Archives of Dermatological Research (2025) 317:535
https://doi.org/10.1007/s00403-025-03998-2

ORIGINAL PAPER

PeiTuQingXin formula alleviated atopic dermatitis symptoms via inhibiting TRADD/TRAF2/RIP1 complex mediated NF-κB signaling pathway activation

Zixuan Cheng[1] · Xin Ma[1] · Feng Luo[1] · Xiumei Mo[2,3] · Junfeng Liu[2,3] · Dachan Chen[2,3] · Fenggen Yan[2,3]

Received: 10 November 2024 / Revised: 31 January 2025 / Accepted: 3 February 2025

Abstract

Atopic dermatitis (AD) is a chronic inflammatory skin disease with recurrent course, and traditional Chinese medicine (TCM) is regarded as an effective treatment. In this study, we aim to evaluate the potential effects and elucidate the mechanism of PTQX formula in alleviating a house dust mite (HDM)-induced AD model. NC/Nga mice were divided into Control, AD model and PTQX-treated and stimulated with HDM oinment. PTQX formula exerted significant anti-inflammatory effects, alleviated dermatitis performance, decreased the serum IgE by more than 2-fold, reduced the secretion level of other inflammatory cytokines and downregulated the Th2 cells ratio in lymph nodes. Treatment with PTQX formula reduced the level of inflammatory cytokines, which was measured by an inflammatory cytokine array kit (RayBio®). Kyoto Encyclopedia of Genes and Genomes pathway enrichment revealed the anti-inflammatory effect was exerted via regulation NF-κB and Toll-like receptor signaling pathway. Data independent acquisition (DIA) proteomics analysis results showed that the expression of totally 149 proteins were regulated by PTQX formula, while the expression of TRADD was significantly downregulated. According to the western blotting analysis, the PTQX group exhibitied an over 2-fold decreased expression of TRADD, TRAF2 and RIP1 compared with the AD group, accompanied by the inhibition of NF-κB signaling pathway activity. Collectively, this finding suggested that PTQX formula may exert effects by inhibiting the expression of the TRADD/TRAF2/RIP1 complex and downregulating the activity of the NF-κB signaling pathway.

Keywords PeiTuQingXin (PTQX) formula · Atopic dermatitis · TRADD · NF-κB signaling pathway

Introduction

Atopic dermatitis (AD) is a chronic dermatitis characterized by severe pruritus and relapse [1], and is a part of atopic march comprising other allergic diseases, including asthma, allergic rhinitis and allergic conjunctivitis. The high prevalence of AD and type 2 inflammatory diseases poses substantial burden on AD patients and their families [2]. However, the treatment of AD remains challenging to clinicians owing to its refractory and recurrent disease course.

AD presents with distinct phenotypes classified by disease severity and trajectory [3]. Recently, AD was further classified into different endotypes based on serum biomarkers [4]. These endotypes present with characteristics of high T-helper type 2 (Th2) cell-associated C-C chemokines level, which induce Th2 cells migration and type 2 inflammation. Thus, the activation of Th2-bias inflammation remains a key mechanism in AD. Currently, dupilumab [5], JAK

✉ Dachan Chen
cdc@gzucm.edu.cn

✉ Fenggen Yan
fenggen_yan@gzucm.edu.cn

1 The Second Clinical School of Medicine, Guangzhou University of Traditional Chinese Medicine, Guangzhou, China

2 State Key Laboratory of Dampness Syndrome of Chinese Medicine, Department of Dermatology, the Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, China

3 Guangdong-Hong Kong-Macau Joint Lab on Chinese Medicine and Immune Disease Research, Guangdong Provincial Hospital of Chinese Medicine, Guangzhou University of Chinese Medicine, Guangzhou, China

Published online: 08 March 2025

inhibitor [6], glucocorticoids and other immunomodulators [7] are important components of AD treatment. However, the potential side effects and weakness in preventing recurrence still are the shortcoming of above treatment.

Traditional Chinese medicine (TCM) has been well-known for alleviating AD, and as a complementary treatment for AD [8] and allergic diseases [9] in Asia for numerous years. PeiTuQingXin (PTQX) formula is a clinically derived formula composed for AD treatment that exerted a considerable therapeutic effect in a previous clinical study [10] and randomized clinical trial [11]. Further animal experiments verified the role of PTQX formula in alleviating dermatitis and inhibiting Th2 cell differentiation [12]. Network pharmacology identified the TNF-α, NF-κB and cytokine interaction signaling pathway as the potential targets of PTQX formula in AD. However, in vivo experiments are necessary to verify these results.

Herein, we established house dust mite (HDM)-induced AD mouse model using NC/Nga mice and treated the AD model with PTQX formula. The dermatitis score, transepidermal water loss (TEWL), scratching behavior, serum inflammatory cytokines levels, and Th1/2/17 cell proportions were used to evaluate the effect of PTQX formula. Moreover, mouse inflammation array and data independent acquisition (DIA) proteomics analysis were performed to identify the mechanisms of PTQX formula in interventing AD mice, with potential targets were further verified by western blotting.

Materials and methods

Preparation of PTQX formula

PTQX formula comprises nine Chinese herbs, including Atractylodis macrocephalae Koidz. 10 g, Pseudostellaria heterophylla (Miq.) Pax 10 g, Dioscorea opposite Thunb. 15 g, Coix lacryma-jobi L.varmayuen (Roman.) Stapf 20 g, Imperata cylindrica Beauv.var.major (Nees) C.E.Hubb. 15 g, Forsythia suspensa (Thunb.) Vahl 10 g, Dictamnus dasycarpus Turcz. 10 g, Pteria martensii (Dunker) 0.3 g, Glycyrrhiza uralensis Fisch. 5 g, at a ratio of 30:30:45:60:45:30:30:1:15. All herbs were purchased from the Second Affiliated Hospital of Guangzhou University of Chinese Medicine. The compounds in PTQX formula were detected using ultra-high-performance liquid chromatography-tandem mass spectrometry and reported in our previous study [12]. The formula was prepared at a concentration of 1.95 g/ml, and the detailed process is described in the Supplementary Material.

Animals

Fifteen male specific pathogen-free NC/Nga mice (9 ~ 33 weeks old, weighing 20 ~ 40 g) were acquired from the Shanghai Model Organisms Center (Shanghai, China). Animal experiments were permitted by the Institutional Ethics Committee of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine, (NO.2022096; batch number: 44829400002008). The mice were maintained in a specific pathogen-free environment at 22 ± 3 °C, 55 ± 5% relative humidity, and a 12-h light/dark cycle. Mice were acclimated for one week and provided standard rodent chow and fresh tap water. The body weights of mice in each group were measured weekly, and all experiments were performed under 3% sevoflurane anesthesia using a TAIJI small-animal anesthesia machine (RWD, Shenzhen, China). All procedures were performed in accordance with the Laboratory Animal Research Committee Guidelines of the Guangdong Provincial Hospital of Chinese Medicine (approval number 2016015) on the usage, welfare and ethics of experimental animals.

Mice were randomly assigned to one of the following three groups ($n = 5$/group): (1) Control group; (2) AD group; (3) PTQX group. After acclimatization, approximately 8 cm^2 of dorsal hair was removed using a shaver and hair removal cream. Mice in the AD and PTQX groups were applied with 150 µl of 4% sodium dodecyl sulfate to the dorsal skin surface 1 h before applicating House Dust Mite (*Dermatophagoides farina*) extract oinment (Biostir AD®, Biostir Inc, Osaka, Japan). Meanwhile, mice in the Control group were applied to 4% sodium dodecyl sulfate, only. The stimulation was performed twice weekly for 3 weeks totally.

For treatment, mice in the PTQX group were fed with prepared PTQX formula daily at a volume of 10 ml/kg (19.48 g of crude drug/kg/day) via intragastric administration, whereas mice in the Control and AD groups were fed distilled water at the same frequency. The interventions were initiated on day 2 and lasted for 3 weeks. The experimental design is shown in Fig. 1A.

The scratching behavior was recorded the day after intervention completed (d21). Subsequently, the mice were sacrificed, and blood, skin, lymph node, spleen samples were collected.

