESR2, GRB2, GSK3B, MAPK14, RAF1, SRC
T cell receptor signaling pathway	8	3.79E-04	GRB2, GSK3B, IL2, ITK, LCK, MAPK14, PDPK1, RAF1
Metabolism of xenobiotics by cytochrome P450	7	4.59E-04	ADH5, GSTA1, GSTA3, GSTM1, GSTM2, GSTP1, GSTT2
Glutathione metabolism	6	5.83E-04	GSTA1, GSTA3, GSTM1, GSTM2, GSTP1, GSTT2

KEGG, Kyoto Encyclopedia of Genes and Genomes

Table 3 Related targets of asthma

PDE4D	HLA-G	IL6R	TNFRSF4	TPSB2	CD274	ADORA2B	PTGIR
HNMT	ADAM33	TYRO3	SERPINB3	VEGFA	IL25	TACR2	CFB
VDR	PLA2G7	MMP12	SERPINB4	SUV39H1	PDCD1LG2	CYP26A1	TACR3
MMP2	IRAK3	GABRA2	FKBP5	HRH1	TPSD1	TRPV1	CTSK
BTK	C5	GAD2	CPN1	FABP4	IL31	TOP2A	CTSL
NR1I2	CCL11	HAVCR1	ADCY9	STAT4	NKAIN2	ROS1	CTSB
ADH5	LTC4S	MUC7	CCL18	MUC5B	JAML	SELE	CTSS
PDE4B	C5AR1	MARCKS	CLCA1	IL11RA	ORMDL1	CXCR2	AOC3
NR3C1	FLG	GABRB2	C3AR1	CX3CR1	ORMDL2	PDE3A	CCR4
NPSR1	IL12B	TPT1	RASGRP4	IL12RB1	TAS2R31	SELENOP	NOCT
IL13	ORMDL3	CCR5	SETDB2	CCR6	ACTG2	SELL	CNOT6
TNF	SCGB1A1	PTEN	POSTN	JAG1	CLC	ADRB2	TLR9
IL4R	TBX21	DAP3	DENND1B	SATB1	MIF	MCL1	UTS2R
IL9	HLA-DRB1	TLR4	CRHR1	JAG2	IL17F	F2	TSLP
ALOX5	HLA-DRB1	GAD1	CD74	CXADR	MYL3	ADRB3	CSF2RB
PTGDR	HLA-DRB1	TNIP1	FCAR	H3C1	ABCA1	PDE4A	PTGER4
TBXA2R	SCGB3A2	C3	ITGB6	CST7	CAV3	HR	TLR7
PTGDR2	MMP9	FCER1A	IL11	ALOX15B	HPS1	ESRRA	NOS2
CYSLTR1	IL13RA1	PRKCA	ALOX15	FFAR2	ALG9	ITGA5	NANOS2
IL4	CHI3L1	IRF4	LTA	KCNMB1	CDHR3	CCL2	SERPINE1
IL5	CMA1	TNFSF10	TRAF5	SIGLEC5	IL5RA	PRSS1	CBR1
IL13RA2	MS4A2	SOCS3	CFTR	CBX5	ANGPT2	PLA2G1B	ITGA4
ALOX5AP	IL9R	TNFSF14	MUC5AC	ICOS	CHRNA7	HAL	KCNMA1
CYSLTR2	CHIA	SART1	SNCA	FCGR2B	ANGPT1	SYK	MAP3K9
SELP	GATA3	BPIFA1	NFKB1	CD209	MC4R	TACR1	ADRA2C
PTAFR	RUNX3	SPRED1	NFKB2	TAS2R14	IL17A	MICU1	BDKRB2
PTGER3	CCL24	GLCCI1	PTGER2	TAS2R10	IL17RA	SCN9A	OPN4
CCR3	SPINK5	USP38	PTPN6	PDE11A	CHRM4	SCN10A	PDE5A
LTB4R2	NOD1	IL10	SPRR2A	HDAC3	CHRM2	TBXAS1	CSF2

