Research Square

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Proteomic analysis of tears and serum for dry eye disease using ovariectomized cynomolgus monkey

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Abstract

Background

Ovariectomized cynomolgus monkey 30 months after surgery was selected as the research object to identify protein changes in tears and serum to provide a reference for the diagnosis and pathogenesis of dry eye in menopausal women.

Methods

Six cynomolgus monkey were randomly divided into an experimental group and a control group (3 in each group). The experimental group underwent bilateral ovariectomy, while the control group underwent sham surgery with their ovaries reserved. Proteomic analysis was performed by LC-MS/MS on tears and serum collected from two groups. Differentially expressed proteins were identified and were performed cluster analysis, which included gene ontology, the Kyoto Encyclopedia of Genes and Genomes pathway and protein-protein interaction.

Results

33 differentially expressed proteins have been identified in tears and17 differentially expressed proteins have been identified in serum. Kyoto Encyclopedia of Genes and Genomes enrichment analysis in tears has discovered Glucagon signaling pathway and neurotrophin signaling pathway may play an important role in the pathogenesis of dry eye. Gene ontology enrichment analysis in serum has discovered insulin-like growth factor binding and growth factor binding in molecular function probably make effort in pathogenesis of dry eye. KEGG analysis in serum has discovered salivary secretion may be the key pathway in pathogenesis of dry eye.

Conclusions

Protein G7PCH4, Q2PG17 and G7PT55 in tears may be the key protein in pathogenesis of dry eyes. Protein G7P1T1, G7PUN9 and G8F302 in serum may play an important role in pathogenesis of dry eyes.

1. Background

Dry eye is a common ocular surface disease. Dry eye sufferers complain of dryness, “gritty”, burning, painful and photophobia. It has a significant impact on vision, quality of life, work efficiency and physical and mental health. Compared with 2007 International Dry Eye Workshop (DEWS)[1], the 2017 DEWS II emphasizes the loss of homeostasis of the tear film is the most prominent feature of dry eye[2].

The tear film is seen as a two-phase model in which the lipid layer covers the aqueous surface of the mucus[3]. The aqueous surface of the mucus contains at least 4 major mucins and over 1500 different proteins and peptides[4]. These proteins play an important role in maintaining tear film homeostasis. Main etiological causes of disrupting tear film homeostasis are instable and hypertonic tear film, ocular

surface inflammation and damage, neurosensory abnormalities[5, 6]. Although studies have shown that proteins in tears change when dry eye occurs[7], it has not been determined which proteins or changes in protein levels contribute to the diagnosis. Further research is needed on the changes of proteins in tear film to identify new biological markers that can be used to diagnose and even predict and treat dry eye.

The incidence of dry eyes is significantly correlated with sex and age[8, 9]. There is high dry eye rate among women in menopause period[10]. Several large studies have shown that a statistically significant age-adjustment increased the risk of dry eyes by at least 50% in women[11–13]. The prevalence of dry eye in menopausal women is much higher than that of young women[14, 15]. The sex-age-related difference in prevalence of dry eye is due in large part to different hormones in the body, such as sex steroids[16], hypothalamic-pituitary hormones[17] and insulin[18].

Among the known dry eye animal models, monkey, as a primate, is highly similar to human in terms of immunity and metabolism[19]. It can simulate the effects of human's physiological changes on the eye surface[20]. Our previous studies have shown that 24 months after surgery,the serum estradiol concentration in the experimental group declined which was consistent with woman menopause physiology and ocular surface changes were observed in the same period time[21]. We also found that high osmolarity was the trigger in pathogenesis of dry eye[21]. In this study, cynomolgus monkey 30 months after surgery was selected as the research object to identify protein changes in tears and serum to provide a reference for the diagnosis and pathogenesis of dry eye in menopausal women. We also conducted bioinformatic analyses, which included gene ontology (GO), the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and protein-protein interaction (PPI).

2. Materials And Methods

2.1 Animals

Six specific pathogen-free female cynomolgus monkeys originally obtained from JOINN Laboratories (Suzhou, China). The cynomolgus aged 4–6 years and weighing 2.9 kg to 4.0 kg were included in the study. JOINN Laboratories provides animals care according to standard operating protocol. Animal use followed the Guidelines on the Care and Use of Animals for Scientific Purposes governed by the State Scientific and Technological Commission. Animals were kept in a room with natural circadian rhythm and temperature 20 to 23 °C and humidity 60–80%. The animals were randomly divided into an experimental group and a control group (3 in each group).

2.2 Sex hormone-related dry eye model

2.2.1 Ovariectomy

All animals fasted for 12 to 20 hours before the surgery. Animals were anesthetized by intramuscular injection of ketamine at 10 mg/kg weight and intravenous injection of pentobarbital at 20 mg/kg weight. Animal was immobilized at supine position on the operating table and was exposed, shaved and

sanitized with anerdian. A 4- to 5-cm vertical incision was made along the midline of lower abdomen to access the abdominal cavity and exposed both ovaries and fallopian tubes. In the experimental animals, both ovaries were removed and fallopian tubes were ligated. In the control animals, both ovaries and fallopian tubes were left attached. All animals had their skin dressing changed three days after surgery and had stitches removed seven days after surgery. Then, keep the monkeys for 30 months.

2.3 Sample collection

Unstimulated tear fluid samples were collected using Schirmer tear test strips. The Schirmer tear test strips were placed in the lower conjunctival fornix of cynomolgus monkeys without anesthesia to collect tear fluid at least 30 mm. The strips were placed in 20 ml centrifuge tubes and stored at − 80 °C until analysis.