Evaluation of dermatitis, TEWL and scratching behavior

For each mouse, skin lesions were imaged weekly using a digital camera (Canon, Tokyo, Japan), and the dermatitis score was evaluated as described previously [12]. The scoring procedure was as follows: (i) erythema/hemorrhage, (ii) edema, (iii) excoriation/erosion, and (iv) scaling/dryness,

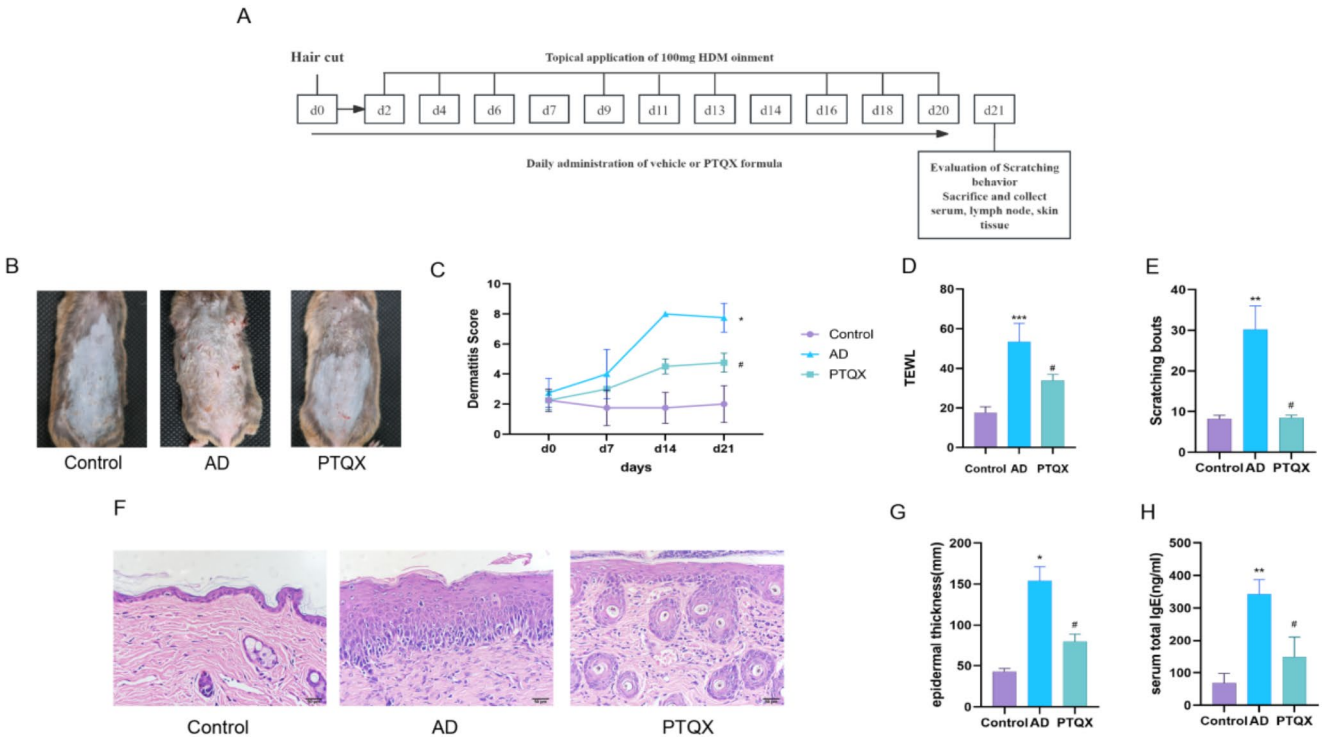

Fig. 1 Therapeutic effects of PTQX formula in the AD mouse model. **A** The experimental schedule of HDM-induced AD mouse model. **B** The dermatitis appearance and **C** score of mice from Control, AD, and PTQX group. **D** The TEWL index and **E** scratching bouts of mice from each group. **F** Representative H&E-stained for skin sections, **G** epidermal thickness, and **H** serum IgE levels of each group. Control: blank group. AD: HDM-induced group. PTQX: PTQX formula treated group. The data are presented as means ± SD ($n = 4$/group). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus the Control group. $^{\#}P<0.05$ versus the AD group. AD, atopic dermatitis; H&E, hematoxylin and eosin; HDM, house dust mite; PTQX, PeiTuQingXin; TEWL, trans-epidermal water loss

were scored 0 (none), 1 (mild), 2 (moderate), or 3 (severe), respectively. The sum of the scores was calculated.

TEWL was measured once weekly using the AS-VT100RS instrument (VAPO SCAN, Tokyo, Japan). Scratching behavior was evaluated using scratching bouts. Briefly, each mouse was placed in a clean box without top and video-recorded. Scratching bouts were defined as episodes in which the mouse lifted its hind paw and scratched more than 3 times until the paw returned to the floor. If the interval time between two scratching bouts was less than 1 s, it was considered one bout. The total number of bouts of scratching the dorsal skin or ear with their paws or limbs in 25 min was calculated.

Tissue collection

After assessing scratching behavior, mice were anesthetized with 5% sevoflurane and euthanized by cervically, death was confirmed by the cessation of breathing, disappearance of reflexes and dilated pupils. Blood samples, skin tissue, and lymph node were collected.

Hematoxylin and eosin (H&E) staining

The harvested skin tissue (1 cm×1 cm) were stored in cold 4% paraformaldehyde and embedded in paraffin following standard procedures. Subsequently, the tissues were prepared, and 4-μm-thick tissue sections were mounted on silane-coated glass slides. Deparaffinized and rehydrated sections were stained with hematoxylin and eosin (H&E) and imaged using a light microscope (Olympus BX53, Tokyo, Japan). Epidermal thickness was measured using the CellSens Standard program (Olympus).

Serum IgE level

The blood samples were centrifuged (14 000 ×g, 4 °C) for 15 min, and the plasma was collected and stored at −80 °C until use. IgE levels of each group were measured using a mouse IgE ELISA kit (ab157718; Abcam, Cambridge, MA, USA) following the manufacturer's protocol.

Flow cytometry

Cell suspensions were isolated from lymph nodes using a sterile 70 μm cell strainer (352350, Falcon, NY, USA), and

then activated using phorbol-12-myristate-13-acetate and Ionomycin (50 ng/ml and 1 μg/ml, respectively) in the presence of BD GolgiStopTM Protein Transport Inhibitor for 6 h. The cells were stained using a mouse Th1/Th2/Th17 Phenotyping kit (560758, BD, NZ, USA) following the manufacturer's protocol. Flow cytometry was performed using the NovoCyte Quanteon device (Angilent, Santa Clara, CA, USA).

Serum inflammatory cytokine measurement

The inflammatory cytokine level of mice from each group were measured from extracted serum using a RayBio® mouse inflammation array (AAM-INF-G1, Norcross GA, USA). Serum samples were diluted 2-fold and incubated in antibody array pools. The array was washed and incubated with biotin-conjugated anti-cytokines for 2 h at room temperature (22 ~ 25°C). Next, the Streptavidin-Flour reagent was diluted into a working solution, added to the array pools, and incubated at room temperature (22 ~ 25°C) for 2 h in light-resistant container. Fluorescence signal intensity was determined using a laser scanner (InnoScan 300 Microarray Scanner, Innopsys, Carbonne, France). Fluorescence intensity was analyzed using the RayBiotech analysis tool. Differentially expressed protein (DEP) analysis and diagram generation were performed using R software (version 4.3.0). The enrichment analysis of Gene Ontology (GO) categories was conducted for biological process (BP), cellular component (CC), molecular functional (MF) annotations. The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was conducted to identify the signaling pathways associated with DEPs.

Data independent acquisition (DIA) proteomics analysis

A Prominence LC-20 A (SHIMADZU, Kyoto, Japan), NanoElute® 2 and timsTOF Pro2 (Bruker, Billerica, MA, USA) were employed to perform the DIA proteomics mass spectrometry. The detailed process was described in the Supplementary Material. Briefly, proteins were extracted from the skin tissue of mice using a lysis buffer, digested with trypsin enzyme and desalted before High pH RP separation, and analyzed using liquid chromatography-tandem mass spectrometry in either data-dependent acquisition (DDA) or DIA acquisition mode. The retention time of the obtained protein data was normalized to the indexed retention time of the peptide. The false discovery rate was set at 1% for the acquired protein data identification based on a Target-decoy model. All analysis were conducted on BGI-tech platform. Bioinformatic analysis was performed using the UniProt protein and NCBI reference sequence databases. DEPs were analyzed using MSstats package in R software. Significant differential protein screening was defined as a Q value<0.05, with a fold change>1.2 or<0.83 as threshold. KEGG pathway and GO term enrichment analyses were conducted to determine the characteristic of DEPs.

