




# Quantitative proteomics analysis of the treatment of asthma rats with total flavonoid extract from chamomile

Qian Li · Shengjun Zhao · Jun Lu · Xiaolong Kang · Gang Zhang ·  
Feicui Zhao · Jihong Nie · Xiaomi Yang · Xuelei Xin · Hongping Zhang ·  
Haji Akber Aisa 

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## Abstract

**Objective** Asthma is a chronic immune disease that has become a serious public health problem. The currently available medications are not ideal because of their limitations and side effects; hence, new target proteins and signaling cascades for precise and safe therapy treatment are needed. This work established an ovalbumin-induced asthma rat model and treated it with total flavonoid extract from the Xinjiang chamomile. The proteins that were differentially expressed in the chamomile extract-treated asthmatic rats and the asthma and healthy rat groups were identified using isobaric tagging followed by LC-MS/MS. Kyoto encyclopedia of genes and genomes

pathway analysis of the differentially expressed proteins was performed.

**Results** Pathways involved in purine metabolism, herpes simplex infection, and JNK phosphorylation and activation mediated by activated human TAK1 were enriched, indicating the intrinsic links between the mechanism of asthma development and treatment effects. Furthermore, we constructed a protein–protein interaction network and identified KIF3A as a potential target protein of chamomile extract that affected the Hedgehog signaling pathway.

**Conclusions** This study may provide new insights into the pathogenesis of asthma and reveal several proteins and pathways that could be exploited to develop novel treatment approaches.

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Q. Li · X. Xin · H. A. Aisa (✉)  
CAS Key Laboratory of Chemistry of Plant Resources in  
Arid Regions, Key Laboratory of Xinjiang Indigenous  
Medicinal Plants Resource Utilization, Xinjiang  
Technical Institute of Physics and Chemistry, Chinese  
Academy of Sciences, 40-1, Beijing Road,  
Urumqi 830011, Xinjiang, China  
e-mail: HajiAkberAisa@outlook.com

Q. Li  
University of Chinese Academy of Sciences,  
Beijing 100049, China

H. Zhang  
Scientific Research Department, The Third Affiliated  
Hospital of Guangxi University of Chinese Medicine,  
Liuzhou 545001, People's Republic of China

Q. Li · S. Zhao · J. Lu · X. Kang · G. Zhang · F. Zhao ·  
J. Nie · X. Yang  
Department of Medicine Research, Hospital of Chinese  
Medicine Affiliated to Xinjiang Medical University,  
Urumqi 830000, People's Republic of China

**Keywords** Asthma · Chamomile · Differential expressed proteins · KIF3A · Hedgehog signaling pathway

## Introduction

Asthma is one of the most common chronic immune diseases, affecting more than 300 million people and killing at least 250,000 people every year worldwide (Fuchs et al. 2017). Asthma is a heterogeneous disease characterized by chronic airway inflammation involving a variety of cell types, such as eosinophils (EOS), mast cells, T lymphocytes, neutrophils, and airway epithelial cells, and cellular components (Wenzel 2012). Asthma patients have different phenotypes. The Th2 immune response is crucial for the molecular mechanism of asthma, so inhibiting Th2 inflammation is an important strategy for the treatment of asthma (Fahy 2015). Glucocorticoids (GCS) are effective drugs for treating asthma that inhibit type 2 inflammation. However, GCS can cause many side effects, including dysphonia, candidiasis, cataracts, osteoporosis and adrenal suppression (Sobieraj and Baker 2018; Wise 2014). Thus, individual treatment is necessary for asthma patients, especially for moderate-to-severe patients with poor symptom control. After the first anti-IgE drug Omalizumab was brought to market, further study of the mechanism of asthma identified more potential target proteins, such as mitogen-activated protein kinase, to provide new insights for the precise treatment of asthma (Khorasanizadeh et al. 2017).

Xinjiang chamomile (*Matricaria chamomilla* L.) is an annual herb and is included in the National Pharmaceutical Standard Uyghur Pharmacopoeia. It has been reported that chamomile extracts play important roles in anti-cancer, sedation, anti-oxidation, anti-spasm, and hypoglycemia (Srivastava and Gupta 2007), and play an especially significant role in paraquat-induced lung injury (Ranjbar et al. 2014). These findings suggest that chamomile extracts may be used in the treatment of lung-related diseases. Moreover, previous research has shown that the inhibition ratios of chemical and immune-induced histamine release when using extracts from eight herbal medicines, including chamomile, are 81 and 85%, respectively, meanwhile, compared with

patients receiving placebo, allergic asthma patients treated with herbal tea showed a decrease in cough frequency and intensity and an increase in FEV1/FVC (Haggag et al. 2003). Our previous studies showed that asthmatic mice treated with *n*-butanol extract from chamomile showed improved lung function, reduced airway hyperresponsiveness, and a significantly shorter breathing interval. Additionally, chamomile extracts could decrease the levels of EOS and malondialdehyde in bronchoalveolar lavage fluid and increase the levels of serum IL-2, IL-10, IL-12 and superoxide dismutase in lung tissue (Qian et al. 2017). Here, we established a rat asthma model and treated it with total flavonoid extract from the Xinjiang chamomile. A proteomics study was performed to investigate the differentially expressed proteins between chamomile extract-treated rats and asthmatic and healthy rats. In this study, we screened a series of potential target proteins that could be used to develop novel treatment strategies in the future and to provide a better understanding of the pathophysiological mechanisms associated with asthma.