1.5 mL Venous blood were obtained from cynomolgus monkeys. The blood were placed in 5 mL sterile tubes and were left overnight at 0 °C. The next day, centrifuged the blood and took the upper serum to store at − 80 °C until analysis.

2.4 Sample preparation

The 10 μL serum was diluted with 30 μL buffer A(Agilent Technologies)to 40 μL. Then, filter with a 0.22 μm-filter. The sample were loaded at a flow rate of 0.125 mL/min and an outflow of 5 to 7.5 min was collected. Then, IAA solution was added to the sample to achieve a final concentration of 50 mmol/L. After the light-avoiding reaction for 40 minutes, the sample was centrifuged at 4℃ at a speed of 12000 rcf for 10 minutes. Added 200 μL 50 mmol /L of NH4HCO3 solution to the sample and centrifuged again at 4℃ at a speed of 12000 rcf for 10 minutes. Replace casing, add 100 μL 50 mmol/L NH4HCO3 solution into the ultrafiltration tube. Added trypsin in accordance with the mass ratio of trypsin to substrate 1:100, kept at 37 ℃ for 4 hours. Continue adding trypsin to sample and kept at 37 ℃ overnight (16 h). Centrifuged at 4 ° c at 12,000 rcf for 10 min, added 100 μL pure water, eddied it and centrifuged at 4℃ at a speed of 12000 rcf for 10 minutes and repeat operation. After desalting with self-filling desalting column, the sample was dried in vacuum at 45℃ for later use.

2.5 LC-MS/MS analysis

The peptides were dissolved in the sample solution (0.1% formic acid, 2% acetonitrile), and then fully oscillating in a vortex, centrifuged at 4℃ at 13200 rpm for 10 min for LC-MS/MS analysis. A 300 μm id × 5 mm id. Chromatographic column was employed. A step linear gradient of mobile phase B from 4 to 10% over 5-min, 10 to 22% for 80 min, 22 to 40% for 25 min, 40 to 95% for 1 min and 95% for another 9 min, at flow rate of 300 nL/min was collected. The separated peptide goes directly to the mass spectrometer (Ultimate 3000, Thermo Fisher Scientific, America) for on-line detection. Settings for mass spectrometer were as follows: for orbitrap, scan range(m/z) = 350–1800, Resolution = 30 k, AGC target = 4e6, Maxium IT = 50 ms, Spectrum data type = profile; for ion trap, Resolution = 17500, AGC target = 20000, Maxium IT = 80 ms. The MaxQuant software was used to search in the UniProt database, for the

protein data collected by the mass spectrometer. The retrieval parameters are as follows: Fixed modifications = Carbamidomethyl (C), Variable modifications = Oxidation (M), Enzyme = Trypsin, Maximum Missed Cleavages = 2, Peptide Mass Tolerance = 20 ppm, Fragment Mass Tolerance = 0.6 Da, Mass values = Monoisotopic, Significance threshold = 0.05.

2.6 Data analysis

2.6.1 Functional enrichments of all identified significant proteins and DEPs in tears and DEPs in serum

Discerning the roles of all identified significant proteins and differentially expressed proteins (DEPs) in tears and DEPs in serum, biological attributes including biological processes (BP), cellular components (CC), and molecular functions (MF) were extracted from GO enrichment analysis. Based on the identified protein ID, Unicus annotation was performed using OmicsBean, and the GO database annotation information of the corresponding protein was obtained from the Uniprot database by mapping. Next, functional annotation of proteins was performed using OmicsBean (http://www.omicsbean.cn) software. P-value□0.05 were considered statistically significant. GO enrichment was analyzed and displayed using histogram. The KEGG pathway enrichment analyses were performed in all identified significant proteins and DEPs in tears and DEPs in serum. The KEGG database is a database that systematically analyzes the metabolic pathways of gene products in cells and the function of these gene products. It integrates data on genomics, chemical molecules, and biochemical systems, including metabolic pathways, drugs, disease, gene sequences, and genomes. The use of this database facilitates the study of genes and expressions as a whole. The KEGG pathway annotation was based on Kobas 3.0 and displayed using histogram, pie chart and bubble chart.

2.6.2 PPI network construction of DEPs in tears and serum

In the current study, PPI network construction might provide insights into the mechanisms of beginning or development of diseases. PPI network and hierarchical clustering were constructed in DEPs in tears and serum. Hierarchical clustering represented the level of DEPs in tears and serum across 6 comparable samples with high expression samples marked in red and low in blue.

3. Results

After proteomic analysis, 33 DEPs have been identified in tears of these cynomolgus monkeys. 7 DEPs are up-regulated proteins in tears of experimental group and the other 26 DEPs are down-regulated. On the level of serum, 17 DEPs have been identified in these cynomolgus monkeys. 11 of them are up-regulated proteins and the other 6 are down-regulated ones.

3.1 GO enrichment assessment of all identified proteins in tears

Protein function annotation results of all identified proteins in tears were classified in Supplementary Fig. 1. GO (biologic process, cellular component and molecular function) analysis of all identified proteins in tears (Fig. 1). Top 10 significant enrichments of biologic process, cellular component and molecular function were shown in blue, red and yellow respectively. In the same category, the P values were all significant and increased from left to right. Changes in biologic processes were significantly enriched in the organonitrogen compound metabolic process. Changes in cellular components were mostly enriched in cytoplasm, intracellular and intracellular part. GO analysis results showed that changes in molecular function were mainly enriched in catalytic activity.