Reverse transcription-quantitative PCR (RT-qPCR) assay

RNA was isolated from skin samples using TRIZOL (15596-018; Ambion, Naugatuck, CT, USA) agent. The cDNA was synthesized using a Reverse Transcription kit (A0010CGQ; EZB, Shanghai, China) and RT-qPCR was performed using a ViiA7 Real-Time PCR system (Thermo Fisher Scientific, Massachusetts, USA) with a SYBR Green Mix (A0012-R2, EZB). Primers used for RT-qPCR were synthesized by Invitrogen. Primers sequence are listed in Supplementary Material. The relative fold change in mRNA expression was calculated using the 2–ΔΔCt method with GAPDH as the housekeeping gene.

Western blotting analysis

The skin tissues of mice were lysed using RIPA Lysis Buffer (P0013B, Beyotime, Shanghai, China) mixed with a phosphatase and protease inhibitor cocktail (PhosSTOP™, 4693159001, Roche Diagnostic, Indianapolis, IN, USA). Protein concentrations were quantified using a BCA Protein Assay Kit (23227, Thermo Fisher Scientific) following the manufacturer's instructions. 40 μg protein was added to SDS-polyacrylamide gel and separated via electrophoresis (BioRad, Hercules, California, USA). The proteins were transferred onto a 0.22-μm PVDF membrane (Merck Millipore, Burlington, MA, USA), blocked with Quickblock Buffer (P0252, Beyotime) and incubated with primary antibodies against Claudin-1, TNF receptor-associated factor 2 (TRAF2), receptor-interacting protein 1 (RIP1) (37-4900, PA5-17500, PA5-20811, Thermo Fisher Scientific), NF-κB, p-NF-κB, IκB, p-IκB (8242 S, 3033 S, 4812 S, 2859 S, Cell Signaling Technology, Danvers, MA, USA), TRADD (15468-1-AP, Proteintech, Wuhan, China), GAPDH (T0004, Affinity, Jiangsu, China). The membrane was then incubated with HRP-conjugated secondary antibodies (1:5000, Cell Signaling Pathway) according to primary antibody species. The ChemiDoc ECL system (Bio-Rad) was used for protein bands detection, and densitometry was performed using ImageJ software (version 1.53, National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

All statistical analyses were conducted using GraphPad Prism software (Version 9, San Diego, CA, USA). Data are expressed as the means ± standard deviation (SD) or median with interquartile range (IQR). The Shapiro-Wilk normality test was used to determine the normality of data distribution of data. The significance of normally distributed data was determined by one-way analysis of variance (ANOVA), the data did not following a normal distribution were analyzed using Kruskal-Wallis test. $P<0.05$ was considered significant.

Results

PTQX formula alleviated HDM-induced AD-like symptoms

Upon examining the development of dermatitis in each group, we found the dorsal skin exhibited severe erythema, edema and dryness (Fig. 1B), while dermatitis score significantly increased in the AD group (Fig. 1C), along with increased TEWL index (Fig. 1D) and scratching behavior (Fig. 1E). Additionally, H&E-stained sections of the AD group exhibited inflammatory cell infiltration and epidermal thickening (Fig. 1F G). Mice in the PTQX group had a milder dermatitis appearance, less inflammatory cells infiltration, and less epidermal thickness than mice in the AD group, as evidenced in H&E-stained sections. In addition, TEWL and scratching behavior were also significantly decreased in the PTQX group. These results demonstrated the effect of PTQX formula in alleviating dermatitis.

PTQX formula reduced serum IgE levels in AD mice

High IgE levels are characteristic of atopic diseases and AD [13]. Herein, we observed that serum IgE levels were significantly elevated in AD mice comparing to the Control mice, whereas PTQX formula intervention significantly reduced IgE secretion (Fig. 1H).

PTQX formula regulated Th2 cell differentiation and Th2 cell-associated chemokine expression in AD mice

Activation of type 2 inflammation is a key mechanism in AD. To evaluate the effect of PTQX formula on regulating type 2 inflammation, we determined the proportion of Th1, Th2, Th17 in the lymph nodes of mice. No significant differences were observed in the proportions of Th1 and Th17 cells between the AD and Control groups, whereas the proportion of Th2 cells increased in the AD group. The differentiation of Th2 and Th17 cells was suppressed in the PTQX group compared with that in the AD group (Fig. 2A B),. Additionally, we quantified gene expression levels of TARC, CCL22 and CCR4, and found that transcription levels of these Th2 cell-associated chemokines were significantly reduced in the PTQX when comparing with the AD group (Fig. 2D), accompanied by the downregulation of GATA3 expression (Fig. 2D E). These results demonstrated the inhibitory effect of PTQX formula on the differentiation and migration of Th2 cells.

PTQX formula recovered the function of skin barrier function in AD mice

Skin barrier dysfunction is a pivotal factor that triggering AD aggravation. The significantly increased epidermal thickness in the AD group was consistent with the elevated TEWL index (Fig. 1H), whereas Claudin-1 expression was significantly reduced (Fig. 2D, E), indicating skin barrier dysfunction in AD mice. Treatment with PTQX formula recovered the epidermal thickness and Claudin-1 expression, verifying the function of PTQX formula in repairing skin barrier.

PTQX formula regulated the gene expression of inflammatory cytokines in AD mice

A series of inflammatory cytokines promote systemic inflammation and AD pathogenesis. Accordingly, we measured secretion levels of serum inflammatory cytokines in each group using a Luminex assay. The AD group exhibited significantly higher inflammatory cytokine expression than the Control, among which the secretion level of interleukin (IL)-1β, eotaxin and fractalkine were significantly elevated. Meanwhile, levels of IL-1β, granulocyte-colony stimulating factor, TCA-3, IL-12 p40/70, fractalkine, TECK and lymphotactin were significantly reduced in the PTQX group (Fig. 3A). According to the KEGG enrichment analysis results, DEPs between PTQX and AD groups were mainly enriched in NF-κB and Toll-like receptor signaling pathways (Fig. 3B), confirming the findings of our previously reported network pharmacology study that PTQX formula may exert anti-inflammatory effects by regulating the function of NF-κB signaling pathway.

Proteomic analysis of skin tissue in AD mice

DEPs in skin tissues of PTQX group and AD groups were analyzed using label-free, quantitative proteomics in a DIA mode. A total of 7182 proteins were identified, among which 743 DEPs differed significantly between the AD