IL33	PHF11	LGALS3	SPRR2B	KCNMB2	CHRM3	LTB4R	ADRB1
PLA2G4A	DPP10	NFKBIA	TNC	LY96	CAMP	ACKR3	CHRM5
STAT6	HSD11B2	SERPINB2	TPSAB1	CXCL16	CHAMP1	ADORA1	MME
ATP2A2	ACE	TYK2					

The targets are shown by their gene names

Figures

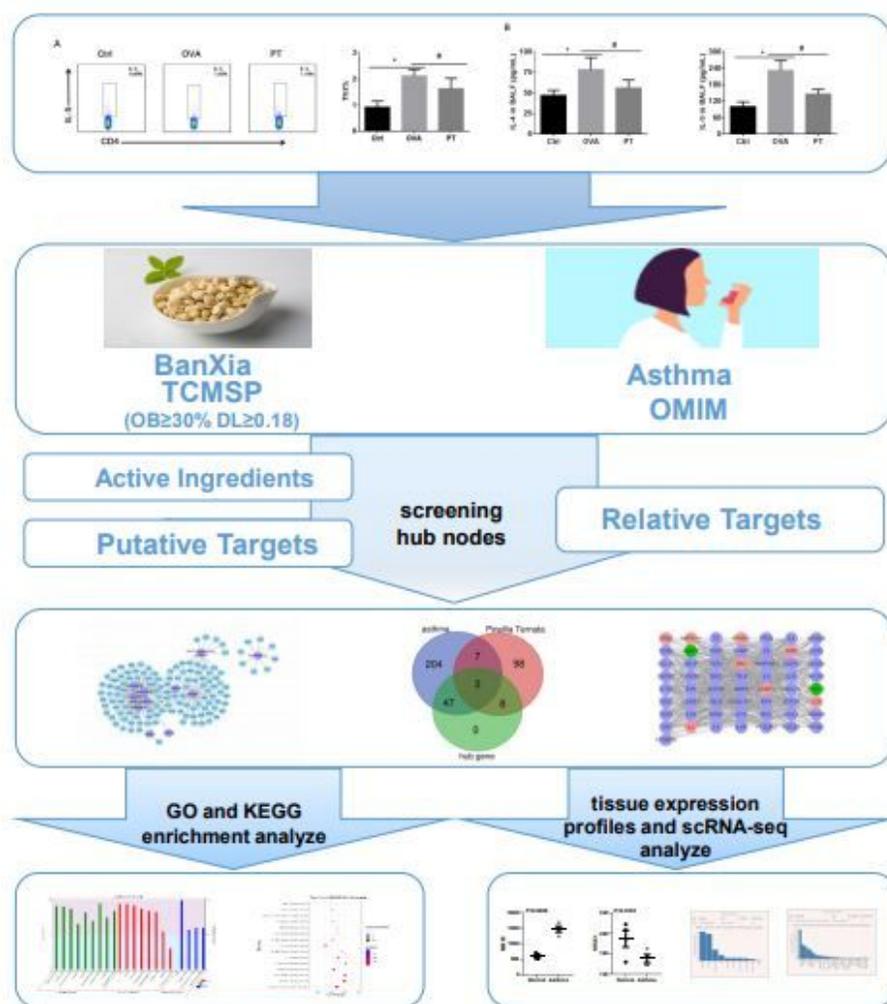


Figure 1

The schematic illustration of network pharmacology analysis for exhibiting pharmacological pathways of PT against asthma