## Materials and methods

### Animals

Clean adult Sprague-Dawley (SD) rats with body weights of  $220 \pm 20$  g were purchased from Xinjiang Laboratory Animal Research Center. All rats were allowed to adapt to the experimental environment (room temperature 20–25 °C, 12 h light–dark cycle) for 1 week and had free access to ovalbumin (OVA)-free food and water. The animal experiments were conducted in accordance with the Guidance Suggestions for the Care and Use of Laboratory Animals from the Ministry of Science and Technology of China and approved by the Ethical Committee of Xinjiang Medical University.

### Extraction of total flavonoids from Xinjiang chamomile

A 70% ethanol extract of 8 kg of Xinjiang chamomile was concentrated and then dissolved in water, followed by three cycles of petroleum ether retraction and decompression recovery. A total of 95 g of extract and liquid layer solution were obtained. After

extracting the liquid layer three times with ethyl acetate and performing decompression recovery, 725 g of extract and liquid layer solution were obtained. Then, the liquid layer was extracted three times with *n*-butanol and by performing decompression recovery, 510 g of extract was obtained. The yield of *n*-butanol extract was 6.37%, and it was used as a therapeutic treatment in this experiment.

#### OVA-induced rat asthma model and treatment

The rats were divided into three groups: N, which was the control group of healthy rats, M, which was the group of rats with OVA-induced asthma, and G, which was the treatment group of asthma-affected rats treated with total flavonoids extracted from the Xinjiang chamomile. The OVA-induced asthma rat model was established according to previous study with some modifications (Li et al. 2016). Each group had three rats. The rats in the M group and the G group were intraperitoneally injected with 1 mg OVA complexed with 200 mg Al(OH)<sub>3</sub> on days 1 and 8. The sensitized rats were intranasally challenged with 1% OVA on day 15, which lasted for 7 days. The rats in the N group were injected with normal saline on days 1 and 8 and received normal saline intranasally during the following 4 weeks. From day 28 to 57, the rats in the N group and the M group were intragastrically perfused with normal saline for 1 h before aerosol inhalation, while the rats in the G group were intragastrically perfused with a dosage of 0.32 g/kg of total flavonoid extracted from the chamomile.

#### Protein extraction

Lung tissue samples weighing 500 mg from three rats in each group were ground and resuspended in 2.5 mL lysis buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 1 mM PMSF, 0.1% SDS, 1 mM EDTA, and 1% proteinase inhibitor), followed by ultrasonic dissolution on ice. After 0.5% IPG buffer (Sigma, USA) was added, centrifugation was performed at 20,000×*g* for 10 min at 4 °C, and the upper layer was collected. The protein concentration of each sample was quantified by a BCA assay, (Beyotime, Shanghai, China) and the samples were preserved at – 80 °C.

#### iTRAQ labeling and LC-MS/MS

A total of 200 µg protein from each group sample was adjusted to the same volume with lysis buffer and a fivefold volume of acetone was added to precipitate the protein overnight at – 20 °C, followed by centrifugation at 20,000×*g* for 15 min at 4 °C. The precipitants were resuspended in 50 µL dissolution buffer (contains 0.5M triethylammonium bicarbonate) and 4 µL reducing reagent (contains 50 mM tris-(2-carboxyethyl) phosphine) from a 4-plex iTRAQ Reagent Kit (AB Sciex, USA) was added for 1 h at 60 °C. Two microliters of cysteine-blocking reagent (contains 200 mM methyl methanethiosulfonate in isopropanol) of the iTRAQ Reagent Kit was then added. The reduced alkylated protein solution and 100 µL dissolution buffer were added into 10k ultrafiltration centrifuge tubes, followed by centrifugation at 20,000×*g* for 20 min three times. Proteins were incubated with trypsin (MS Grade, Thermo Fisher Scientific, USA, trypsin: protein = 1:50) in the tubes at 37 °C overnight then spun to bring the protein digest to the bottom of the tube. Each vial of iTRAQ Reagent-4plex was prepared to room temperature and centrifuged to bring the solution to the bottom, then dissolved in 150 µL isopropanol. The contents of iTRAQ-4plex 114 vial were transferred to the tube of peptide fragments from the N group, 115 to the tube of M group, and 116 to the tube of G group. After incubation at room temperature for 2 h, 100 µL water was added to terminate the reaction. The contents of each labeled sample were combined into one tube and dried in a vacuum concentrator, then redissolved in buffer A (2% acetonitrile, 98% H<sub>2</sub>O, pH 10.0). After centrifugation at 20,000×*g* for 10 min, the supernatant was loaded onto an RP C18 column. Reverse-gradient separation (buffer B: 90% acetonitrile, 10% H<sub>2</sub>O, pH 10.0) was performed using HPLC (Shimadzu, LC20AD). The eluent was loaded onto a LC–MS system (NCS3500, Dionex, USA; Q Exactive, Thermo Scientific, USA). Buffer A of liquid chromatography was 0.1% formic acid and 99.9% H<sub>2</sub>O, and buffer B was 0.1% formic acid and 99.9% acetonitrile. Mass spectrum full scan range was *m/z* 350–1600. The parent ions were fragmented by high-energy c-trap dissociation (HCD) and sequenced by secondary mass spectrometry, and isobaric tag was used for quantification.

