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Egg white peptides increase the supplementation of amino acids *in vivo* in inflammation period and show a positive effect on the intestinal barrier in childhood

Qi Yang[a,b], Jingbo Liu[a,b], Siwen Lyu[a,b], Ting Li[a,b], Xuanting Liu[a,b], Zhiyang Du[a,b], Xiaomin Shang[a,b], Ting Zhang[a,b], Huifang Ge[c,*], Chunyu Xi[b,*]

[a] *Jilin Provincial Key Laboratory of Nutrition and Functional Food, Jilin University, Changchun 130062, China*
[b] *College of Food Science and Engineering, Jilin University, Changchun 130062, China*
[c] *State Key Laboratory of Tea Plant Biology and Utilization, School of Tea and Food Sciences and Technology, Anhui Agricultural University, Hefei 230036, China*

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ABSTRACT

Bioactive peptides have various excellent biological activities and serve as functional foods to prevent chronic diseases in the human body. This article investigated the regulatory effect of egg white peptides (EPs) on the intestine barrier of young mice with colitis. The results showed that the intake of EPs could protect the intestines from inflammation damage. Besides, the expression of tight junction proteins and mucin is upregulated. Markedly, the intake of EPs can increase the concentration of amino acids in the serum of young mice, which is crucial for nutritional supplementation during intestinal inflammation process. Proteomics analysis implied that EPs can regulate protein expression in the intestine, involving multiple inflammatory pathways including phosphoinositide 3-kinase (PI3K)-protein kinase B (AKT) and mitogen-activated protein kinase (MAPK) signaling pathway. This demonstrated the health benefits of bioactive peptides and provides a theoretical basis for the development of animal derived proteins as functional foods.

1. Introduction

Recently, the public has increasingly attached importance to intestinal health, and the gut has become a research hotspot in the field of food nutrition. The key role of the intestine barrier is to strictly prevent the entry of harmful substances while absorbing essential nutrients, maintaining a certain balance between these two reactions[1-2]. Once the intestinal barrier is disrupted, the intestine immune system would receive chemical signals to activate defense mechanisms, triggering an inflammatory response. Inflammatory bowel disease (IBD) is one of the most common intestinal diseases, which can be divided into Crohn disease (CD) and ulcerative colitis (UC). Its clinical manifestations include diarrhea, bloody stools, acute stomachache, which bring a serious threat to human health, especially for children in the younger age group[3]. The data showed that the incidence rate of IBD in children is increasing year by year, which has become one of the public health problems[4]. UC patients during childhood have similar clinical characteristics compared to adult patients, but the progression of the disease is faster and more severe, even delaying the growth and adolescence of children[5]. Children's gut microbiota and intestine immune systems are at a critical stage of development, and it's crucial for nutritional intake and physical development during childhood. Most of the drugs currently used to treat UC are mainly antibiotics and immunosuppressants. Although

* Corresponding authors.
E-mail address: ghfjulia@163.com (H.F. Ge); xichunyu@jlu.edu.cn (C.Y. Xi)
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drugs can reduce the symptoms of the disease or control its progression, long-term drug interventions seriously affect the physical and mental health and growth of young children. Therefore, finding natural and safe food derived active substances to protect the intestinal health of adolescents is urgent, and it is of great significance to promote the application of food derived active substances in the field of human health.

Accumulating evidence has shown that dietary components, including macronutrients, vitamins, and minerals, are regulators of intestine barrier function[6-7]. For example, dietary protein intake provides the body with essential amino acids (EAA), maintains intestinal integrity, inhibits pro-inflammatory signaling pathways and protects cells from apoptosis and oxidative stress. Therefore, it is significant to develop bioactive peptides from animal protein sources as functional foods to aid in the prevention of chronic diseases for children in the younger age group[8-9]. The structure of egg white protein is very similar to human serum albumin and contains all 8 EAAs. Therefore, egg whites have become the main object of comprehensive utilization and deep processing of eggs, and are considered as one of the good sources for the preparation of bioactive peptides[10]. These bioactive peptides are not only safe for consumption but also have the nutritional properties of proteins, and are more beneficial to human absorption and utilization than intact proteins. It has been proved that egg white peptides (EPs) have various functional activities, such as, anti-inflammatory, antioxidant, antibacterial and immunomodulatory activities. However, the regulatory mechanism of intestinal barrier function in young mice is still unclear, which seriously limits the development of related products[11-13].

Therefore, this study takes young mice as the research object, and constructs a colitis model by dextran sodium sulfate (DSS) to clarify the repairing effect of EPs on intestine barrier. In addition, the change of amino acids level was determined by high performance liquid chromatography (HPLC) to investigate the effect of EPs intake on nutritional supplementation of mice. Finally, proteomics technology was applied to reveal the mechanism of EPs on repairing intestine barrier. The study will provide a theoretical basis for the product development of bioactive peptides, and expand its application in the field of functional foods.

2. Materials and methods

2.1 Materials

Alcalase and DSS (molecular weight: 40 000 kDa) was purchased from Sigma-Aldrich (Shanghai) Trading Co., Ltd. (China). Lipopolysaccharide (LPS) and *D*-lactic acid (*D*-LAC) kit were purchased from Shanghai Enzyme-Linked Biotechnology Co., Ltd. (China). Amino acid standard kits and AccQ-Tag Ultra Derivatization Kit were purchased from Waters (Milford, MA, USA). Thermo Fisher Scientific Co., Ltd. (USA) provided acetonitrile. All reagents are analytical grade.

2.2 Preparation of EPs

Firstly, heat the 8% egg white protein solution at 90 ℃ for 10 min, during which a stirrer should be added to continuously stir to ensure uniform heating. Then cool the solution to 56 ℃, adjust the pH to 10 with 1 mol/L NaOH. Next, add 12% (*m*/*m*) alcalase of egg white protein mass for 3 h enzymatic hydrolysis. Then, heat the solution to 90 ℃ for 10 min to inactivate the enzyme. The supernatant obtained by centrifuging at 9 000 r/min for 10 min was passed through an ultrafiltration membrane to obtain EPs with a molecular weight below 1 kDa.