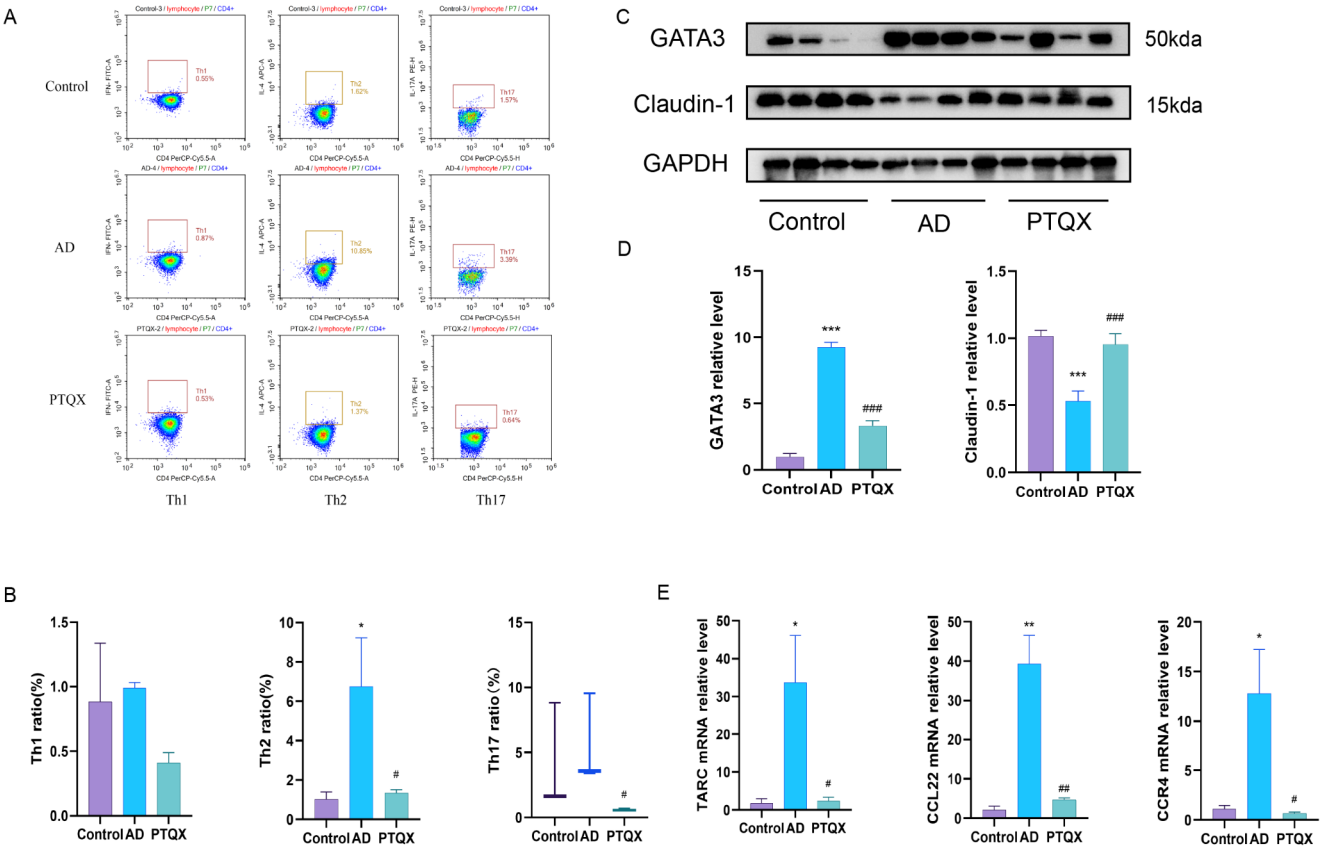

Fig. 2 The effect of PTQX formula in regulating type 2 inflammation, Th2 cell associated chemokines and Claudin-1 expression. **A** Representative flow cytometry images and **B** proportions of Th1, Th2 and Th17 cells in the lymph nodes of NC/Nga mice from Control, AD and PTQX group. **C** The mRNA expression of TARC, CCL22 and CCR4 in the skin tissue. D Representative western blotting images and E analysis for GATA3 and Claudin-1 expression. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus the Control group. $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ versus the AD group. AD, atopic dermatitis; PTQX, PeiTuQingXin

and Control groups (566 upregulated and 177 downregulated) (Fig. 4C). Compared with the AD group, the PTQX group had 51 upregulated and 100 downregulated proteins (Fig. 4D). A Venn diagram was plotted to display DEPs between each group, and 49 DEPs were found overlapped in AD versus Control and PTQX versus AD groups (Fig. 4B). In the clustering analysis of DEPs, the heatmap displayed significant difference in the comparison between each group (Fig. 4E). GO term enrichment analysis and annotation was conducted and shown in Fig. 5A. According to the KEGG pathway enrichment analysis in the overlapped DEPs (Fig. 5B), D-Amino acid metabolism, Biotin metabolism and Nitrogen metabolism signaling pathways were the top-ranking clusters. However, KEGG pathway interaction analysis (Fig. 5C) revealed that more DEPs were enriched in the ECM-receptor interaction and Adipocytokine signaling pathway according to the KEGG pathway interaction analysis. These clusters contained DEPs including TNFR1 Associated Death Domain (TRADD) and NF-κB inhibitor beta (IκB), which participate in regulating the NF-κB signaling pathway. Other overlapping DEPs include the Keratin subtype, Guanine nucleotide-binding proteins subunit α i3 (Gnαi3), and integrinα (ITGα) subtype, thereby indicating the potential role of PTQX formula in regulating skin barrier function, protein synthesis and immune response.

PTQX formula regulated the expression of TRADD/TRAF2/RIP1 and the NF-κB signaling pathway in AD mice

Western blotting was performed to further verify the function of PTQX formula in regulating TRADD and NF-κB signaling pathway. Because TRADD plays an important part in the TNF signaling pathway and exerts biological functions when complexed with TRAF2 and RIP1, we detected the expression of TRAF2 and RIP1 simultaneously. Treatment with PTQX formula significantly reduced the expression of TRADD, TRAF2 and RIP1 (Fig. 6A, B). Furthermore, treatment with PTQX formula suppressed the significantly reduction phosphorylation levels of IκB and NF-κB indicating NF-κB signaling pathway activation was inhibited in PTQX group (Fig. 6C, D). These results confirmed the

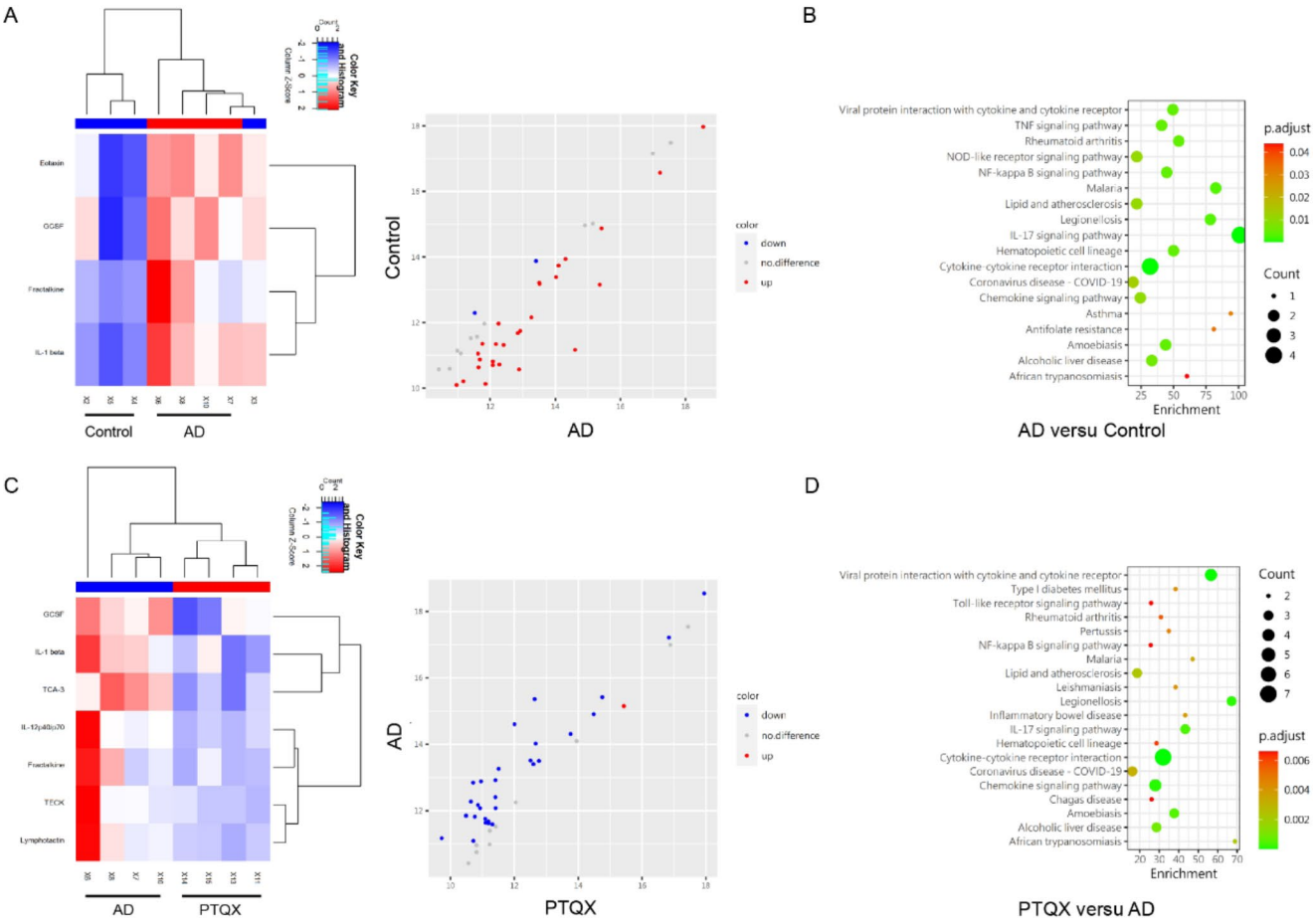

Fig. 3 Expression levels of inflammatory cytokines expression level detected using RayBio® mouse inflammation array. **A** The clustering heatmap of the DEPs and **B** KEGG pathway enrichment analysis result of AD versus Control group and **C** **D** PTQX versus AD group, respectively. AD, atopic dermatitis; DEPs, differentially expressed proteins; KEGG, Kyoto Encyclopedia of Genes and Genomes; PTQX, PeiTuQingXin

effect of PTQX formula in downregulating TRADD expression and NF-κB signaling pathway activation recognized as the downstream signaling pathway of the TRADD complex.