3.2 KEGG pathway assessment of all identified proteins in tears

The KEGG enrichment analysis of all identified proteins in tears was analyzed. Different KEGG pathway enrichment categories and subcategories were illustrated in different colors (Fig. 2A). The number after each column was the number of proteins in the category. KEGG pathways of all identified proteins in tears were also shown in pie chart (Fig. 2B) and bubble chart (Fig. 2C). Changes in KEGG pathway analysis were mainly in protein processing in endoplasmic reticulum, proteasome, neurotrophin signaling pathway, glycolysis, estrogen signaling pathway, Epstein-Barr virus infection and endocytosis.

3.3 GO enrichment assessment of DEPs in tears

The numbers of up-regulated and down-regulated DEPs in tears were shown in red and green respectively (Supplementary Fig. 2A). Protein function annotation results of DEPs in tears were classified in Supplementary Fig. 2B. GO (biologic process, cellular component and molecular function) analysis of DEPs in tears. Top 10 significant enrichments of biologic process, cellular component and molecular function were shown in blue, red and yellow respectively. In the same category, the P values were all significant and increased from left to right. Changes in biologic processes were significantly enriched in the actin filament-based process and carbohydrate metabolic process. Changes in cellular components were mostly enriched in intracellular part, cytoskeleton and cytoplasm. GO analysis results showed that changes in molecular function were mainly enriched in isomerase activity, intramolecular transferase activity, actin binding and intramolecular transferase activity.

3.4 KEGG pathway assessment of DEPs in tears

The KEGG enrichment analysis of DEPs in tears was analyzed. Different KEGG pathway enrichment categories and subcategories were illustrated in different colors (Fig. 4A). The number after each column was the number of proteins in the category. KEGG pathways of all identified proteins in tears were also shown in pie chart (Fig. 4B) and bubble chart (Fig. 4C). Changes in KEGG pathway analysis were mainly

in protein processing in Glucagon signaling pathway, neurotrophin signaling pathway, Rap1 signaling pathway.

3.5 PPI network establishment of DEPs in tears

We have constructed the PPI network of DEPs in tears. A network of the DEPs and their associated pathways was constructed (Fig. 5A). Hierarchical partitioning of DEPs in tears was set up visually (Fig. 5B). It represents the level of DEPs in tears across 6 comparable samples with high expression samples marked in red and low in blue.

3.6 GO enrichment assessment of DEPs in serum

The numbers of up-regulated and down-regulated DEPs in serum were shown in red and green respectively (Supplementary Fig. 3). GO (biologic process, cellular component and molecular function) analysis of DEPs in serum (Fig. 6). Top 10 significant enrichments of biologic process, cellular component and molecular function were shown in blue, red and yellow respectively. In the same category, the P values were all significant and increased from left to right. Changes in biologic processes were significantly enriched in the glucose metabolic process, hexose metabolic process and monosaccharide metabolic process. Changes in cellular components were mostly enriched in extracellular region. GO analysis results showed that changes in molecular function were mainly enriched in insulin-like growth factor binding and growth factor binding.

3.7 KEGG pathway assessment of DEPs in serum

The KEGG enrichment analysis of DEPs in serum was analyzed. Different KEGG pathway enrichment categories were illustrated in different colors (Fig. 7A). The number after each column was the number of proteins in the category. KEGG pathways of all identified proteins in serum were also shown in pie chart (Fig. 7B). Changes in KEGG pathway analysis were mainly in salivary secretion, glycosaminoglycan biosynthesis-keratan sulfate and glycosaminoglycan biosynthesis-lacto and neolacto series.

3.8 PPI network establishment of DEPs in serum

We have constructed the PPI network of DEPs in serum. A network of the DEPs and their associated pathways was constructed (Fig. 8A). Hierarchical partitioning of DEPs in serum was set up visually (Fig. 8B). It represents the level of DEPs in serum across 6 comparable samples with high expression samples marked in red and low in blue.

4. Discussion

Dry eye is a multifactorial disease with complicated pathophysiological process, various symptoms and causes. Dry eye is a disease of ocular surface, which including the cornea, conjunctiva, eyelids, tear film, lacrimal glands, and the meibomian glands[22]. The disorder of the function or injury of different parts

will cause the change of ocular surface state. Disrupted tear film homeostasis is the key link of the onset of dry eye[5]. Trouble is, there are also many underlying causes result in disrupted tear film homeostasis[23, 24]. The clinical symptoms of dry eye are various, from redness, painful, gritty eyes to visual impairment[25]. The difference between patients is huge. Therefore, dry eye is lack of a unified standard for diagnosis and classification which was a stumbling block for the diagnosis. Above all, it is urgent to identify effective biomarkers for diagnosis.

Proteomic analysis can provide theoretical basis of pathogenesis of disease. In the study, 33 DEPs have been identified in tears. 7 of DEPs in tears are up-regulated proteins and the rest are down-regulated proteins. Similarly, 17 differentially expressed proteins have been identified in serum. 11 of DEPs in serum are up-regulated proteins and the rest are down-regulated proteins. KEGG enrichment analysis in tears has discovered Glucagon signaling pathway and neurotrophin signaling pathway may play an important role in the pathogenesis of dry eye. Gene ontology (GO) enrichment analysis in serum has discovered insulin-like growth factor binding and growth factor binding in molecular function probably make effort in pathogenesis of dry eye. KEGG analysis in serum has discovered salivary secretion may be the key pathway in pathogenesis of dry eye.