2.3 Animal treatment

The young mice (4 weeks, (15.00 ± 2.00) g) were raised in the Experimental Animal Center of the First Hospital of Jilin University and the animal use license number was SYXK2019-0012. The mice were divided into 4 groups after 7-day acclimation: control cheek group (CK, Normal eating and drinking; administrated with saline during the whole 14 days), model group (M, drinking 3% DSS freely from 8th to 14th day and administrated with saline during the whole 14 days), low-dose of EPs group (EPs-L, drinking 3% DSS freely from 8th to 14th day and administrated with 200 mg/kg EPs by gavage during the whole 14 days), and high-dose of EPs group (EPs-H, drinking 3% DSS freely from 8th to 14th day and administrated with 400 mg/kg EPs by gavage during the whole 14 days). The experiment protocol was presented in Fig. 1A. After 2-week treatment, the mice in each group were anesthetized with pentobarbital and dissected by cervical dislocation. The colon tissues and blood were collected for further use.

2.4 Assessment of disease activity index (DAI)

The body weight was documented during the two weeks. Stool state and fecal bleeding was observed from the 8th day. The criteria can be found in were referenced from Ge et al.[14], and DAI was calculated by formula below:

$$\mathrm{DAI} = \frac{\text{Weight loss score} + \text{Fecal trait score} + \text{Fecal blood score}}{3}$$

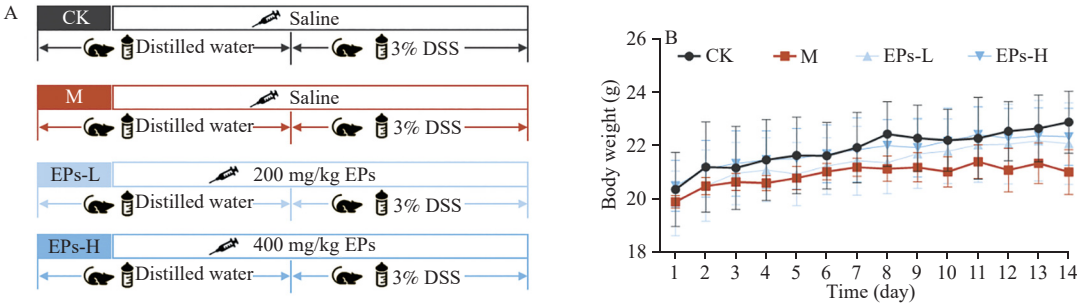

Fig. 1 Improvement effect of EPs on colitis mice. (A) Animal treatment process. (B) Body weight change in each group. (C) DAI. (D) Colon length of mice in each group. (E) Masson staining of colon tissue. (F) Collagen area in each group. (G) Colon fibrosis score in each group. *P < 0.05; **P < 0.01; ***P < 0.001 represent significance between each group compared with M group. Same as below.

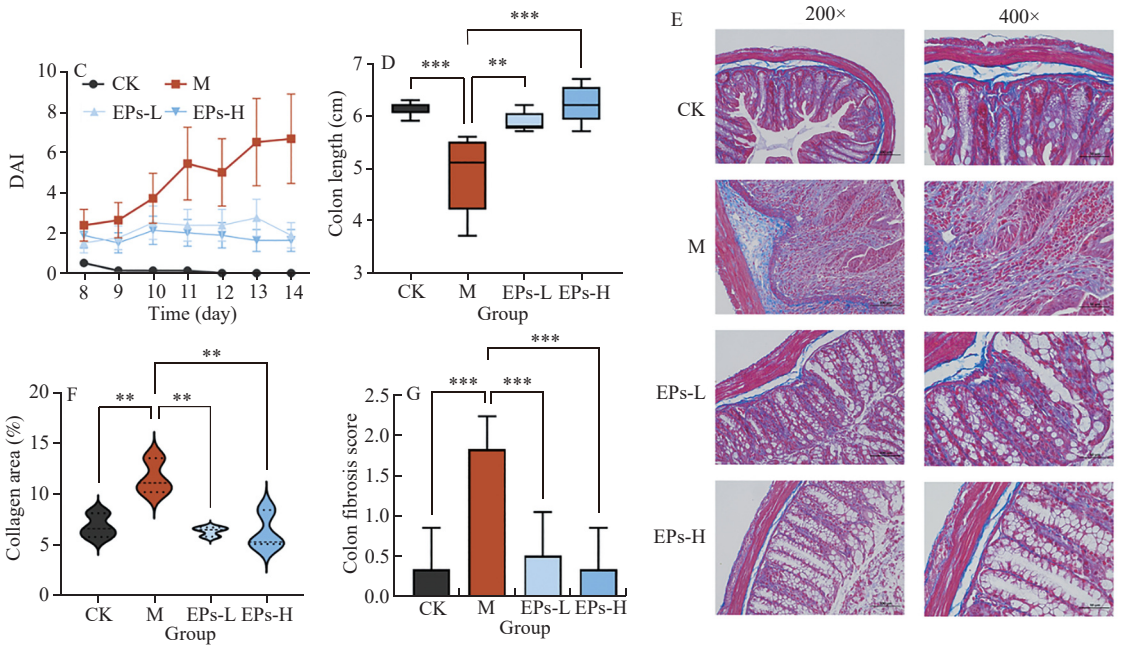

Fig. 1 (*Continued*)

2.5 Masson staining

Using paraffin to make colon tissue into paraffin slices, which were cleaned with distilled water before being stained for 10 min with hematoxylin working solution and completely washed. Then ponceau acid fuchsin solution were used to stain the slices (10 min). Following that, the slices were immersed in a 1% phosphomolybdic acid solution (5 min). After that, the slices were then stained with aniline blue for 5 min. Finally, the slices were submerged in a 0.2% glacial acetic acid solution for a time before being dehydrated in 95% ethanol and treated with dimethylbenzene hyaluronic acidification. The collagen area was calculated by software ImageJ.

The colon fibrosis scores were evaluated by criteria from Wang et al.[15] based on the Masson staining. The sores were listed as follows: 0, no obvious hyperplasia of collagen fiber, and no obvious thickening of lamina propria; 1, collagen fibers slightly increased, and slight thickening of the lamina propria; 2, collagen fibers obviously increased and visible, and there was macroscopic evidence of thickening of the lamina propria.

2.6 Immunofluorescence staining

Before being immersed in xylene for 20 min, colon tissues were deparaffinized. Slices were submerged in citrate buffer for 5 min before being immersed in ethanol concentrations of 100%, 95%, 85%, and 75%. The slices were then washed for 3 min in phosphate buffered saline (PBS) before being submerged in a sodium borohydride solution for 30 min.

The slices were then immersed in Sudan black dye solution for 5 min. Then they were blocked for 30 min before being incubated in the first antibody. After 24 h, a secondary antibody was applied. In addition, the slices were stained with 4′,6-diamidino-2′-phenylindole (DAPI) solution (37 °C). The final slices were examined by a fluorescence microscope. Detailed information is available in the process of Wang et al.[16].