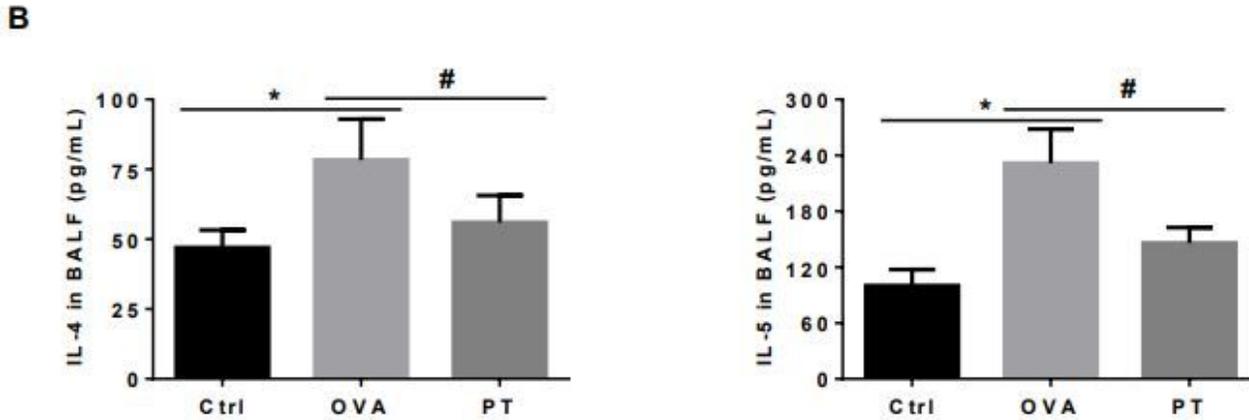
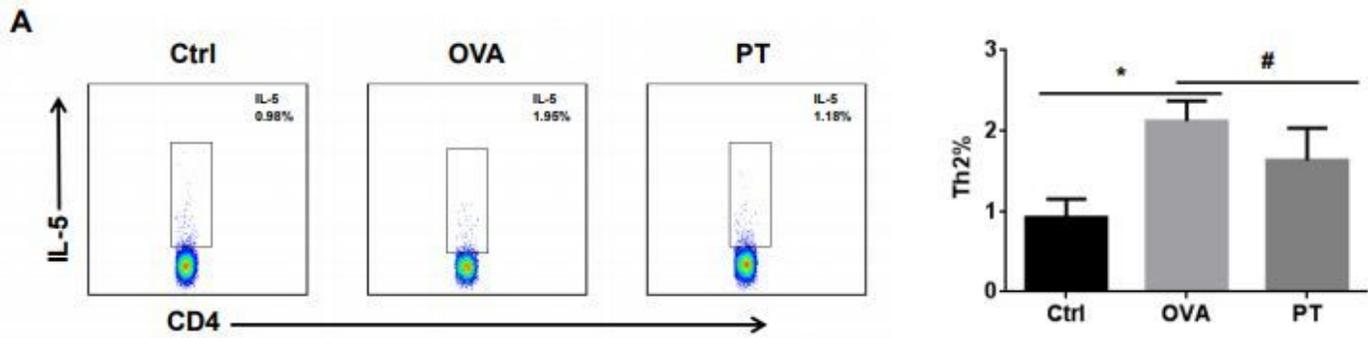


Figure 2

Alleviated allergic reactions in the treatment of PT. (A) The percentages of TH2 cells (CD45+ CD3+CD4+ IL5+) in lymphocytes by FACS; (B) The concentration of IL-4 and IL-5 in BALF. Data shown were normalized to the reference gene GAPDH. Values are expressed as mean \pm SEM. * P value < 0.05 compared to control group; # P value < 0.05 compared to model group.