## Bioinformatics analysis

Protein identification was conducted with Sequest algorithm, which is included in Proteome Discover Version 2.1 (Thermo Fisher Scientific) by searching the *Rattus norvegicus* protein database in NCBI (<https://www.ncbi.nlm.nih.gov/protein>). A maximum of two missed restriction sites in trypsin specific digestion was used as the fixed parameter, and oxidation and phosphorylation of methionine were specified as variable parameters in the search process. The mass tolerance for parent ion was 15 ppm, and 0.02 Da for daughter ion. Reliable peptides with FDR < 1% were selected as filter parameters for qualitative identification of proteins. Specific peptides were used for relative quantitative analysis of proteins between different samples. The differentially expressed proteins (DEPs) between the G group and M group, M group and N group, and G group and N group were identified according to a fold change > 2 (upregulated) or < 0.5 (downregulated) and an FDR < 0.05 using *limma* package of R software. The quantitative data were analyzed with GraphPad Prism 7.0 (GraphPad Software Inc., USA). The kyoto encyclopedia of genes and genomes (KEGG) database was used for the biological interpretation of the target proteins identified in this study. The functional enrichment analysis was conducted by using DAVID 6.8 ( $P < 0.05$ ). Protein–Protein interaction (PPI) data from the STRING database were used to generate the network maps in Cytoscape (<http://www.cytoscape.org>) for interaction pairs with combined scores higher than 0.4. Based on the protein–protein interaction database within the HPRD database, the asthma-related protein network in the GAD database was used to identify the target proteins of total flavonoids extracted from the chamomile.

## Western blot

Total protein was extracted by adding lysis buffer. The cells were centrifuged at 13,000 rpm for 10 min at 4 °C. The supernatants were collected and protein concentrations were measured using BCA assay (Beyotime, China). Proteins were separated by SDS-PAGE, transferred to PVDF membranes (Roche, U.S.A.), and incubated with one of the following primary antibodies: Casp3 monoclonal antibody (Thermo Fisher Scientific, USA), Rap1b monoclonal antibody (Santa Cruz, USA), Mmp9 monoclonal

antibody (Thermo Fisher Scientific, USA), Nme3 polyclonal antibody (Fine Test, China), Kif3a polyclonal antibody (Thermo Fisher Scientific, USA) and  $\beta$ -actin polyclonal antibody (Thermo Fisher Scientific, USA). The membranes were treated with the appropriate horseradish peroxidase-conjugated secondary antibody (Thermo Fisher Scientific, USA) and washed with PBS.