Glucagon is a hormone that secreted by pancreatic cells. Its function is increasing blood glucose, by promoting glycogenolysis and gluconeogenesis. Glucagon regulates insulin and sustains glucose homeostasis in human body[26]. The glucagon binds to a G Protein Coupled Receptor (GPCR) in the cell membrane. Then, adenylate cyclase is activated to increase cAMP in cells which activates the protein kinase A to phosphorylate and activates enzymes in the target cell[27]. Protein G7PCH4 and Q2PG17 were involved in the pathway. First of all, high glucose is toxic to epithelial cells of meibomian gland[24]. The damage of meibomian gland is one of the important causes of dry eye[28]. Secondly, glucagon induced phosphorylation of insulin receptors. Insulin receptors were found in lacrimal and ocular surface tissue[29]. At the same time, animal studies have shown that insulin loss causes lacrimal gland shrinkage, corneal innervation, and decreased tear volume[30, 31]. In human, insulin resistance was observed with oral estrogen and progesterone replacement therapy in menopause women[32]. Glucagon signaling pathway play an important role in dry eye.

Neurotrophins is a family of proteins that regulates the differentiation and survival of nerve cells. It is activated by engaging special receptors, such as Trk tyrosine kinase receptors and p75 neurotrophin receptor[33]. The neurotrophin signaling pathway is regulated by connecting multiple intracellular signaling cascades[34]. Protein G7PT55 and Q2PG17 were involved in the pathway. Neurotrophin (e.g. NGF, GDNF) is one of the chemical mediators responsible for the inflammatory environment of the ocular surface[35]. Increasing sustained neural activity by reducing the stimulation threshold of sensory at nerve endings. At the same time, these neurotrophins are key reverse messengers that induce changes in gene expression[36]. Inflammatory environment of the ocular surface plays an important role in the pathogenesis and long-term course of DED and is a major driver of injury, and regeneration of peripheral sensory nerves[37].

Growth factor is a protein that stimulates cell growth, proliferation, healing, and differentiation. It's a signal molecule between cells. It works by binding to receptors[38]. Protein G7P1T1 and G7PUN9 were involved in both insulin-like growth factor binding and growth factor binding molecule function. Among them, insulin-like growth factor (IGF) has been studied in the pathogenesis of dry eye. The IGF-binding protein also known as a carrier protein for IGF-1. It mediates growth hormone (GH). The receptors of GH and IGF-1 and associated signaling pathways have been found in lacrimal and ocular surface tissues[39]. There is evidence that these hormones can affect tissue development and wound healing[40].

GH binds to a predimerized GH receptor on the cell membrane to activate the intracellular Janus-kinase 2 (JAK2) pathway, which can further activate Signal Transducers and Activators of Transcription (STATs) [29]. It regulates the transcription of GH, including IGF-1. GH induces cells to secrete IGF-1, which can enhance and supplement the effect of GH. IGF-1 signaling is initiated by binding to membrane bound insulin-like growth factor receptor (IGF-1R). IGF-1R phosphorylates and activates insulin receptor substrate (IRS)-1 which actives the cascade of phosphoinositide 3-kinase (PI3K)/Akt pathway. It is an important regulator of cell cycle progression and cell survival[41].

Studies have shown that GH/IGF-1 axis regulates the growth and function of the meibomian gland[42]. IGF-1 may be an autoimmune target in Sjogren's syndrome[18]. Sex hormones play an important role in regulating GH and IGF-1. There are a lot of kinds of growth factors. Markedly, nerve growth factor and its receptor levels are upregulated after ocular injury[43]. Epidermal growth factor and transforming growth factor were found elevated in the protein level of hydro-deficient dry eye.

Salivary secretion is a nerve-mediated reflex. It is stimulated by neurotransmitters released from autonomic nerve endings. Protein G8F302 was involved in the pathway. Sympathetic fibers are found surrounding the arteries and arterioles in the lacrimal stroma[44]. The sympathetic nerve relaxes the blood vessels in lacrimal gland with the purpose of changing the blood flow and increasing the secretion at the same time[45]. Sympathetic neurotransmitters can also directly induce the secretion, including proteins, electrolytes and water. Glycosylation of mucin was observed in dry eye disease[46]. Salivary acidification of MUC16 detected in patients with dry eyes is correlated with severity of the disease[47].

In this study, we constructed a dry-eye animal model of deovariated cynomolgus monkeys and performed proteomic analysis in the level of tears and serum. Protein G7PCH4, Q2PG17 and G7PT55 in tears may be the key protein in pathogenesis of dry eyes. Protein G7P1T1, G7PUN9 and G8F302 in serum may play an important role in pathogenesis of dry eyes. Similar pathways and molecular function have been found in human being. It may provide suspicious biomarkers for the diagnosis and reveal the potential pathogenesis of dry eyes in menopausal women. Notably, the differences between cynomolgus monkeys and menopausal women should be taken into consideration. Our study provides potential biomarkers in protein level and useful dry-eye animal models for the further study.

Abbreviations

DEWS: Dry Eye Workshop; GO: gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: pathway and protein-protein interaction; DEPs: differentially expressed proteins; BP: biological processes; CC: cellular components; MF: molecular functions; GPCR: G Protein Coupled Receptor; IGF: insulin-like growth factor; GH: growth hormone.

Declarations

Ethics approval: All experiments were approved by the Institutional Animal Care and Use Committee (ACU18-1006). Animal use followed the Guidelines on the Care and Use of Animals for Scientific Purposes governed by the State Scientific and Technological Commission.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interest.