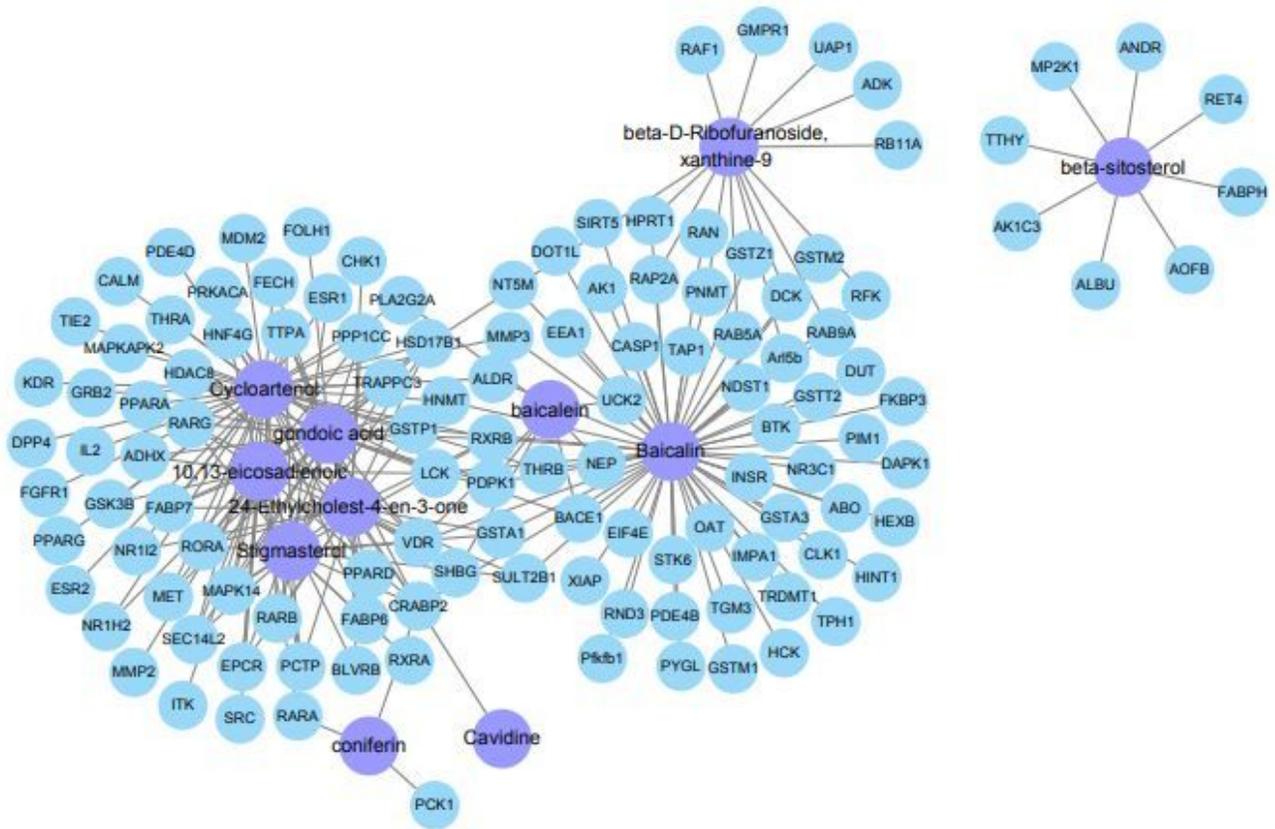


Figure 3

Component-Target network (C-T Network). The network of 11 active ingredients of PT and 115 putative targets was generated with Cytoscape version 3.7.2. All protein targets are represented by their gene symbol