Discussion

AD is characterized by severe itchiness and a chronic phase, leading to a tremendous disease burden on patients and their families. With the annual increase in the incidence of AD, research on AD treatment has become an urgent issue. TCM has been applied in AD treatment for thousands of years and is associated with fewer side effects. AD is characterized by complex trigger factors and pathogenesis, while TCM formulas are regarded to exert effects through multiple mechanisms, which may be an advantage in AD therapy. Several studies have elucidated the efficiency and mechanisms of TCM formulas in treating AD, such as regulating the relative abundance of the skin-dominant microflora [14], or exerting a negative regulatory effect on the TLR4/MyD88/NF-κB signaling pathway [15]. In a randomized controlled study, medicine-treated group exhibited significant decrease in the mean SCORAD comparing with the control [10], and the application of TCM was proved to decrease corticosteroids exposure, manifested by shorter usage duration and frequency of visit [16]. Besides, several innovative administration carriers are developed to acquire better effect of TCM agents in AD [17, 18], displaying tremendous vitality of the traditional therapeutic method.

In the current study, we confirmed the effect of PTQX formula in an HDM-induced AD mouse model, as evidenced by improvements in clinical performance, serum inflammatory cytokines, skin barrier function and type 2 inflammation in mice treated with PTQX formula. The statistically significant reduction in serum inflammatory cytokine levels in the PTQX group indicated the anti-inflammatory effect of PTQX formula. Simultaneously, KEGG pathway enrichment analysis revealed the involvement of the TLR and NF-κB signaling pathways in mediating the effects of

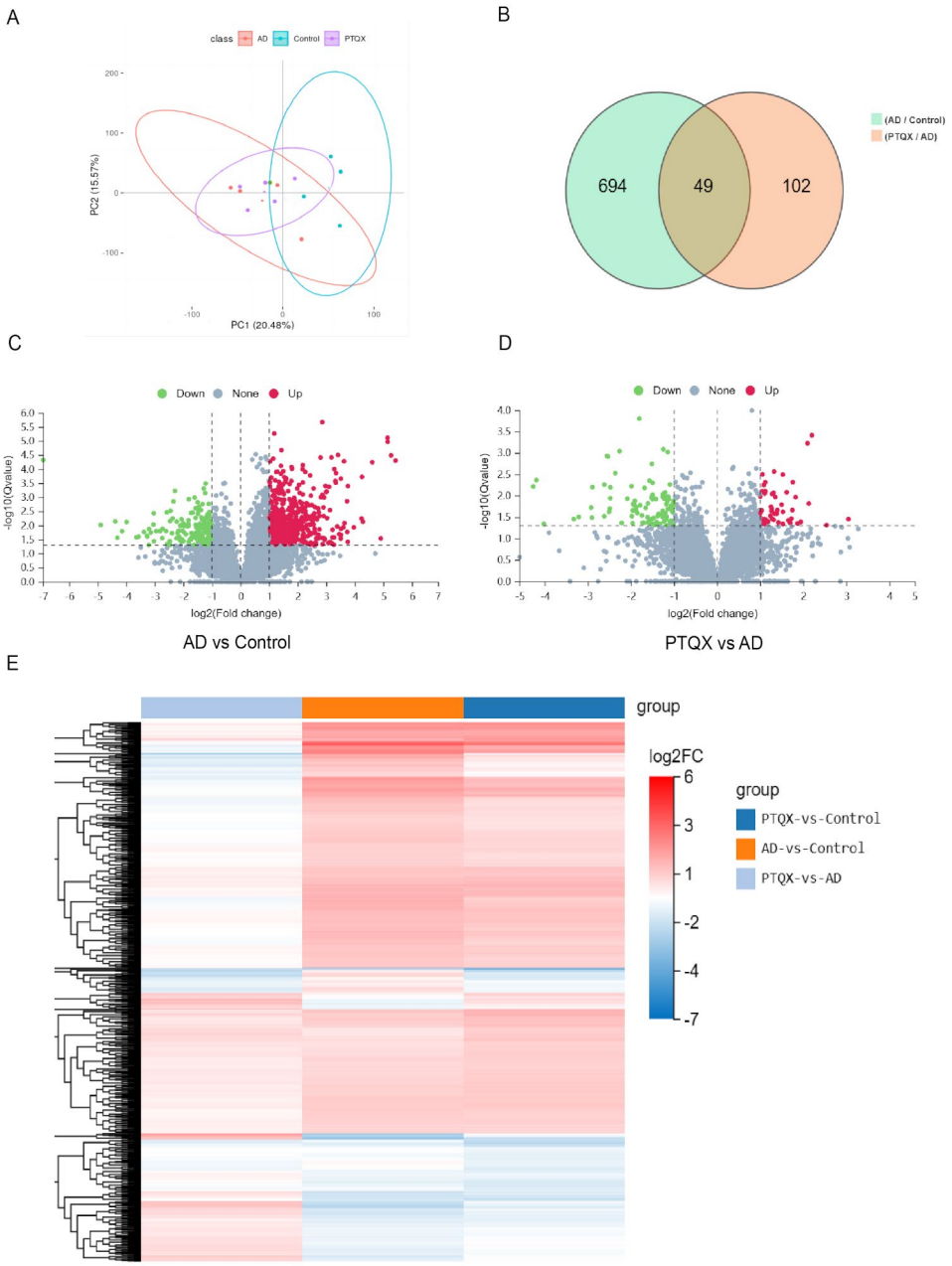

Fig. 4 The DIA proteomic analysis of skin tissue in the NC/Nga mice from each group. **A** The principal component analysis (PCA) result of the identified proteins. **B** The overlap of the DEPs between different group comparisons is represented using a Venn diagram. **C** The volcano diagram for AD versus Control and **D** PTQX versus AD group, and **E** clustering heatmap of the DEPs. AD, atopic dermatitis; DEPs, differentially expressed proteins; DIA, data-independent acquisition; PTQX, PeiTuQingXin

PTQX formula, thereby supporting the previously reported conclusion of our pharmacology research.

In addition to inflammatory cytokines, intervention with PTQX formula significantly suppressed transcription levels of several Th2 cell associated chemokines, including TARC and CCL22. The upregulation of TARC and CCL22 mediate Th2 cell migration from lymph nodes towards skin tissue [19], also serve as predictive factors for AD development [20] and disease severity [21]. Meanwhile, as the specific receptor of TARC and CCL22, CCR4 participates in the process of Th2 immune response [22] and promote Th2 migration in other atopic diseases. Our findings indicate another possible mechanism through which PTQX formula mediate type 2 inflammation, except for directly inhibit Th2 cell differentiation.