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Authors' contributions: XXY and YX should be considered similar in author order of this manuscript, who conducted the experiments, analyzed the data, and drafted the article; YW and CL helped the completion of the experiment and the analysis of the data; XFZ as the correspondence author of this manuscript, designed the study and finally approved the version. All authors read and approved the final manuscript.

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Supplemental Figures

Supplementary Figure 1. Protein function annotation results of all identified proteins in tears.

Supplementary Figure 2. Protein function annotation results of DEPs in tears. (A) The numbers of up-regulated and down-regulated DEPs in tears were shown in red and green respectively. (B) Protein function annotation results of DEPs in tears were counted.

Supplementary Figure 3. The numbers of up-regulated and down-regulated DEPs in serum.

Figures

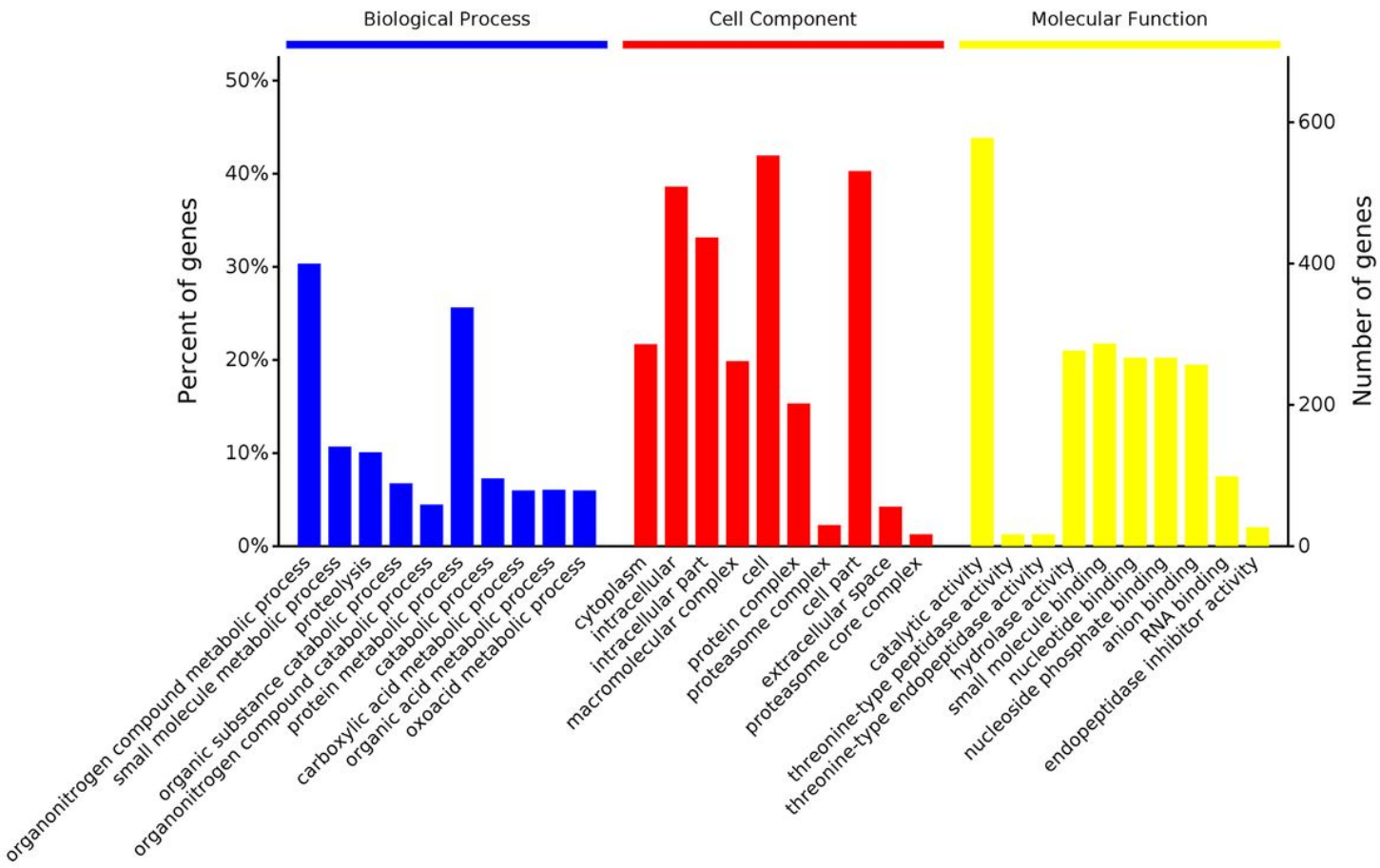


Figure 1

GO (biologic process, cellular component and molecular function) analysis of all identified proteins in tears. Top 10 significant enrichments of biologic process, cellular component and molecular function were shown in blue, red and yellow respectively. In the same category, the P values were all significant and increased from left to right. Changes in biologic processes were significantly enriched in the organonitrogen compound metabolic process. Changes in cellular components were mostly enriched in cytoplasm, intracellular and intracellular part. GO analysis results showed that changes in molecular function were mainly enriched in catalytic activity.