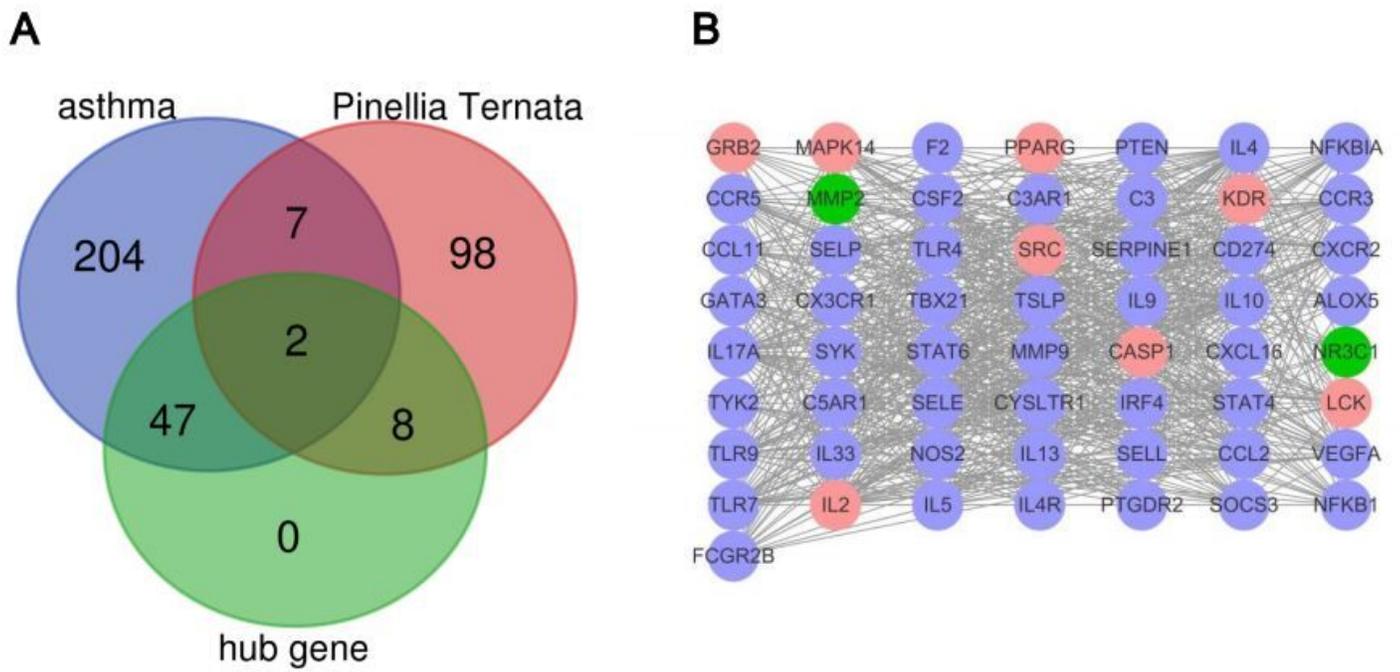


Figure 4

Network of drug and disease targets. (A) The targets of asthma and compounds; (B) Network of hub nodes. The property of the purple nodes are asthma-related targets; the property of the pink nodes are component-related targets, and the property of the green nodes are common targets of asthma and components.