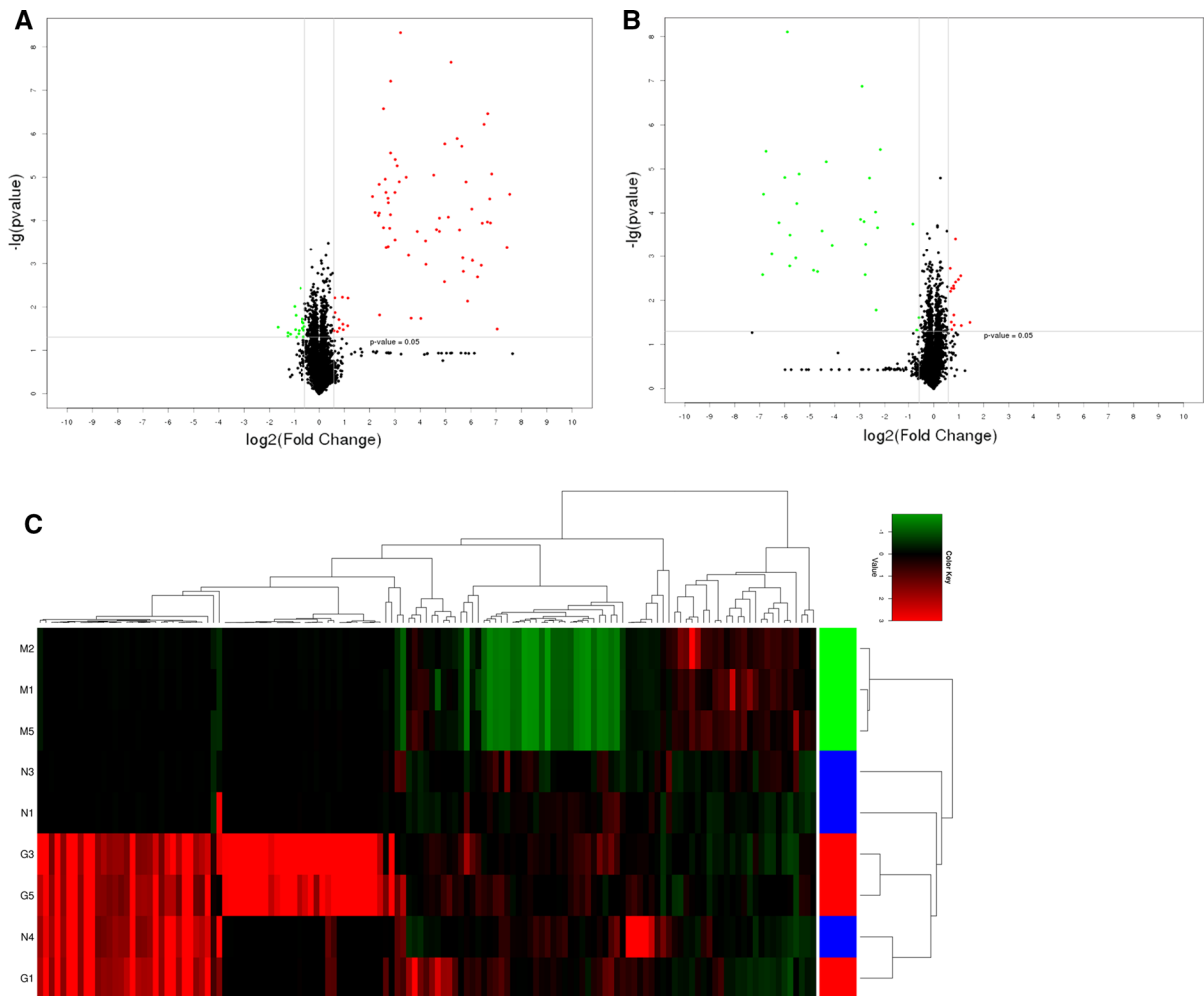
## Results

### Identification of differentially expressed proteins

Lung tissues collected from healthy rats (N group), OVA-induced asthma rats (M group), and asthmatic rats treated with total flavonoid extract from chamomile (G group) were used for iTRAQ assessment and LC-MS/MS. The peptide length distribution is shown in Fig. S1A, and the molecular weights and coverage distribution of the proteins are shown in Fig. S1B. The main finding is the identification of the differentially expressed proteins (DEPs) between the G group and M group and the M group and N group. As shown in Fig. 1a, b, proteins with a fold-change > 2 or < 0.5 and  $P < 0.05$  were selected. A total of 89 DEPs were identified between the G and M groups, among which 71 proteins were significantly upregulated and 18 proteins were downregulated in the G group. Forty-six DEPs were identified between the M group and the N group, among which 15 proteins were significantly upregulated and 31 proteins were downregulated in the M group. Notably, only three upregulated proteins and two downregulated proteins were found in the G group compared with the N group, which showed that after total flavonoid extract treatment, the protein expression level in asthma rats was mostly consistent with that in healthy rats; this also indicated the effect of flavonoid extract on asthma treatment. Moreover, the hierarchical cluster analysis of the DEPs shown in Fig. 1c suggested that the G and N groups were more similar, while the M group was distinguished from those two groups.

### KEGG enrichment analysis

KEGG pathway enrichment analysis was performed to determine the biological processes in which the DEPs



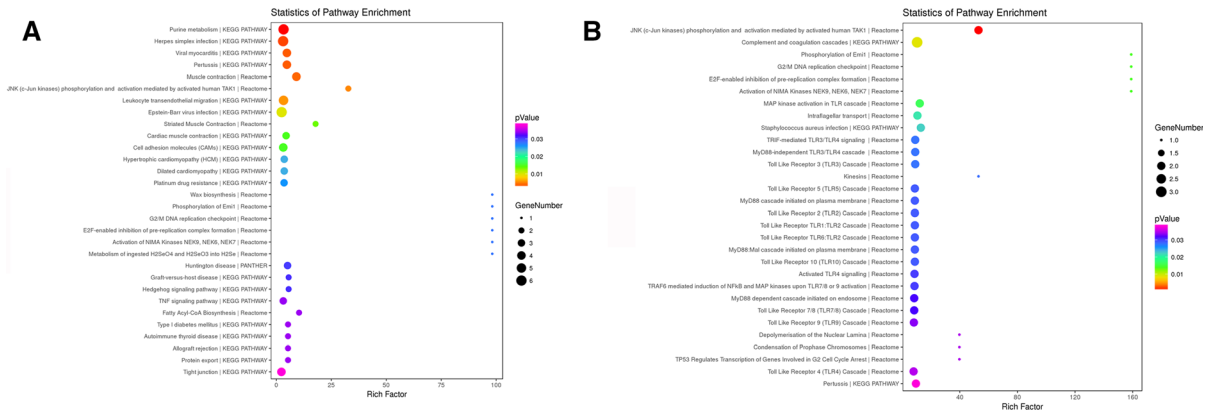
**Fig. 1** Volcano plots and heatmap of the DEPs. In the volcano plot, the X-axis is fold-change ( $\log_2$ ) and the Y-axis is  $P$  value ( $-\log_{10}$ ). Green dots (fold-change  $< 0.5$ ) represent the down-regulated proteins, and red dots (fold-change  $> 2$ ) represent the

up-regulated proteins. **a** is DEPs between G and M group and **b** is DEPs between M and N group. In the heatmap **c**, red color represents the up-regulated proteins and green color represents the down-regulated proteins

were involved. The DEPs between the G and M groups were significantly enriched in pathways involved in purine metabolism, herpes simplex infection, viral myocarditis, and pertussis (Fig. 2a). The DEPs between the M and N groups were significantly enriched in pathways involved in JNK phosphorylation and activation by TAK1, the complement and coagulation cascades, and Toll-like receptor-related cascades (Fig. 2b). Moreover, the DEPs between the G and M groups were highly enriched in pathways involved in JNK phosphorylation and activation mediated by TAK1, which were also enriched in the comparison of the M and N groups.