DIA proteomic analysis is widely used to identify targets and mechanisms of therapeutic agents. We identified ECM-receptor interaction and Adipocytokine signaling pathway were the potential target signaling pathways of PTQX formula, and found that the expression of TRADD and IκB, enriched in the above signaling pathways, was significantly inhibited in the PTQX group when compared with that in the AD group. TRADD is a potential adaptor molecule for TNF signaling, which participates in mediating inflammation, immune response through NF-κB, mitogen-activated protein (MAP) kinase or other pathways. After activated

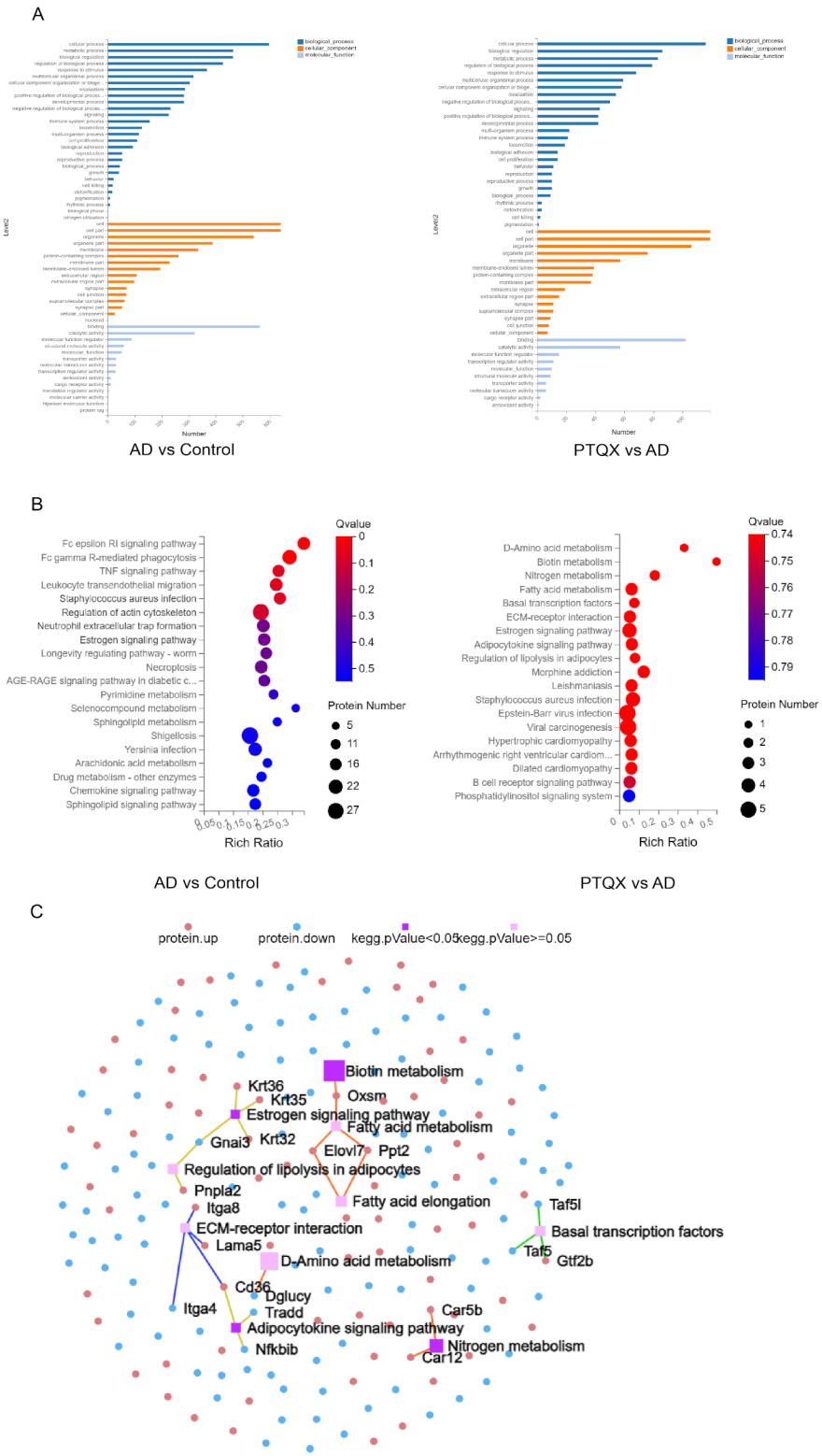

Fig. 5 Identification of the potential targets and mechanism of PeiTuQingXin (PTQX) formula in treating AD. **A** The gene Ontology (GO) term enrichment analysis and **B** the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis result between AD versus Control group, PTQX and AD group, respectively. **C** The interaction of the proteins and top10 enriched pathway in the comparison between PTQX versus AD group

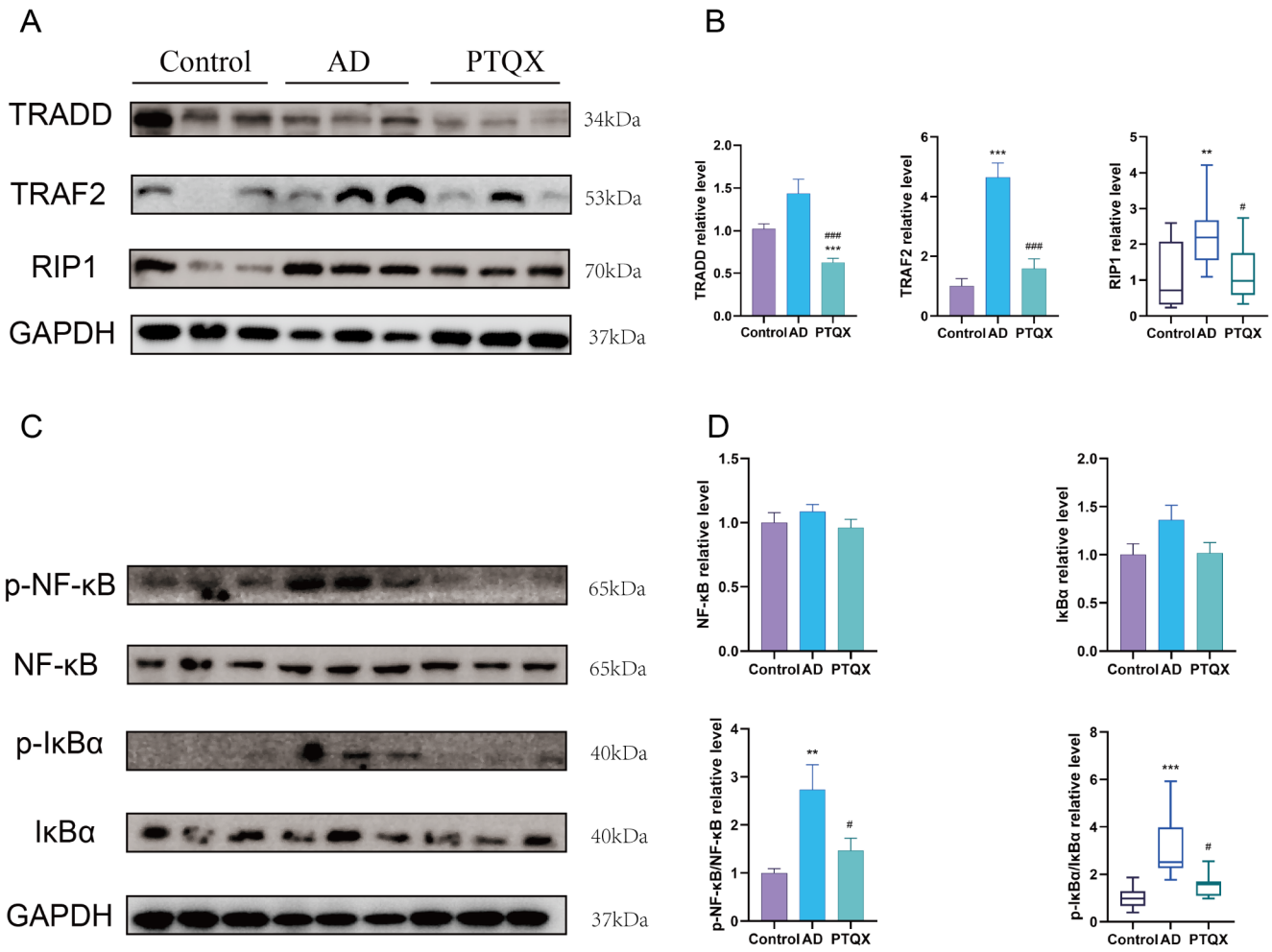

Fig. 6 Effects of PeiTuQingXin (PTQX) formula in regulating the expression of TRADD/TRAF2/RIP1 complex, and activity of NF-κB signaling pathway. **A** Western Blotting images and **B** protein expression level of TRADD, TRAF2, and RIP1 in the skin tissues of mice from each group. **C** Representative western blotting images and **D** phosphorylation levels of IκB and NF-κB in the skin of mice from each group. $^{**}P<0.01$, $^{***}P<0.001$ versus the Control group. $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ versus the AD group. AD, atopic dermatitis

and recruited by TNFR1, TRADD interact with TRAF2 and RIP1 to form complex and further promote the NF-κB activation initiated by RIP1 ubiquitination [23]. Thus, we proposed the TRADD/TRAF2/RIP1 complex as a target of PTQX formula. To the best of our knowledge, a link between TRADD/TRAF2/RIP1 complex and AD pathogenesis has not been reported. However, TRAF2 and RIP1 were shown to participated in regulating Th2 cell differentiation from CD4+T cells. Specific knocking down of TRAF2 in CD4+T cells resulted in the defect of cell proliferation and function mediated by T cell receptor (TCR) signaling [24], and downregulation of both TRAF2 and RIP1 completely inhibit the activation of NF-κB signaling pathway [25]. Considering the vital role of TRADD/TRAF2/RIP1 complex in mediating NF-κB signaling pathway, and the downregulated IκB level in PTQX group revealed by DIA analysis, we conducted western blotting analysis and found significantly reduced expression levels of TRADD, TRAF2, RIP1 in PTQX group, as well as the phosphorylation levels of IκB and NF-κB.