A

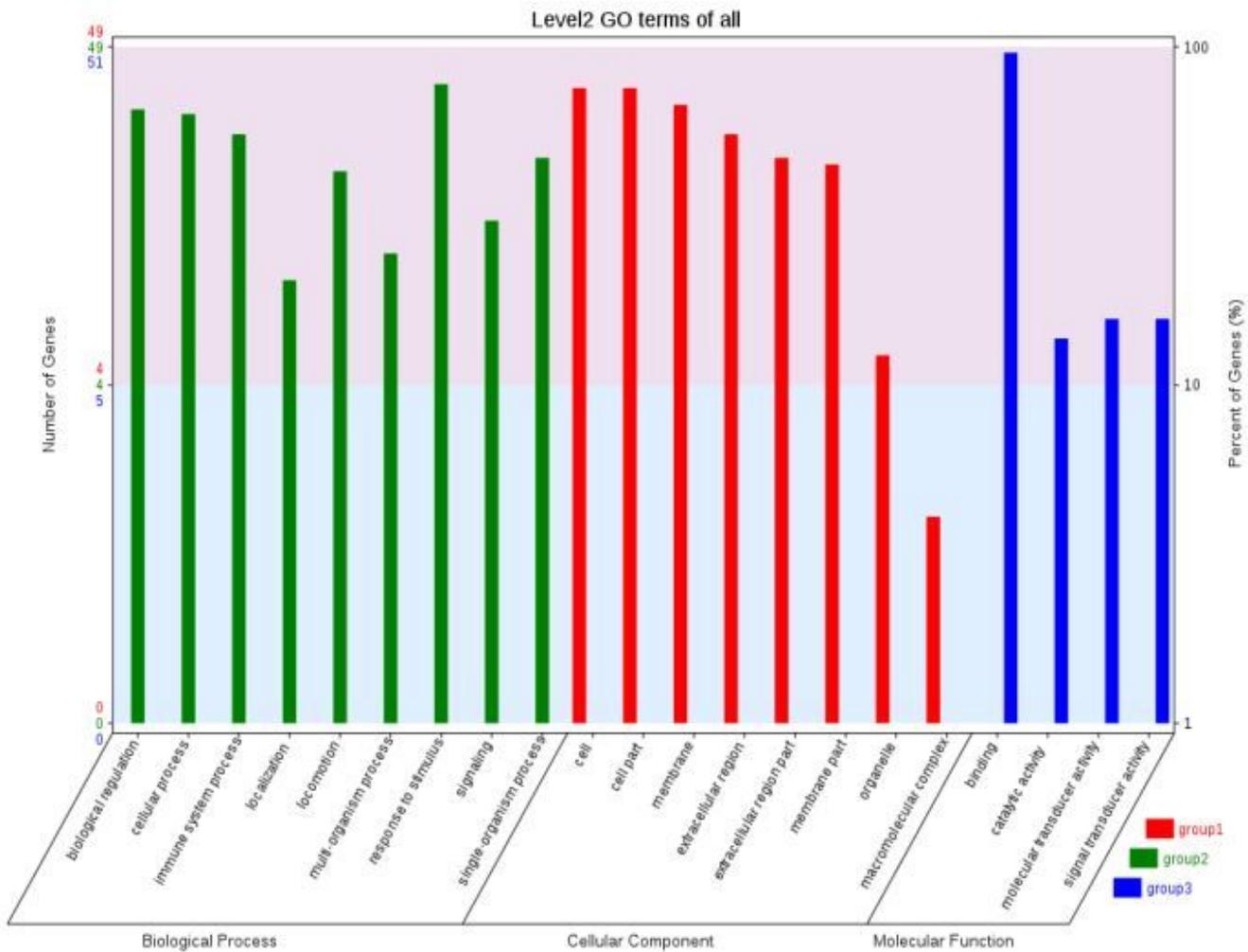
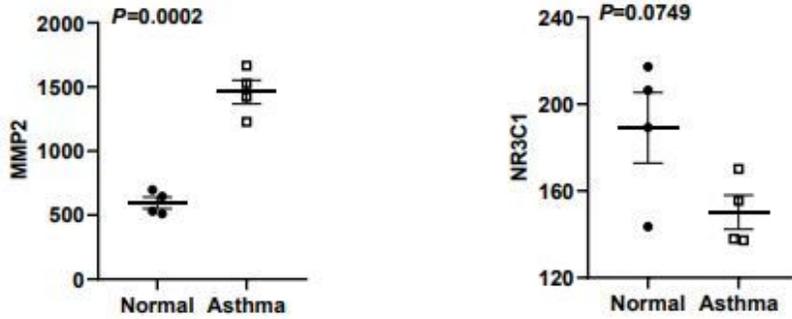
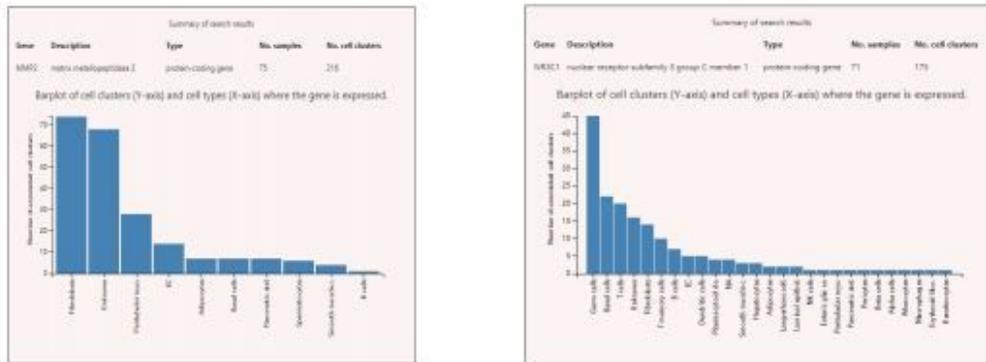


Figure 5

Enrichment analysis of the hub genes for *Pinellia Ternatea* in the treatment of asthma. (A) The GO enrichment analysis of hub nodes; (B) The top15 KEGG pathway enrichment analysis of hub nodes.

A**B****Figure 6**

Gene expression levels of MMP2 and NR3C1 in tissue and single cells. (A) Gene expression levels of lung tissue in GSE6858; (B) Expression of genes in multiple cell types of human from scRNA-seq datasets, include non-adult and non-primary samples (embryo, fetal, post-natal development, cell lines)