### PPI network analysis

Protein–protein interaction network analyses of the differentially expressed proteins between the G and M groups and the M and N groups were conducted by using the STRING database, as shown in Fig. 3. The red dots represented the up-regulated proteins and the green dots represented the down-regulated proteins. The high-degree hub nodes included caspase 3 (*Casp3*), the Ras-related protein Rap-1b (*Rap1b*), matrix metalloproteinase 9 (*Mmp9*), nucleoside diphosphate kinase 3 (*Nme3*), class III  $\beta$ -tubulin (*Tubb3*), and kinesin family number 3A (*Kif3a*). Among these high-degree hub nodes, *Casp3*, *Rap1b*,



**Fig. 2** KEGG enrichment analysis of DEPs. Significantly enriched KEGG pathways of proteins between G and M group (a) and proteins between M and N group (b)

and *Mmp9* were up-regulated in G group compared with M group, while *Nme3*, *Tubb3*, and *Kif3a* were down-regulated in M group compared with N group.

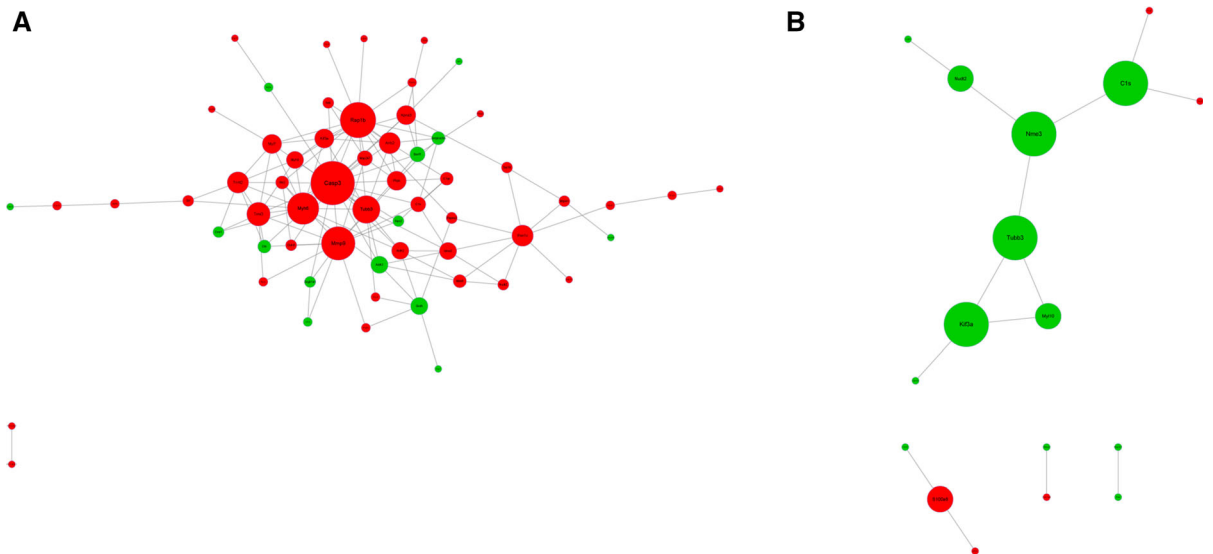
Western blot

In order to further confirm the differentially expressed proteins between the G and M groups and the M and N groups, western blot was conducted (Fig. 4a, b). *Casp3*, *Rap1b*, and *Mmp9* were upregulated in G group compared to those in M group. Meanwhile, *Nme3*, *Tubb3* and *Kif3a* were also upregulated in N group compared to those in M group. The expression

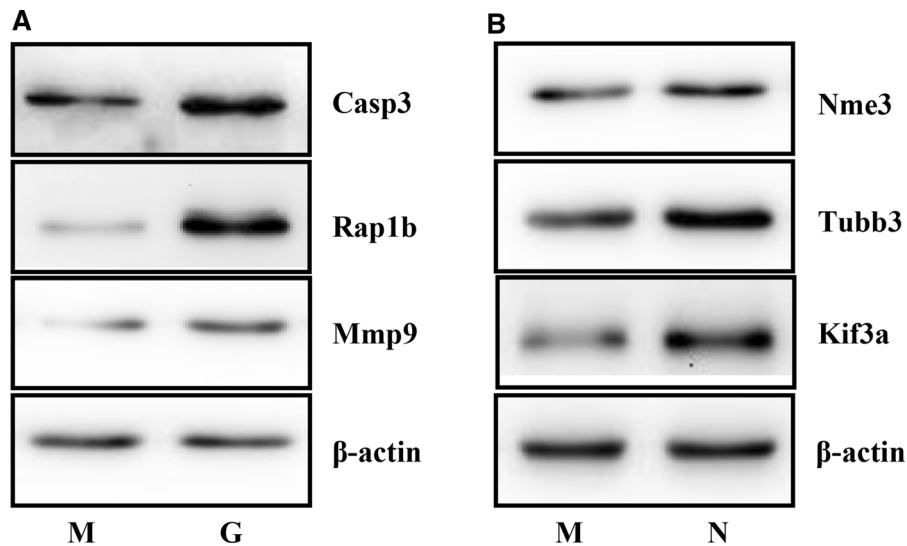
of proteins were almost 2–5.5 times higher in G group than those in M group (Fig. S2A), and the expressions of proteins were about 1.5–2.5 times higher in N group than those in M group (Fig. S2B). This result strongly proved the differentially expressed proteins by PPI network analysis.