2.7 Alcian blue and periodic acid Schiff (AB-PAS) staining

Based on the method of Shimizu. et al.[17], the tissues in each group were sectioned and embedded in paraffin. After that, the slices were dyed before being examined under a microscope.

2.8 Evaluation of intestinal permeability

Intestinal barrier breakdown was extremely related to intestinal permeability. LPS and *D*-LAC have been widely used as indicators of intestinal permeability. Based on the kit instructions, enzyme-linked immunosorbent assay (ELISA) kits were used to determine the concentration of LPS and *D*-LAC.

2.9 Amino acids determination in mice serum by HPLC

Firstly, acetonitrile (1:3, *V/V*) was added in mouse serum and centrifuged at 3 500 × *g* for 20 min to eliminate impurities. After that, the supernatant was nitrogen-concentrated to 10 μL and then mixed with derivative reagents (AQC, 20 μL) and borate buffer (70 μL). The combined solutions were then incubated for 10 min at 55 °C. The samples were then tested using an HPLC instrument. The mobile phase component and gradient was referenced from Yang et al.[18]. The final detection wavelength was 248 nm.

2.10 Proteomic analysis

Firstly, add protein lysis solution to the sample and shake it 3 times using a high-throughput tissue grinder for 40 s each time. The lysis process was carried out on ice, and after 30 min, the solution was centrifuged at 12 000 × *g* for 30 min. Then the supernatant was collected for protein concentration measurement. Next, take 100 μg protein and add triethylammonium bicarbonate buffer to achieve a final concentration of 100 mmol/L. Then tris-(2-carboxyethyl)-phosphine

was added to achieve a final concentration of 10 mmol/L and react at 37 ℃ for 1 h. Next, add iodoacetamide to the solution (final concentration was 40 mmol/L) and react in dark at room temperature for 40 min. Then add pre-cooled acetone to each tube and precipitate for 4 h, and centrifuge the solution at 10 000 × *g* for 20 min to obtain sediment. Dissolve the sample thoroughly with 100 μL triethylammonium bicarbonate buffer and hydrolyze the solution with trypsin overnight at 37 ℃. Finally, acetonitrile was added to the tandem mass tags (TMT) reagent and incubate at room temperature for 2 h. Then add hydroxylamine to the solution and react at room temperature for 30 min, and dry the sample using a vacuum concentrator. The sample passes through a C_{18} chromatographic column (2.1 mm × 150 mm, 1.7 μm, Waters, USA) separation for 90 min (flow rate: 300 nL/min). Subsequently, a liquid phase gradient elution was performed. The mobile phase component and gradient was referenced from Wen et al.[19]. The *t* test function in R language was used to calculate the *P*-value of the significance of differences between samples.

Differentially expressed proteins (DEPs) was identified by the thresholds of fold change (FC) > 1.2 or < 0.83. Gene Ontology (GO) annotation analysis and functional clustering analysis from three aspects: biological processes (BP), cellular components (CC), and molecular functions (MF) was performed (http://geneontology.org/). The Kyoto Encyclopedia of Gene and Genes (KEGG) database was applied to analyze the pathways involved in DEPs (http://www.genome.jp/kegg/).

2.11 Statistical analysis

Data are displayed as the mean ± standard deviation. One-way analysis of variance with Tukey's honest significant difference were used to calculate the *P* value.

3. Results

3.1 Improvement effect of EPs on colitis symptoms

Due to the exploration of the preventive and protective effects of EPs on the intestinal barrier throughout the experiment, the consumption of DSS and the administration of EPs by gavage were conducted simultaneously. The weight of the mice in the CK group increased gradually from an initial 20.33 g to 22.86 g on day 14, while the weight of the colitis mice increased only from 19.88 g to 20.99 g, indicating that colitis slowed the mice growth. In contrast, after gavage of EPs on day 8, the mice in EPs-L and EPs-H group gained weight compared to the M group, indicating that additional protein intake improved the growth retardation caused by colitis (Fig. 1B). Throughout the entire modeling process, the body weight, rectal bleeding, and fecal morphology of each group of mice were monitored daily, and DAI was calculated based on these indicator values. During the modeling period, the M group mice experienced severe rectal bleeding, with an increase in DAI (Fig. 1C), indicating that the M group had already developed acute colitis due to DSS and animal model was successfully constructed. The EPs treatment groups also showed similar conditions to the M group during the modeling period, but their morbidity symptoms were significantly improved compared to the M group (Fig. 1C), suggesting that EPs intake could alleviate the pathological condition of colitis pups. In addition, colitis resulted in shortened colonic length in young mice, but the symptoms were reduced and the length was restored after EPs intervention, with the most significant effect in the EPs-H group (Fig. 1D).

In Fig. 1E, it can be observed that cells in the CK group are arranged closely, the crypt structure is clearly visible, and collagen is regularly distributed in the submucosa. However, the intestinal cells in the M group were significantly damaged, with significant collagen accumulation, disappearance of crypt structures and inflammatory infiltration, and significant damage to muscle fiber structures. Compared to the CK group (6.84%), due to the increased expression of collagen fibers in the mucosa, submucosa, and muscle of the M group, the collagen area in M group increase into 11.63% ($P < 0.01$) (Fig. 1F). In addition, the intestinal wall was thickened in M group, and colon fibrosis score increased significantly ($P < 0.001$) (Fig. 1G). After ingestion of EPs, the collagen area significantly decreased to 6.35% and 6.27%, respectively ($P < 0.01$). The results showed that EPs has a certain inhibitory effect on colon fibrosis.

3.2 Effect of EPs on intestinal barrier structure of young mice with colitis

Disruption of intestinal barrier structure is a direct result of DSS-induced colitis[20]. Therefore, we performed immunofluorescence analysis of zona occludens-1 (ZO-1) and mucin-2 (MUC-2) expression in the intestine (which are important tight junction (TJ) protein and mucin in protecting intestinal barrier). The results indicated that the expression level of ZO-1 and MUC-2 was markedly lower in M group (Figs. 2A and B). Colitis leads to the destruction of the intact structure of the intestine. The expression of ZO-1 in EPs-L was not significant compared with M group. However, 400 mg/kg EPs intervention resulted in a 18.45% restoration of ZO-1 expression (Fig. 2C). Notably, the expression of MUC-2 was 1.50-fold higher than that of the M group (Fig. 2D). These results suggested that EPs can maintain the integrity of the intestinal barrier by regulating the expression of TJ proteins and mucins.