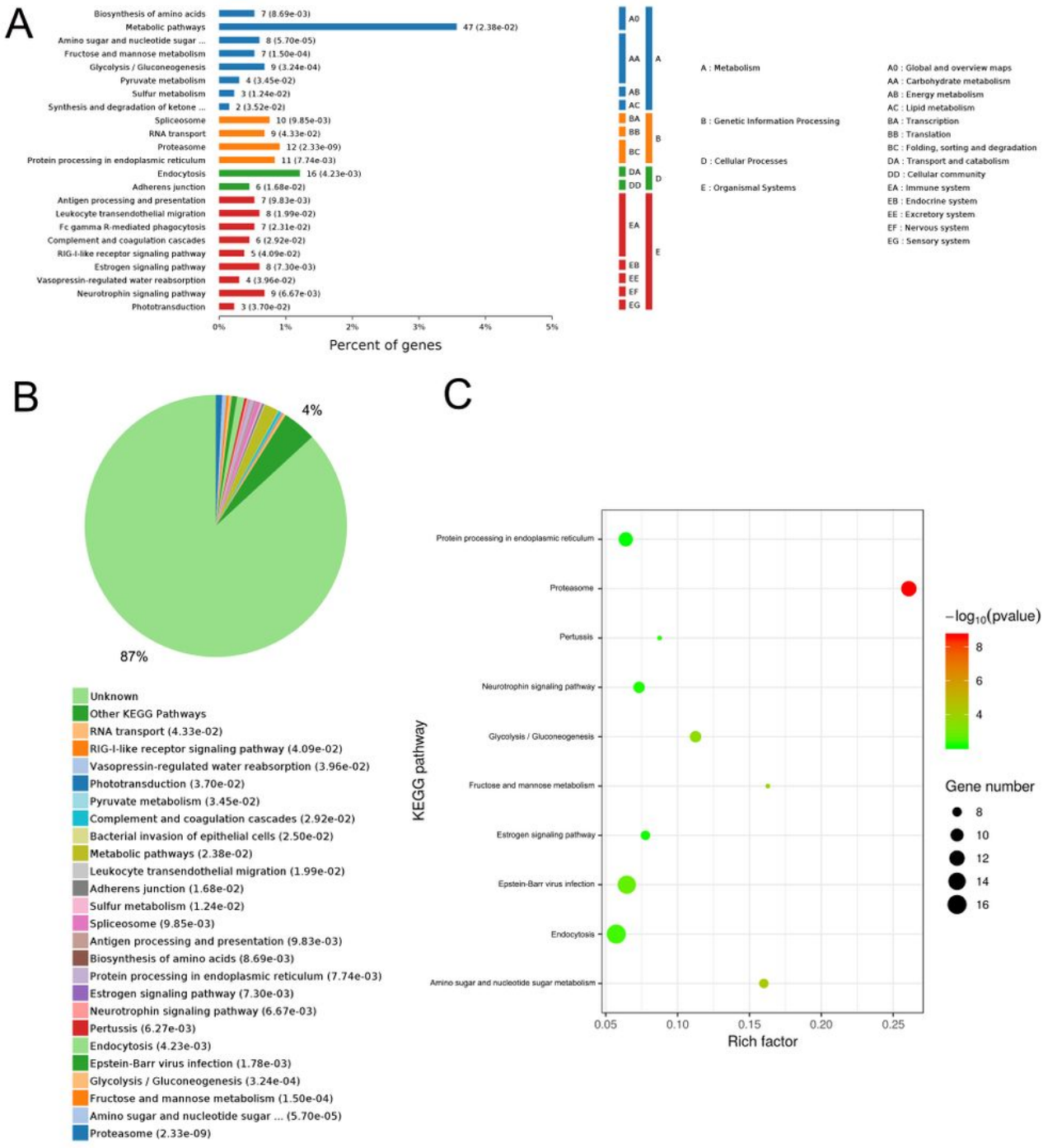


Figure 2

The KEGG enrichment analysis of all identified proteins in tears. (A) KEGG pathway enrichment categories. The abscissa was the percentage of genes enriched, and the ordinate was different enrichment categories and subcategories in different colors. The number after each column is the number of proteins in the category. (B) KEGG pathways of all identified proteins in tears in pie chart. (C) KEGG pathways analyses of all identified proteins in tears in bubble chart.