Screening of potential target proteins for flavonoids

To identify the potential target proteins of flavonoids in the treatment of asthma, 15 DEPs were identified in both the G vs. M group and the M vs. N group (shown



**Fig. 3** Protein–protein interaction (PPI) network of differential expressed proteins between G and M group (a) as well as M and N group (b). Red dots represent the up-regulated proteins and green dots represent the down-regulated proteins



**Fig. 4** Western blot analysis. Differential expressed proteins between G and M group (a) as well as M and N group (b)

in Table 1). The heatmaps of these proteins in the three groups are shown in Fig. 5. Among these 15 proteins, 14 were upregulated (fold-change > 2) between the G and M groups and downregulated (fold-change < 0.5) between the M and N groups. Mt2A was downregulated between the G and M groups and upregulated between the M and N groups. Moreover, 7 proteins were found in the PPI network in both the G vs. M group and the M vs. N group, and the node degrees of these proteins in the two networks are shown in Table 2. Notably, Kif3a had the highest node degree in the two networks containing the seven proteins, indicating that Kif3a may be a potential target protein of flavonoids in asthma treatment.

## Discussion

Asthma is a chronic inflammatory lung disorder caused by both genetic and environmental factors. It has become a public health concern in China, as the prevalence of asthma in people over 14 years old has reached 1.24%, and the poor control of asthma results in increased health care costs and societal burden. To identify new target proteins for treatment and further understand the molecular mechanisms of asthma, we performed a high-throughput proteomic analysis of

asthma rats treated with total flavonoid extract from the Xinjiang chamomile. In this study, we reported the differentially expressed proteins between the flavonoid-treated rats and asthma rats and the asthma rats and healthy rats. Then, we analyzed the functions of these DEPs using KEGG enrichment analysis and identified the core related genes using the STRING database.

The pathway enrichment analysis of the DEPs between the flavonoid treatment group and the asthma group showed that pathways involved in purine metabolism, herpes simplex infection, viral myocarditis, pertussis, muscle contraction, and JNK phosphorylation and activation mediated by active human TAK1 were significantly enriched. Purine has been regarded as an important neurotransmitter that has a great influence on the central and peripheral nervous system. Studies have shown that purine can regulate the cleanliness of airway mucosal cilia, the fluid volume of the airway surface, and the defensive function of the airway (Sabater et al. 1999; Winters et al. 2007). As an inflammatory mediator, purine is involved in a variety of inflammatory reactions. The binding of purines to their receptors, which exist in almost all tissues and organs, affects the development of asthma. Among these receptors, the P1 receptor has both anti-inflammatory (A2a, A3) and pro-inflammatory (A2b) effects (Folkesson et al. 2012; Fredholm et al. 2011), while P2 mainly promotes inflammation (Fabbretti and Nistri 2012; Hausmann and Schmalzing