3.3 Effect of EPs on intestinal permeability of young mice with colitis

AB-PAS staining was used to assess the integrity of the colonic mucus layer. Mucus layer of mice in M group was obviously destroyed, indicating that mucin secretion was downregulated caused by DSS (Fig. 3A). Besides, the number of goblet cells were decreased. However, gavage of EPs during DSS treatment can alleviate this condition, reduce the degree of mucus layer damage. The number of goblet cells in the EPs-H group is like that in the CK group, implying that EPs have a protective effect on the intestinal mucus layer (Fig. 3B). The level of *D*-LAC and LPS in serum are important indicators to assess the level of intestinal permeability. The results in Fig. 3C showed that the LPS concentration was significantly increased in the M group ($P < 0.01$), which was 23.30% higher than that of the CK group. The *D*-LAC level showed a similar trend, with an increase of 60.06% in the M group mice in contrast to the CK group (Fig. 3D). However, the *D*-LAC concentration was reduced by 29.05% and 32.61% in the EPs-L and EPs-H groups, respectively, in comparison with the M group. The results implied that EPs could inhibit the progression of UC, reduce intestinal permeability, and restore intestinal barrier function in young mice.

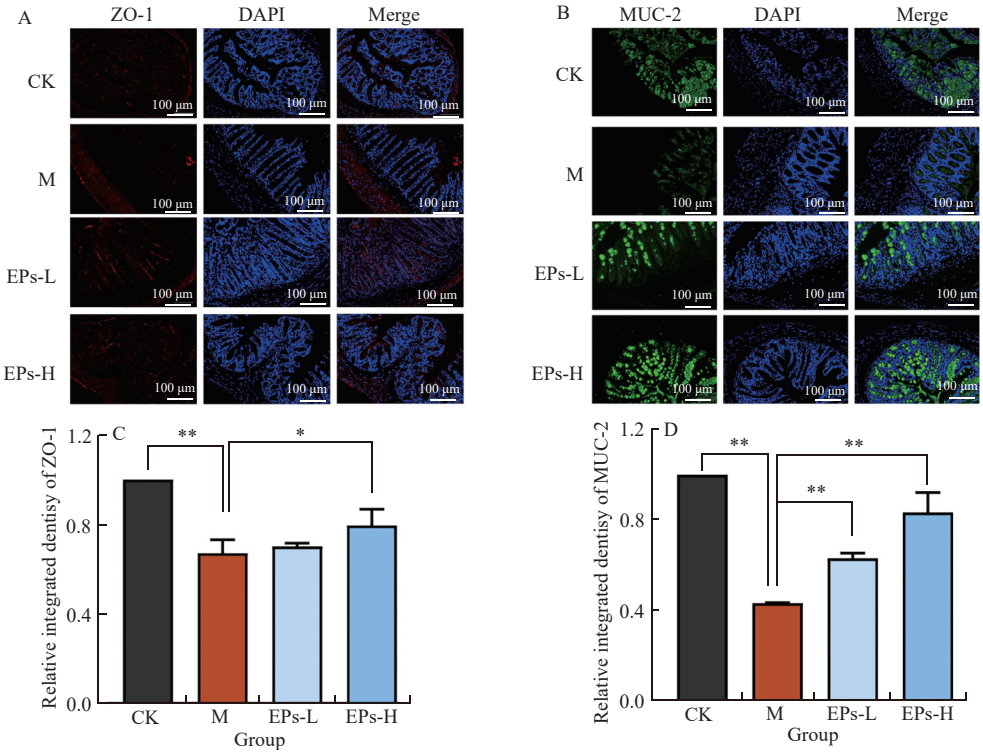

Fig. 2 Protective effect of EPs on intestinal barrier structure of young mice with colitis. (A) Immunofluorescence staining of ZO-1. (B) Immunofluorescence staining of MUC-2. (C) Relative integrated density of ZO-1. (D) Relative integrated density of MUC-2.

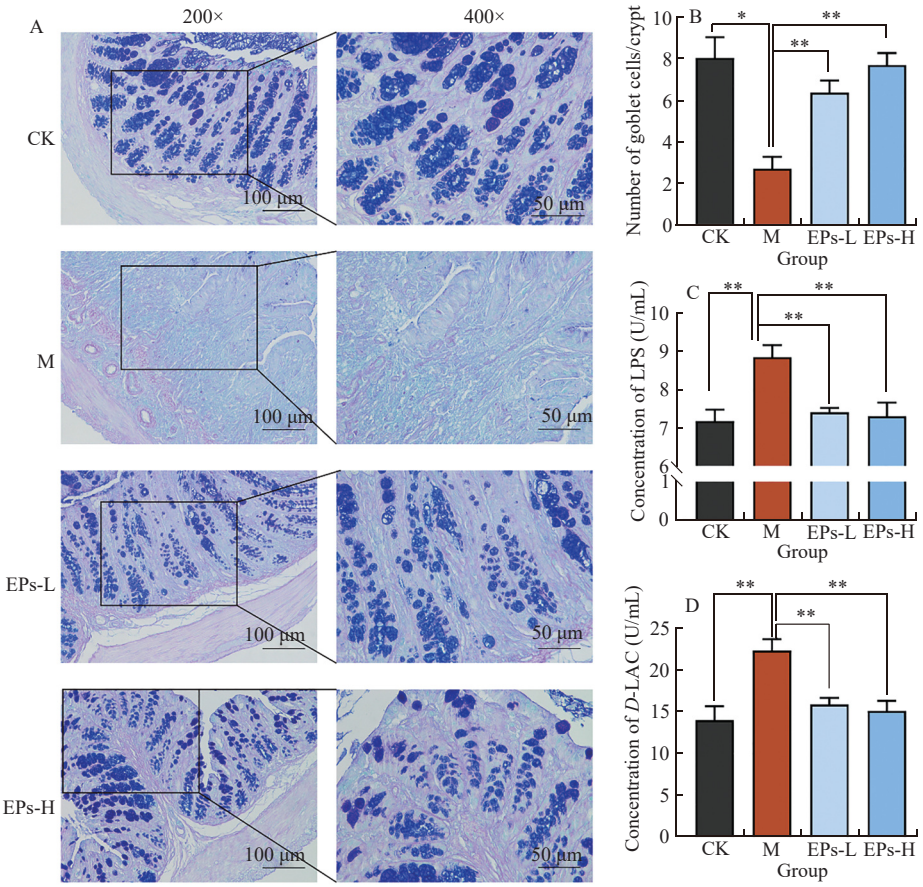

Fig. 3 Effect of EPs on goblet calls and intestinal permeability of young mice with colitis. (A) AB-PAS staining of colon tissue in each group. (B) Number of goblet cells in each group. (C) LPS level in mice serum. (D) *D*-LAC level in mice serum. **P* < 0.05; ***P* < 0.01 represent significance between each group compared with M group.