NF-κB signaling pathway is a well-known pathway mediating the proliferation, differentiation and survival of immune cells, and plays a role in regulating type 2 inflammation. During CD4+T cell differentiation, NF-κB upregulates the expression of IL-4 by binding to its promoter cooperating with nuclear factor of activated T cells (NFAT) [26]. Knockdown of NF-κB subunit p50 reportedly dysregulates GATA3 expression in CD4+T cells [27], indicating the crucial role of NF-κB in mediating Th2 differentiation. Therefore, we proposed the downregulated effect of PTQX formula on Th2 cells may occur via NF-κB signaling pathway (Fig. 7).

However, TCM formulas induce diverse effects in vivo to obtain disease alleviation, and the precise effect of PTQX formula in immune cells remains to be elucidated. The results of our previous experiments in DNCB-induced AD

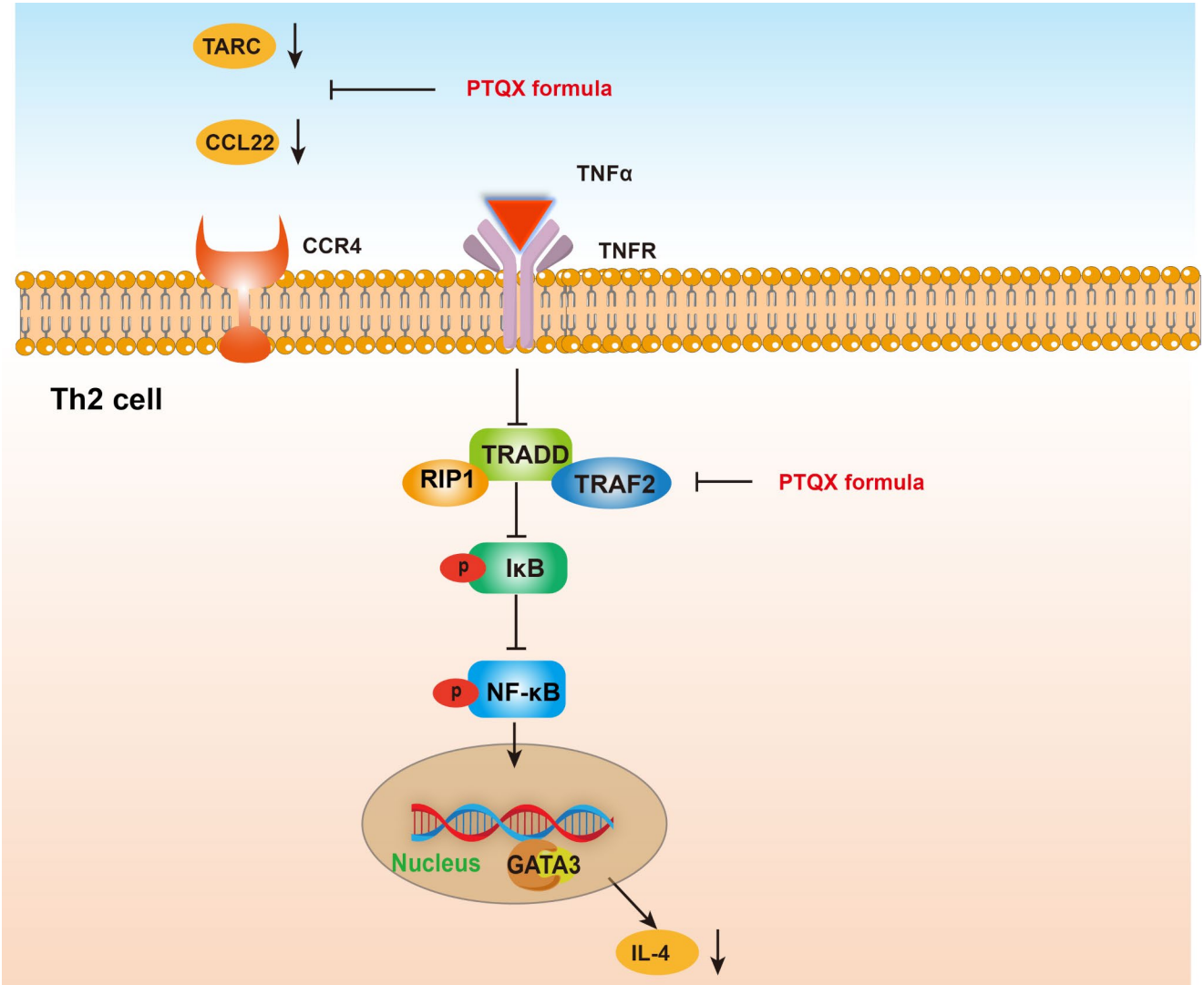

Fig. 7 The proposed mechanism of PeiTuQingXin (PTQX) formula in regulating Th2 cells

model indicates the effect of PTQX formula that regulating immune response in different AD model, and whether the NF-κB signaling pathway play a key role in this process needed further investigations. Our previous mass spectrometry analysis has identified the active material of PTQX formula, such as Forsythoside, Isoliquiritin, Liquirtigenin, Glycyrrhizin, et al. [12]. Considerable parts of these components exert regulating effect on inflammation and NF-κB signaling [28–30], as well as AD. In addition to NF-κB signaling, serveral pathways were reported being regulated by the monomer derived from PTQX formula, including JAK/STAT3 [31] and JNK/p38 MAPK [32] signaling pathway both involving in cell proliferation, cell apoptosis and immue response. Intringuingly, recent research reported the inhibition of TRADD expression induce downregulation of MAPK activity and cell apoptosis. This result may suggest another potential mechanism of PTQX formula though related experiments are necessary.

The effeicacy of PTQX formula in alleviating AD has been validated through long-term clinical application. In the following research, we intend to further identify the potential effective component in the serum of mice fed with PTQX formula, since the components in formula undergo complicate metabolic process in vivo. Verifying the target of PTQX formula and the identified component in regulating TRADD complex expression, as well as the effects upon blocking the activity of NF-κB signaling both in mouse models and Th2 cells, could provide better insight into the effect of PTQX formula.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00403-025-03998-2.

Acknowledgements This work was supported by Guangdong-Hong Kong-Macau Joint Laboratory on Chinese Medicine and Immune Disease Research, Guangzhou University of Chinese Medicine (2020B1212030006); Guangdong Provincial Key Laboratory of Chinese Medicine for Preventions and Treatment of Refractory Chronic Diseases (2018B030322012 and MB2020KF04); Municipal schools (colleges) jointly funded projects(2024A03J0544); State Key Laboratory of Dampness Syndrome of Chinese Medicine, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine (SZ2021ZZ17; SZ2021ZZ39; YN2024GZRPY055); Key-Area Research and Development Program of Guangdong Province (2020B1111100010), the Guangzhou Basic and Applied Basic Research Foundation (2023A03J0747; 2023B03J0562); National Administration of Traditional Chinese Medicine Inheritance and innovation "hundred and ten million" talent project (Qihuang project) Qihuang scholar project (Guo Zhong Yi Yao Ren Jiao Han [2018] No. 284), and the Chen Da-Chan famous Medical studio project of Guangdong Provincial Hospital of Traditional Chinese Medicine (NO. Y0096).

Author contributions Chen Dacan designed the experiments, supervised all research, and revised the manuscript. Cheng Zixuan conducted the experiments, analyzed the data, wrote this manuscript and prepared figures. Ma Xin and Luo Feng conducted the experiments. Yan Fenggen, Liu Junfeng, and Mo Xiumei provided funding and contributed to the interpretation of data and editing the manuscript. All authors reviewed the results and approved the final version of this manuscript.