**Table 1** The 15 DEPs both in G vs. M group and M vs. N group

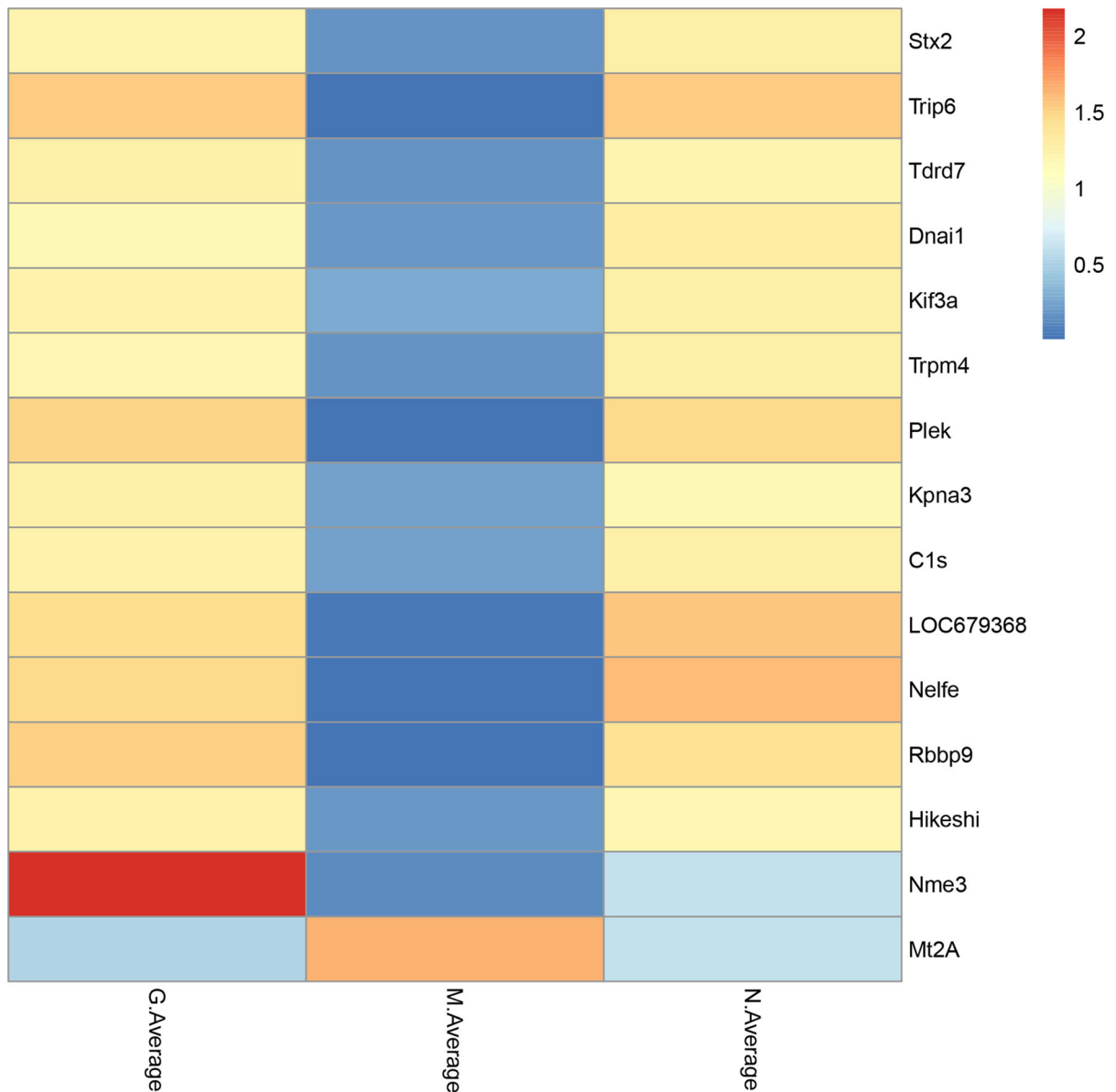
Gene_symbol	Gene_ID	Unique peptides	G-vs-M.FC	G-vs-M P value	M-vs-N.FC	M-vs-N P value
Stx2	25130	3	7.101492135	6.21311E-08	0.133332682	1.34848E-07
Trip6	686323	2	91.84231284	6.04218E-07	0.010921374	0.000890672
Tdrd7	85425	2	7.089497033	2.75385E-06	0.145303023	0.002598116
Dnai1	500442	2	6.123531415	1.10528E-05	0.147358153	0.000517105
Kif3a	84392	2	4.320260841	2.77934E-05	0.221565738	3.61875E-06
Trpm4	171143	2	6.662461778	3.85555E-05	0.140910085	0.000157248
Plek	364206	4	65.66631609	5.40846E-05	0.01563184	1.56121E-05
Kpna3	361055	4	5.197801563	6.65944E-05	0.205179788	0.000215759
C1s	192262	3	5.083262558	7.61286E-05	0.193782011	9.52868E-05
LOC679368	679368	2	27.03253625	8.7329E-05	0.034729437	0.002059531
Nelfe	294258	2	109.5310003	0.00011298	0.008455209	0.002595468
Rbbp9	29459	6	46.88701963	0.000163904	0.023254908	1.30184E-05
Hikeshi	293103	3	6.28252355	0.000415002	0.164076999	1.60436E-05
Nme3	85269	2	5.247926227	0.015374695	0.196959259	0.016515253
Mt2A	689415	2	0.316644555	0.029411613	2.7368962	0.031548621

2012; Rodrigues et al. 2014). In a metabolic pathway analysis performed by Yu et al., purine metabolism pathways were the most significantly influenced pathways in asthma mice. The decreased level of plasma uric acid suggests that inflammation responses inhibited the activity of xanthine oxidase, and the increased level of inosine suggested that inflammatory cells induced ATP breakdown, resulting in the over-expression of ADA during the development of asthma (Yu et al. 2016). Consistent with these findings, our study showed that chamomile extract had a remarkable effect on purine metabolism; thus, the DEPs involved in purine metabolism, such as P2 receptors, could be target proteins for clinical diagnosis and treatment. In addition to purine metabolism, herpes infection was also associated with asthma according to the pathway enrichment analysis. Kim et al. identified patients with herpes who had a history of asthma before the index date of herpes infection and found that asthma significantly increased the risk of herpes infection in children (Kim et al. 2013). Additionally, Kim et al. performed a population-based case-control study and found a significant association between a history of asthma and the risk of herpes in adults, and they also found that the impact of asthma on the risk of viral infection or immune dysfunction might extend beyond the airways (Kwon et al. 2016). Although the

potential biological mechanisms involved in the association between asthma and herpes are still unclear, our results support the theory that asthma might be an unrecognized risk factor for herpes. Chamomile extract may play a role in the treatment of asthma by affecting proteins involved in herpes infection.

Most of the enriched pathways involving differentially expressed proteins in the asthma group and healthy rat group were Toll-like receptor (TLR)-related cascades. TLRs are pathogen-associated molecular pattern receptors that play a pivotal role in priming innate cells and regulating their functions. Most clinically relevant allergens induce allergic airway responses via either the direct activation of TLR4 or the indirect binding and stimulation of TLR4 (Bryant et al. 2015). Endosomal TLRs such as TLR3, 7, 8 and 9 have been shown to be associated with pro-inflammation and asthma exacerbation via their ability to shift the immune response from a Th2 to a Th1 response (Bezemer et al. 2012; Kaiko et al. 2013). Our results reinforce the fact that TLRs are mediators involved in the pathogenesis of asthma, and these receptors as well as their ligands may be potential targets for the development of novel treatments for therapy. On the other hand, we noticed that pathways involved in JNK phosphorylation and activation