3.4 Modulation of EPs on amino acid concentration in the serum of young colitis mice

We further determined the serum amino acid concentrations to assess the effect of EPs on amino acid supplementation *in vivo*. As can be seen in Figs. 4A-C, compared with M group, the total amino acid (TAA) concentration, EAA, and nonessential amino acid (NEAA) in the serum of mice increased after EPs intake. Especially in the EPs-H group, the levels of TAA and EAA were even higher than those in the CK group. Afterwards, we further analyzed the level of lysine (Lys), tryptophan (Trp), and glutamine (Gln), which were altered markedly. As shown in the Figs. 4D and F, in comparison to the CK group, Lys and Trp concentration reduced caused by DSS, indicating a decrease in EAA related to metabolic and immune responses in colitis mice. After treatment by EPs, concentrations of these two amino acids significantly increased in the EPs-H group. In addition, the concentration of Gln significantly increased after intervention with EPs-H (Fig. 4E).

3.5 Protein functional annotation analysis

Considering the repair effect of EPs on the intestinal barrier of young mice, proteomics analysis on samples from the EPs-H group was carried out. The following "EPs" groups represent the EPs-H group. BLAST2GO (2.5.0) was applied for GO function annotation. Identified proteins are associated with cellular processes, metabolic process, and immune system process in the BP (Fig. 5A). MF mainly includes binding, molecular function regulator, and biological activity, such as catalytic activity, transporter activity and ATP dependent activity. The CC part mainly focuses on cellular anatomical entity and protein containing complexes.

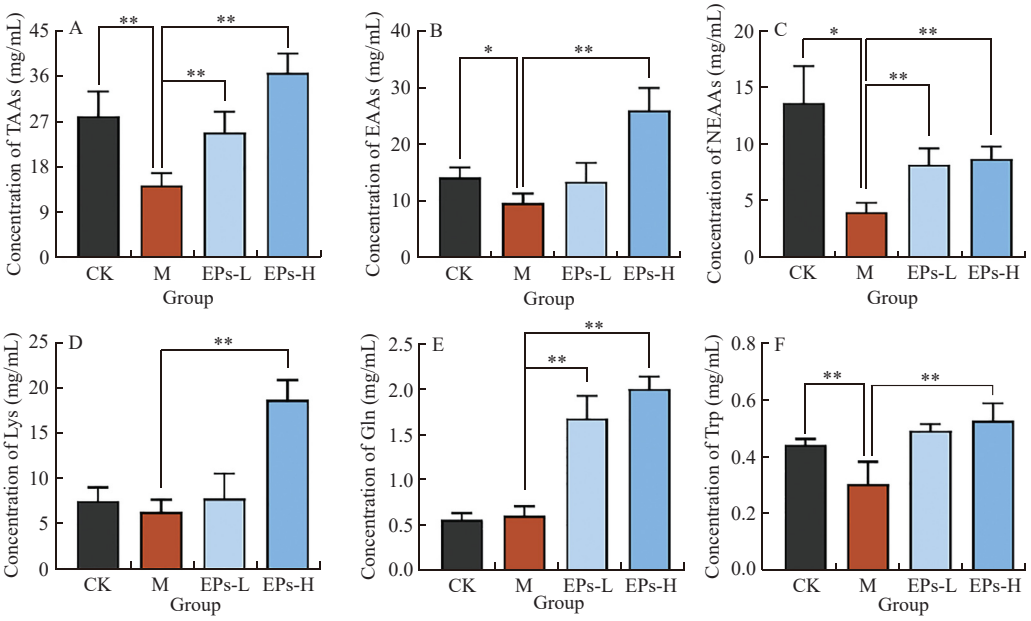

Fig. 4 Effect of EPs on serum amino acid concentration in young mice with colitis. (A) TAAs concentration. (B) Concentration of EAAs. (C) Concentration of NEAAs. (D) Lys concentration. (E) Gln concentration. (F) Trp concentration.

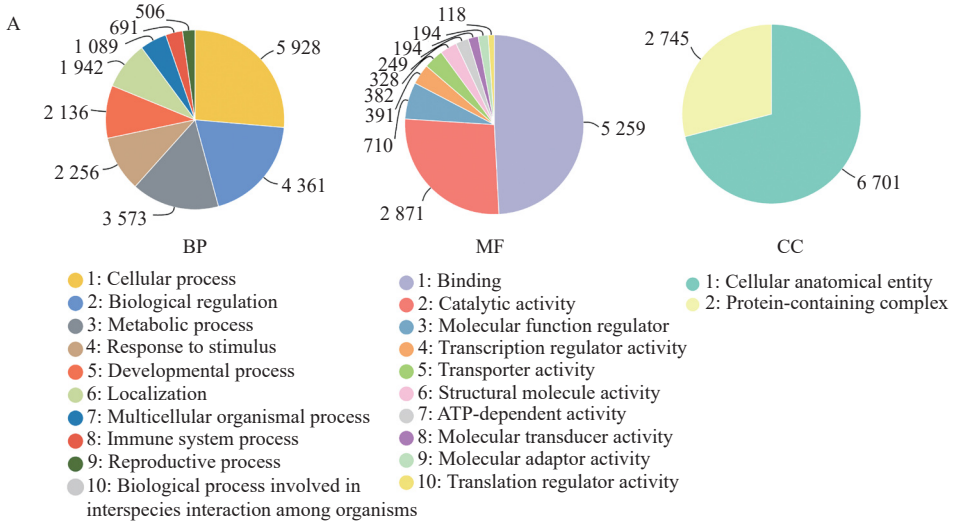

Fig. 5 Protein functional annotation analysis. (A) GO functional annotation of identified proteins in colon tissue. (B) KEGG functional annotation of identified proteins in colon tissue.