Data availability The original data and materials presented in this research are included in the article or Supplementary Material. Other data will be available on request.

Declarations

Ethics approval and consent to participate Ethics Committee approval for animal experiment was obtained from the Institutional Ethics Committee of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine (NO.2022096).

Competing interests The authors declare no competing interests.

Patient consent for publication Not applicable.

References

1. Ständer S (2021) Atopic dermatitis. N Engl J Med 384:1136–1143. https://doi.org/10.1056/NEJMra2023911
2. Dong W-L, An J, Yu M et al (2021) The prevalence and year lived with disability of atopic dermatitis in China: findings from the global burden of disease study 2019. World Allergy Organ J 14:100604. https://doi.org/10.1016/j.waojou.2021.100604
3. Bosma AL, Ascott A, Iskandar R et al (2022) Classifying atopic dermatitis: a systematic review of phenotypes and associated characteristics. J Eur Acad Dermatol Venereol 36:807–819. https://doi.org/10.1111/jdv.18008
4. Bakker DS, Nierkens S, Knol EF et al (2021) Confirmation of multiple endotypes in atopic dermatitis based on serum biomarkers. J Allergy Clin Immunol 147:189–198. https://doi.org/10.1016/j.jaci.2020.04.062
5. McCann MR, Kosloski MP, Xu C et al (2024) Dupilumab: mechanism of action, clinical, and translational science. Clin Transl Sci 17:e13899. https://doi.org/10.1111/cts.13899
6. Guttman-Yassky E, Irvine AD, Brunner PM et al (2023) The role of Janus kinase signaling in the pathology of atopic dermatitis. J Allergy Clin Immunol 152:1394–1404. https://doi.org/10.1016/j.jaci.2023.07.010
7. Lazar M, Zhang AD, Vashi NA (2024) Topical treatments in atopic dermatitis: an expansive review. J Clin Med 13:2185. https://doi.org/10.3390/jcm13082185
8. Hon KL, Chu S, Leung AKC, Wong A (2022) Atopic dermatitis: conventional and integrative medicine. Curr Pediatr Rev 18:84–96. https://doi.org/10.2174/1573396317666210716152325
9. Wang Z, Wang Z-Z, Geliebter J et al (2021) Traditional Chinese medicine for food allergy and eczema. Ann Allergy Asthma Immunol 126:639–654. https://doi.org/10.1016/j.anai.2020.12.002
10. Liu J, Mo X, Wu D et al (2015) Efficacy of a Chinese herbal medicine for the treatment of atopic dermatitis: a randomised controlled study. Complement Ther Med 23:644–651. https://doi.org/10.1016/j.ctim.2015.07.006
11. Gu SX, Mo X, Zhang AL et al (2018) A Chinese herbal medicine preparation (Pei Tu Qing Xin) for children with moderate-to-severe atopic eczema: a pilot randomized controlled trial. Br J Dermatol 179:1404–1405. https://doi.org/10.1111/bjd.16988
12. Yan F, Zhang J, Li X et al (2019) Therapeutic effects of Chinese Herbal Formula (PTQX) on NC/Nga mice with atopic Dermatitis-Like skin lesions. Evid Based Complement Alternat Med 2019:8359252. https://doi.org/10.1155/2019/8359252
13. Lu H-F, Chou C-H, Lin Y-J et al (2024) The genome-wide association study of serum IgE levels demonstrated a shared genetic background in allergic diseases. Clin Immunol 260:109897. https://doi.org/10.1016/j.clim.2024.109897
14. Zeng B, Liu X, Zhou Y et al (2024) Effect of a topical traditional Chinese herbal medicine on skin microbiota in mouse model of atopic dermatitis. Heliyon 10:e33240. https://doi.org/10.1016/j.heliyon.2024.e33240
15. Nie J, Jiang X, Wang G et al (2024) Yu-Ping-Feng-San alleviates inflammation in atopic dermatitis mice by TLR4/MyD88/NF-κB pathway. J Ethnopharmacol 329:118092. https://doi.org/10.1016/j.jep.2024.118092
16. Chen H-Y, Lin Y-H, Wu J-C et al (2015) Use of traditional Chinese medicine reduces exposure to corticosteroid among atopic dermatitis children: a 1-year follow-up cohort study. J Ethnopharmacol 159:189–196. https://doi.org/10.1016/j.jep.2014.11.018
17. Li M, Xu Y, Yu Y et al (2024) Transdermal delivery of natural products against atopic dermatitis. Chin J Nat Med 22:1076–1088. https://doi.org/10.1016/S1875-5364(24)60681-3

18. Tan L, Wu S, Liu L et al (2025) Bacterial cellulose based gel of glycyrrhizic acid gel for atopic dermatitis: design, optimization, in vitro and in vivo investigation. Int J Biol Macromol 286:138425. https://doi.org/10.1016/j.ijbiomac.2024.138425
19. Imai T, Nagira M, Takagi S et al (1999) Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. Int Immunol 11:81–88. https://doi.org/10.1093/intimm/11.1.81
20. Rinnov MR, Halling A-S, Gerner T et al (2023) Skin biomarkers predict development of atopic dermatitis in infancy. Allergy 78:791–802. https://doi.org/10.1111/all.15518
21. Kataoka Y (2014) Thymus and activation-regulated chemokine as a clinical biomarker in atopic dermatitis. J Dermatol 41:221–229. https://doi.org/10.1111/1346-8138.12440
22. Chu Y-T, Liao M-T, Tsai K-W et al (2023) Interplay of chemokines receptors, toll-like receptors, and host immunological pathways. Biomedicines 11:2384. https://doi.org/10.3390/biomedicines11092384
23. Li Z, Yuan W, Lin Z (2020) Functional roles in cell signaling of adaptor protein TRADD from a structural perspective. Comput Struct Biotechnol J 18:2867–2876. https://doi.org/10.1016/j.csbj.2020.10.008
24. Villanueva JE, Walters SN, Saito M et al (2017) Targeted deletion of Traf2 allows immunosuppression-free islet allograft survival in mice. Diabetologia 60:679–689. https://doi.org/10.1007/s00125-016-4198-7
25. Wagner J, Vredevoogd D, Yu X et al (2025) TRAF2 and RIPK1 redundantly mediate classical NFκB signaling by TNFR1 and CD95-type death receptors. Cell Death Dis 16:35. https://doi.org/10.1038/s41419-024-07325-x
26. Köck J, Kreher S, Lehmann K et al (2014) Nuclear factor of activated T cells regulates the expression of interleukin-4 in Th2 cells in an all-or-none fashion. J Biol Chem 289:26752–26761. https://doi.org/10.1074/jbc.M114.587865
27. Das J, Chen CH, Yang L et al (2001) A critical role for NF-kappa B in GATA3 expression and TH2 differentiation in allergic airway inflammation. Nat Immunol 2:45–50. https://doi.org/10.1038/83158
28. Yue Y, Wang J, Tian J (2024) Glycyrrhizic acid promote remyelination after peripheral nerve injury by reducing NF-κB activation. Neurosci Lett 843:138009. https://doi.org/10.1016/j.neulet.2024.138009
29. Xu J, Yin P, Liu X, Hou X (2023) Forsythoside A inhibits apoptosis and autophagy induced by infectious bronchitis virus through regulation of the PI3K/Akt/NF-κB pathway. Microbiol Spectr 11:e0192123. https://doi.org/10.1128/spectrum.01921-23
30. Li Y, Song W, Tong Y et al (2021) Isoliquiritin ameliorates depression by suppressing NLRP3-mediated pyroptosis via miRNA-27a/SYK/NF-κB axis. J Neuroinflammation 18:1. https://doi.org/10.1186/s12974-020-02040-8
31. Wu Q, Mo X, Lin Y et al (2022) Inhibitory effects of isoliquiritin on an atopic dermatitis model through the CD177/JAK2/STAT pathway in vitro and in vivo. Ann Transl Med 10:980. https://doi.org/10.21037/atm-22-3989
32. Lee H-S, Kim E-N, Jeong G-S (2020) Oral Administration of Liquiritigenin Confers Protection from atopic dermatitis through the inhibition of T cell activation. Biomolecules 10:786. https://doi.org/10.3390/biom10050786