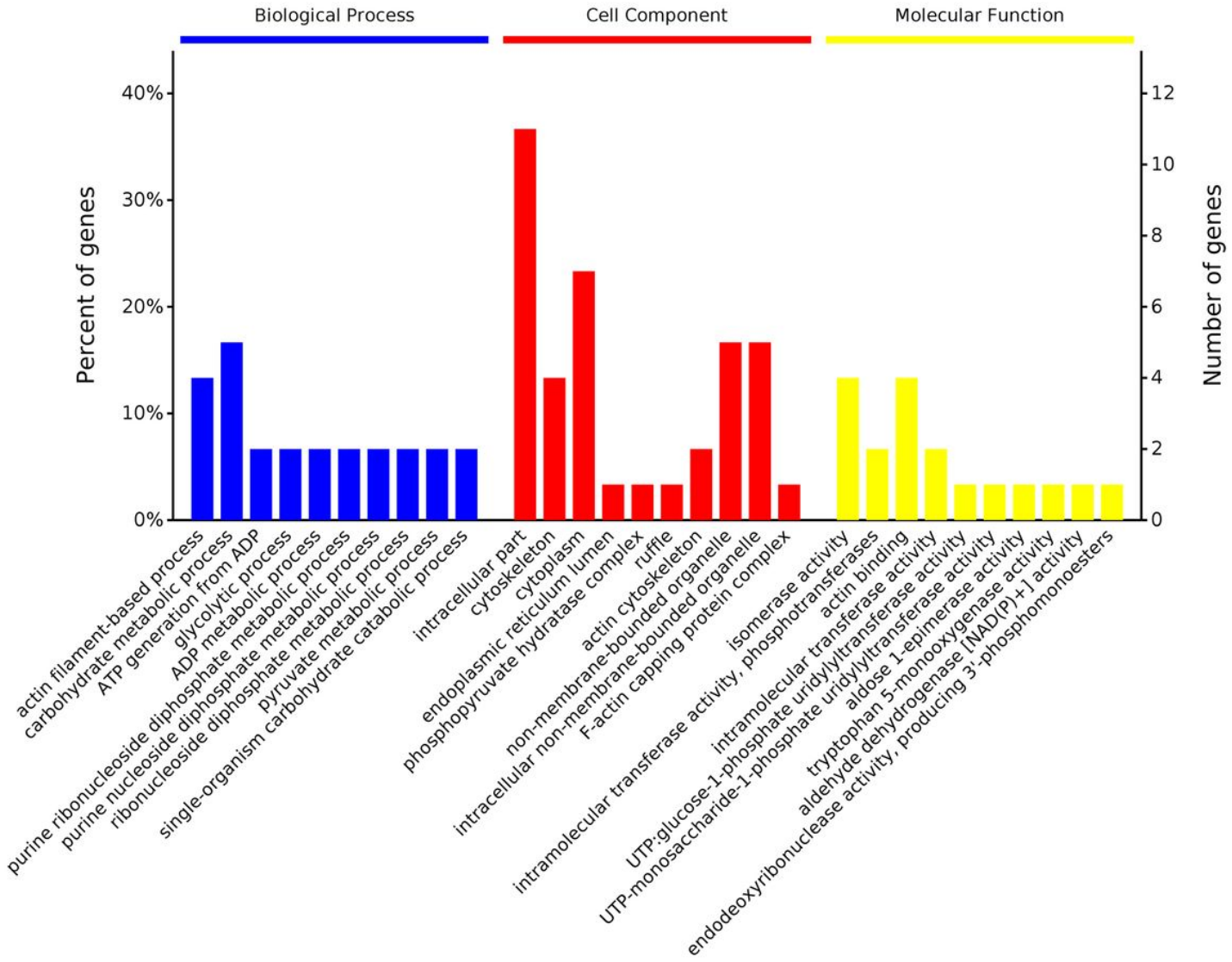


Figure 3

GO (biologic process, cellular component and molecular function) analysis of DEPs in tears. Top 10 significant enrichments of biologic process, cellular component and molecular function were shown in blue, red and yellow respectively. In the same category, the P values were all significant and increased from left to right. Changes in biologic processes were significantly enriched in the actin filament-based process and carbohydrate metabolic process. Changes in cellular components were mostly enriched in intracellular part, cytoskeleton and cytoplasm. GO analysis results showed that changes in molecular function were mainly enriched in isomerase activity, intramolecular transferase activity, actin binding and intramolecular transferase activity.