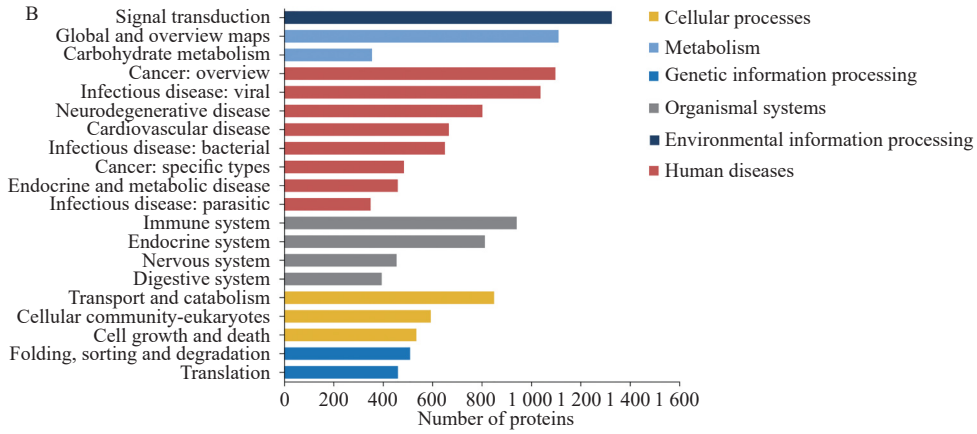

Fig. 5 (*Continued*)

Using KOBAS (2.1.1) for protein KEGG functional annotation analysis. The top 20 KEGG pathways was shown in Fig. 5B according to protein numbers. The highest ranking is signal transduction in environmental information processing, with 1 326 proteins involved. In addition, several human diseases-related pathways were also enriched, including infectious disease: viral and neurodegenerative disease, etc. The important signaling pathways annotated by KEGG above were further counted, and the top 20 pathways are listed (Table 1). In this study, the key pathways enriched in the samples mainly included metabolic pathways, inflammation regulation related pathways (phosphoinositide 3-kinase (PI3K)-protein kinase B (AKT) and mitogen-activated protein kinase (MAPK) signaling pathway), and viral infection. These pathways involved in inflammatory responses are usually interconnected, which was essential for intestine regulation.

Table 1
Top 20 important KEGG signaling pathways.

Pathway	Number of proteins
Metabolic pathways	1 106
Pathways of neurodegeneration-multiple diseases	529
Pathways in cancer	439
Amyotrophic lateral sclerosis	429
Alzheimer disease	363
Endocytosis	347
Human papillomavirus infection	341
Huntington disease	336
Prion disease	304
Proteoglycans in cancer	300
PI3K-AKT signaling pathway	299
Salmonella infection	292
Regulation of actin cytoskeleton	281
Focal adhesion	279
Parkinson disease	277
Human cytomegalovirus infection	273
Chemical carcinogenesis-reactive oxygen species	269
MAPK signaling pathway	263
Diabetic cardiomyopathy	260
Thermogenesis	259

3.6 Enrichment analysis of DEPs

The principal component analysis (PCA) showed that each sample is independent and the clustering degree between the same group is relatively high (Fig. 6A). Compared to the CK group, DSS intervention changed the composition and distribution of protein expression in the colon of young mice. The CK group was separated from the M group in the PC2 direction, indicating that the protein expression and composition in the colon would be markedly altered under DSS intervention. There are also differences between the EPs group and the M group, suggesting that intervention with EPs could alleviate the protein expression imbalance caused by UC. Fig. 6B showed that there are 309, 278 and 76 DEPs in the three sets, respectively. The volcano map showed that compared with the CK group, 155 DEPs were upregulated and 154 were downregulated in M group (Fig. 6C). After EPs intervention, 163 DEPs were upregulated and 115 DEPs were downregulated compared to the CK group (Fig. 6D). EPs intake upregulated 39 DEPs and downregulated 37 DEPs compared to the M group (Fig. 6E). This indicated that EPs can regulate the expression of specific proteins in colon tissue.

UC is a typical model of intestinal inflammation, and to further examine the pathways involved in inflammation regulation, immune system and amino acid metabolism and other related pathways in each experimental group, the KEGG pathways involved in above process were screened and the top 20 KEGG pathways in terms of protein number were analyzed. The results in Fig. 6F implied that the PI3K-AKT pathway was enriched with the most DEPs, followed by MAPK signaling pathway and TJ proteins. Therefore, KEGG enrichment analysis was performed on 299 proteins involved in the PI3K-AKT signaling pathway (Fig. 7A). The results indicated that proteins on the PI3K-AKT signaling pathway could be significantly enriched in multiple signaling pathways simultaneously, including MAPK signaling pathway and Ras signaling pathway. Thus PI3K-AKT signaling pathway is a key role in regulating intestinal barrier by EPs in young mice, and correlation heat map analysis was performed on 299 DEPs (Fig. 7B). The results showed that after intestinal disruption in young mice, the protein in cluster 1 was significantly upregulated in M group, and after intake of EPs, the protein expression returned to the same level as CK. Proteins with significant differences, such as Q9U10, Q99M47, Q05144, and P05144, participate in multiple signaling pathways (Fig. 7C). It further proves that PI3K-AKT signaling pathway is much important in repairing the intestinal barrier regulated by EPs.