**Fig. 5** The heatmap for the 15 proteins. Red color represents the up-regulated proteins and blue color represents the down-regulated proteins

mediated by active human TAK1 were enriched among DEPs between both the G and M groups as well as the M and N groups. The JNK signaling pathway is an important branch of the mitogen-activated protein kinase (MAPK) pathway, which plays a significant role in cellular functions related to inflammation that include cell growth, differentiation and apoptosis (Pearson et al. 2001). Increasing evidence has demonstrated the increased level of

JNK in the airways of asthma patients (Demoly et al. 1992). JNK also plays a role in airway remodeling by suppressing TGF- $\beta$ -stimulated airway smooth muscle cell migration (Xie et al. 2005). Our results suggest that the JNK signaling pathway is involved in the pathological mechanism of asthma and that chamomile extract may have an effect on asthma therapy through JNK inhibition. Since JNK inhibition showed promising results in the suppression of airway

**Table 2** The degree nodes of the 7 DEPs both in the PPI network of G vs. M group and M vs. N group

Gene	Degree_G vs M	Degree_M vs N
Kif3a	7	3
Plek	7	1
Kpna3	7	1
C1s	5	3
Rbbp9	1	1
Nme3	6	3
Mt2A	2	1

remodeling in an asthmatic animal model, it is reasonable to develop drugs targeting the JNK pathway. To the best of our knowledge, SP600125 is currently the only JNK inhibitor tested in vivo for asthma. SP600125 has been proven to suppress DNA overincorporation in airway smooth muscle, inhibit goblet cell hyperplasia and airway smooth muscle cells (Eynott et al. 2003; Nath et al. 2005). However, further research is needed to clarify the underlying molecular mechanism, and drugs that more precisely target the JNK signaling pathway are needed.

To identify the precise protein target of the chamomile extract, we analyzed the PPI network and found that *Kif3a* represented the top high-degree hub node among the DEPs in both the G and M groups as well as in the M and N groups. *Kif3a*, which encodes the motor subunit of the cilia component kinesin-2, is a susceptibility site for many immune diseases, including asthma. Kovacic et al. found that KIF3A was significantly downregulated in the nasal epithelium of asthmatic children and dust mite-induced asthmatic mice and identified KIF3A as a novel candidate target for asthma (Kovacic et al. 2011). Giridhar et al. observed that *Kif3a* knockout mice exhibited greater sensitivity to dust mite extracts, increased airway hyperresponsiveness, and greater Th2-mediated eosinophilic inflammation. After exposure to gas allergens, KIF3A can inhibit type 2 inflammation and regulate airway epithelial cell function related to the development of asthma (Giridhar et al. 2016). Therefore, KIF3A is crucial for the development of inflammation in type 2 asthmatic mice and the integrity of the airway epithelial structure. However, no drug targeting KIF3A for the treatment of asthma has been reported. Our results showed that the total flavonoid

extract from the Xinjiang chamomile can increase the level of KIF3A, which suggests that chamomile extract may play an anti-inflammatory role by targeting KIF3A. It is noteworthy that the Hedgehog signaling pathway, which regulates development and many diseases, was enriched in the chamomile treatment group. The transduction of Hedgehog signals depends on primary cilia, whose deletion results in sonic Hedgehog (sHH) gene activation failure (Goetz and Anderson 2010), and KIF3A is part of the ciliary motor protein and is necessary for the formation of primary cilia. Previous studies have shown that the mutation or knockout of *Kif3a* can block Hedgehog signal transduction, thus affecting bone development and tumorigenesis (Barakat et al. 2013; Koyama et al. 2007). Moreover, the blockage of the Hedgehog signaling pathway also leads to abnormal lung function (Watkins et al. 2003). Thus, we speculated that chamomile extract regulates the sHH signaling pathway through KIF3A during the treatment of asthma, which requires further validation and support.

While the inhalation of corticosteroids is currently a mainstay treatment for asthma, a proportion of patients do not respond well to this treatment, and corticosteroids are not ideal inhibitors of airway remodeling because of their debilitating adverse effects. Therefore, there is an urgent need for novel therapeutic alternatives and the precise targeting of proteins related to the molecular mechanisms responsible for the asthmatic phenotype. Our results revealed differentially expressed proteins in an asthmatic animal model during chamomile extract treatment, and the pathway analysis allowed a better understanding of the mechanisms associated with asthma pathology and chamomile extract effectiveness, which provides a rationale for the targeting of proteins that may underlie a novel therapeutic approach for asthma.

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**Supporting information** Fig. S1—Lung tissues proteins identified by LC-MS/MS. (A) is peptides length distribution and (B) is protein molecular weight and coverage distribution.

Fig S2—Quantitative analysis of protein expression between G and M group. (A) as well as M and N group (B) by Image J software. The expression was normalized to  $\beta$ -actin. The data were reported as mean  $\pm$  SD, n = 3. \*\*significant difference, P < 0.01; \*significant difference, P < 0.05.

**Author contributions** QL and JZ made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data; JL, XK and HZ performed the experiments; GZ and FZ have been involved in drafting the manuscript or revising it critically for important intellectual content; JN, XY and XX given final approval of the version to be published. HAA agreed to be 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.

**Data availability** The data that support the findings of this study are available from the corresponding author upon reasonable request.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. (Laboratory Animal Ethics Committee of Xinjiang Medical University, NO: IACUC20161018-01).

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