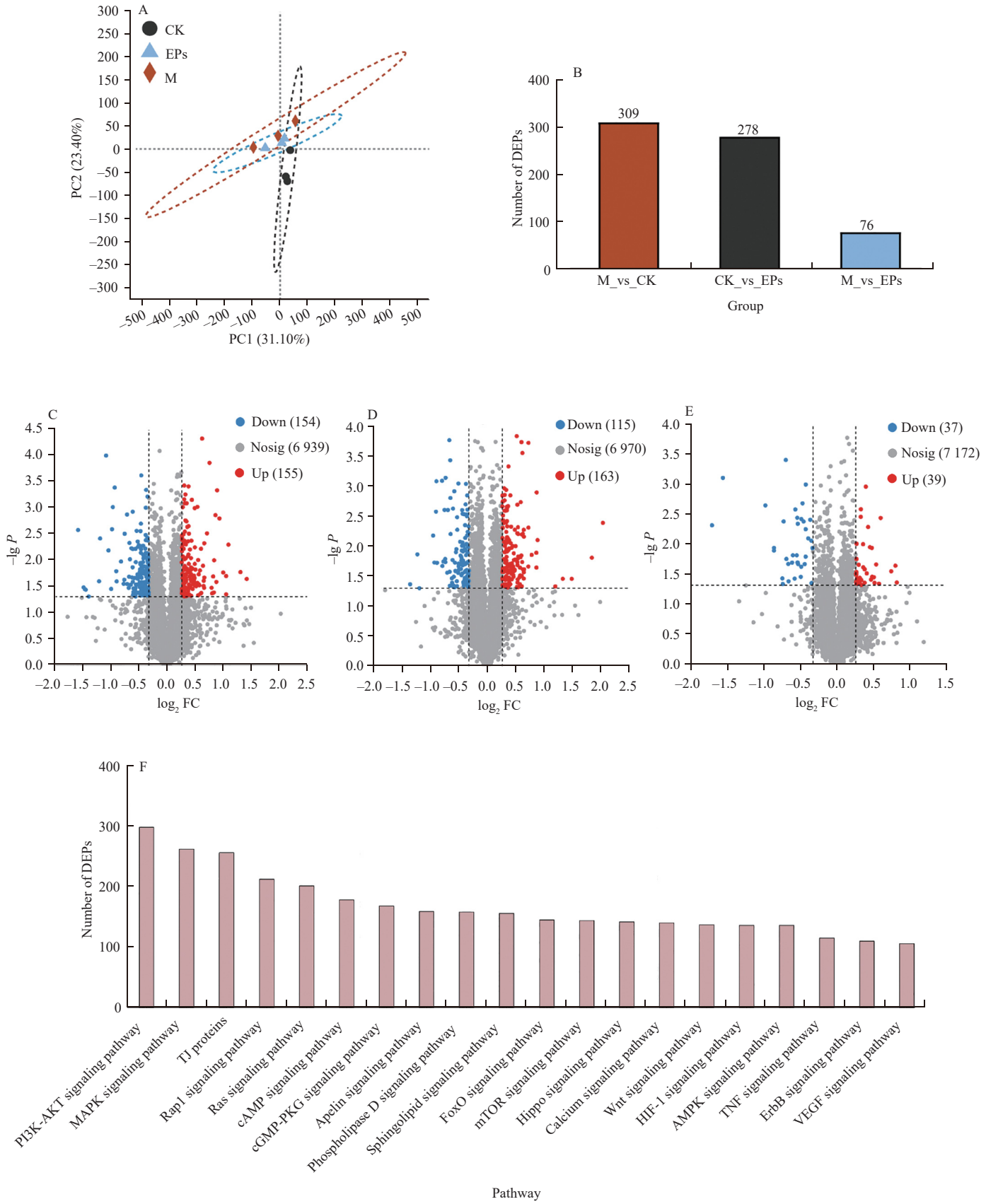

Fig. 6 Enrichment analysis of DEPs. (A) PCA in each group. (B) Number of DEPs identified in each comparative group. (C) Volcano map of M_vs_CK set. (D) Volcano map of CK_vs_EPs set. (E) Volcano map of M_vs_EPs set. (F) The top 20 pathways involved in inflammation regulation, immune system, and amino acid metabolism in terms of DEPs numbers.

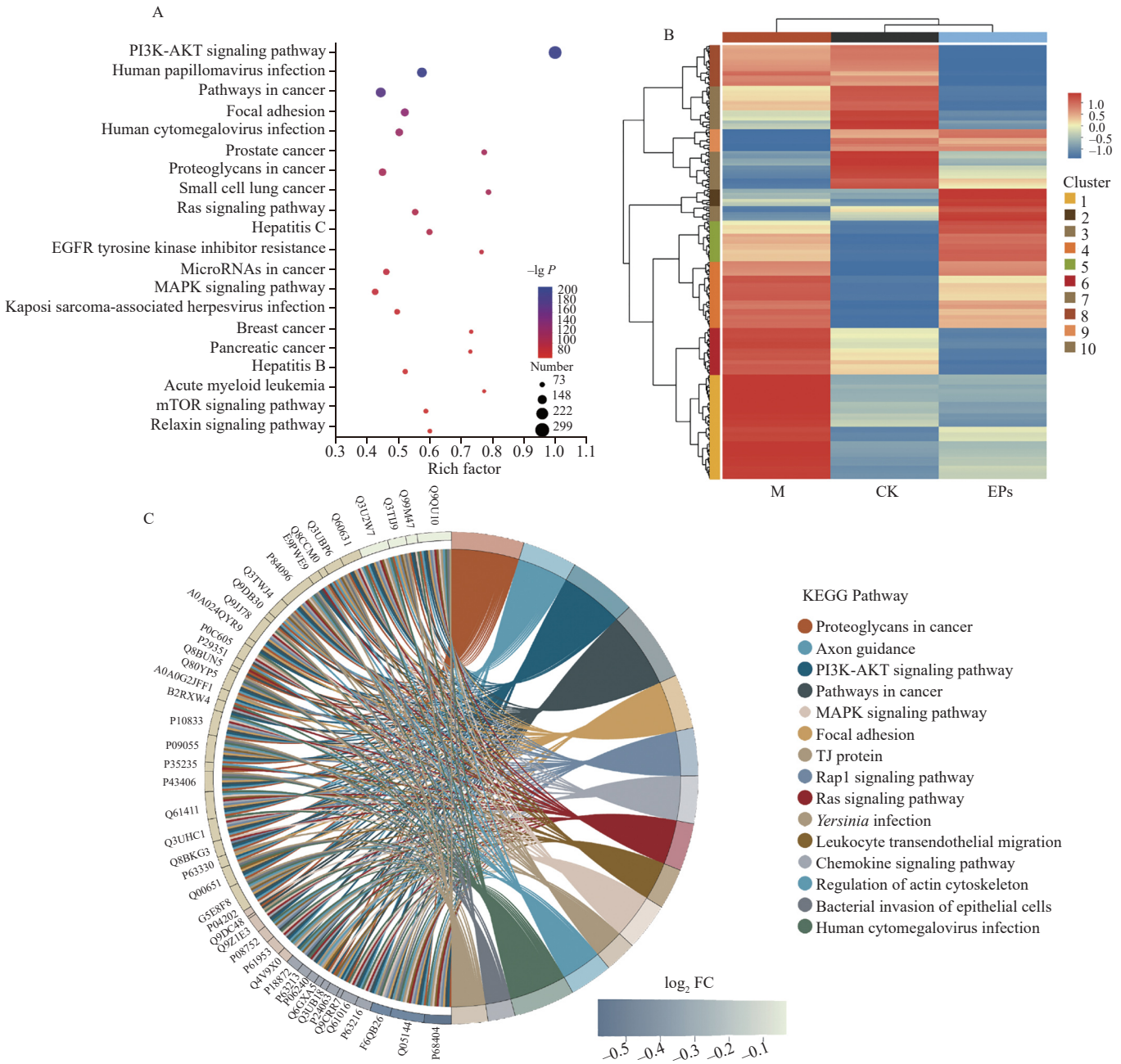

Fig. 7 Pathway analysis regulated by EPs. (A) KEGG enrichment analysis. (B) Heatmap of DEPs in each group. (C) The relationship between DEPs in PI3K-AKT cluster 1 and related signaling pathways.

4. Discussion

Chronic intestinal diseases such as colitis destroy the intestinal barrier in children and seriously affect their growth and nutrient intake. Egg white, as one of the natural high-quality protein sources, is of great advantage for intestinal tract[18,21]. DAI is an important indicator to evaluate the severity of colitis from three aspects: body weight, fecal status and bleeding[22]. EPs intake decreased DAI and increased colonic length. Fibrosis is the pathological and physiological repair mechanism of abnormal deposition of extracellular matrix in connective tissue after tissue injury[23]. Due to extensive inflammatory infiltration, these enlarged areas include many inflammatory cells. Excessive proliferation of fibroblasts and excessive deposition of submucosal collagen are also markers of intestinal fibrosis[24]. Our results showed that DSS-induced colitis would trigger intestinal fibrosis, which was similar to the finding of Wang et al.[15]. The intake of EPs could reduce collagen deposition and improve the pathological state of the colon. In addition, Ge et al.[14] also found that EPs reduced the levels of inflammatory cytokines, indicating the potential ability of EPs to prevent intestinal inflammation. TJ proteins regulate and enhance the selective permeation of the intestinal epithelial barrier to avoid causing abnormalities in the body's immune response[25]. Whereas the loss of TJ protein is the major cause leading to the disorder of intestine structure and balance induced by DSS[20]. The intake of EPs increased the TJ expression, thereby improving the intestinal barrier integrity. What's more, the mucus layer, as an

important component of the intestinal barrier, is the first line of defense for preventing bacteria and toxins from directly contacting the intestinal epithelium[26-27]. Mucin secreted by goblet cells is the main component of the mucus layer (chemical barrier), which contributes to the regulation of immune system of the body (activating immune cells, presenting antigens, and producing anti-inflammatory cytokines)[28-29]. The increment of goblet cells regulated by EPs is of great significance for enhancing the intestinal barrier function. When the intestine is damaged, LPS in the intestine can further promote inflammation. Increasing of LPS level would further exacerbate inflammation, which has been reported in the patients with irritable bowel syndrome[30]. Additionally, when the intestinal barrier is disrupted, *D*-LAC, as a metabolite of intestinal bacteria, would enter to the peripheral blood circulation and is one of the markers of intestinal permeability[31]. Overall, EPs intake reduced the intestinal permeability and improved the intestine structure of young colitis mice.

Amino acids are one of the main nutrients in the body. They are not only essential for maintaining the integrity of the intestine, but also promote the growth of gut microbiota[32]. It has been shown that diet rich in amino acids can affect the accumulation of dendritic cells in the intestinal lamina propria of mice with chronic colitis, prevent intestine dysfunction and maintain the intestine integrity[33]. Nutrient intake during the period of intestinal inflammation is crucial for the recovery of the intestine. After intervention with EPs, the TAA concentration in mouse serum significantly increased. Trp is the EAA in mammals, which involves various metabolic pathways under the action of gut microbiota and closely interact with the immune system. A diet containing Trp can reduce the mRNA expression of inflammatory cytokines, such as interleukin-6, interleukin-1β and tumor necrosis factor-α. This may be closely related to the Aryl hydrocarbon receptor[34]. There is evidence that Trp can regulate the TJ protein expression between intestinal cells and increase gut microbiota diversity[35]. Gln is the most abundant free AA in the host, which can directly or indirectly maintain the integrity of the intestinal barrier and intestinal function. Increasing the level of dietary Gln can also increase the concentration of other amino acids in the blood, such as aspartate, glutamic acid, and alanine[36]. Besides, supplementing Gln can help improve colitis symptoms, maintain normal intestinal structure and barrier function[37]. EPs significantly increased the level of Lys, Trp, and Gln *in vivo*. In summary, EPs, as a nutritional supplement, are expected to play an important role in the process of colon mucosal repair.

The development of proteomics technologies provides a new perspective on cellular processes in health and disease, offering the possibility to explore the deep molecular mechanisms involved in intestinal repair processes[38]. The results showed that EPs regulate the expression of proteins in the colon and that these differential proteins are involved in several BP such as cellular process and immune system process. The PI3K-AKT pathway plays a crucial role in the repair of the intestinal barrier, which is responsible for various cellular processes, such as survival, proliferation, differentiation, apoptosis, and autophagy[39-40]. Previous study has shown that PI3K-AKT signaling pathway are crucial for maintaining intestinal barrier function in infants and young children in terms of the immune regulation and anti-inflammatory response[41]. We have previously demonstrated through network pharmacology, bioinformatics, and molecular docking that the PI3K-AKT signaling pathway is a potential target in alleviating intestinal inflammation. Importantly, the egg white derived bioactive peptide AFKDEDTQ can bind to related target proteins[42]. It is reported that endotoxin inhibits intestinal cell migration by activating PI3K-AKT signaling pathway, resulting in obstruction of intestinal barrier recovery and development of neonatal necrotizing enterocolitis, indicating that PI3K-AKT is an important way to alleviate intestinal barrier function in infants and children[43]. Recent studies have shown that the peptide YFYPEL obtained from casein can alleviate intestinal inflammation by regulating PI3K-AKT signaling pathway, and enhance intestinal cell migration, indicating that animal derived protein derived peptides have the potential to prevent gastrointestinal diseases in children[41].

These EPs used in the article with identified sequences have been proven to have anti-inflammatory activity[44]. Except for peptides that may be fully absorbed, peptides are cleaved and degraded into smaller molecules or free amino acids after digestion in the gastrointestinal tract. These amino acids are involved in various physiological activities in the body, including inflammation regulation. However, the structure of EPs is diverse, and their anti-inflammatory function is closely related to molecular weight, charge, amino acid composition, peptide structure and sequence. In the future, it is necessary to further clarify the structure-activity relationship of EPs and the mechanism by which they exert biological activity.

5. Conclusion

This study demonstrated that additional intake of EPs can improve the growth retardation caused by colitis in young mice and restore the intestinal barrier function, mainly manifested in the recovery of mucin and TJ protein expression. In addition, the intake of EPs could increase the EAA and NEAA levels *in vivo*, which is of great significance for amino acid supplementation and energy supply during the intestinal barrier repair process in childhood. Proteomics showed that EPs can alleviate the imbalance of protein expression caused by colitis and restore the protein expression in the intestine, which involves multiple signaling pathways with PI3K signaling pathway as the core. In summary, the study provides a theoretical foundation for the development of EPs as functional foods and expands the application of bioactive peptides in the prevention of chronic diseases. In the future, the structure-activity relationship between EPs and target proteins should be further considered and investigated.

Conflict of interest

The authors declare no competing financial interest.

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