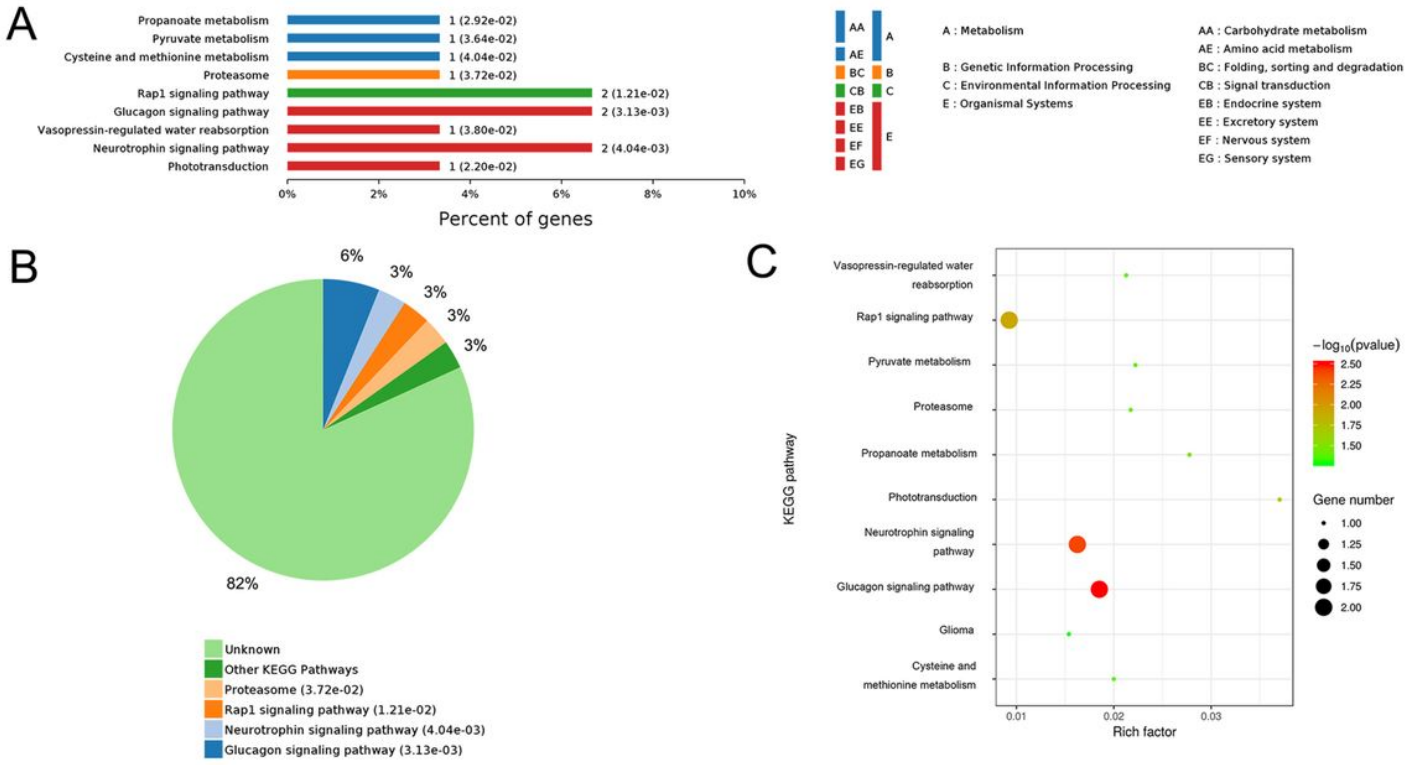


Figure 4

The KEGG enrichment analysis of DEPs in tears. (A) KEGG pathway enrichment categories. The abscissa was the percentage of genes enriched, and the ordinate was different enrichment categories and subcategories in different colors. The number after each column is the number of proteins in the category. (B) KEGG pathways of DEPs in tears in pie chart. (C) KEGG pathways analyses of DEPs in tears in bubble chart.

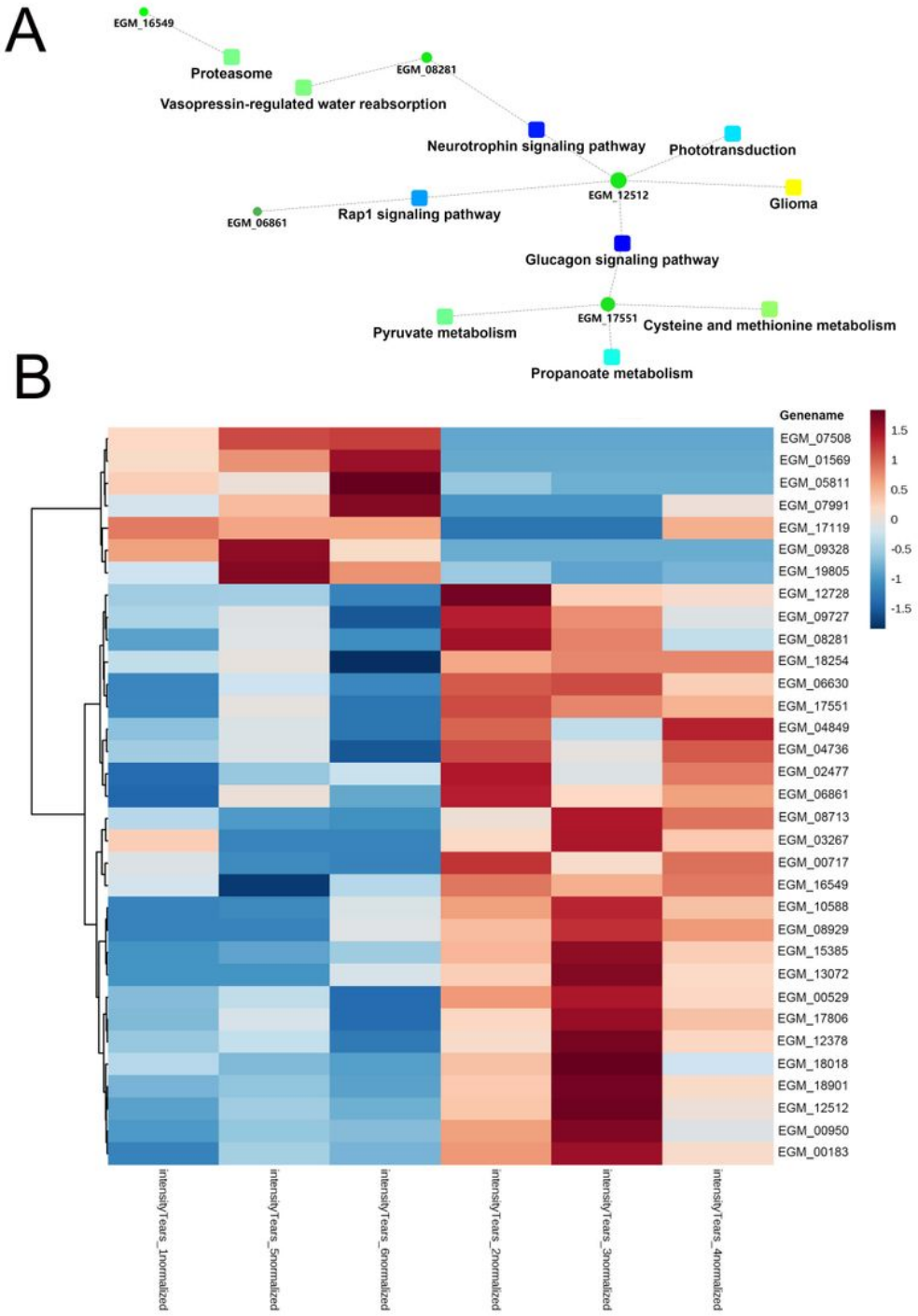


Figure 5

Functional annotations and hierarchical clustering of DEPs in tears. (A) The PPI network of DEPs in tears was constructed. (B) Hierarchical partitioning of DEPs in tears was set up visually. It represents the level of DEPs in tears across 6 comparable samples with high expression samples marked in red and low in blue.

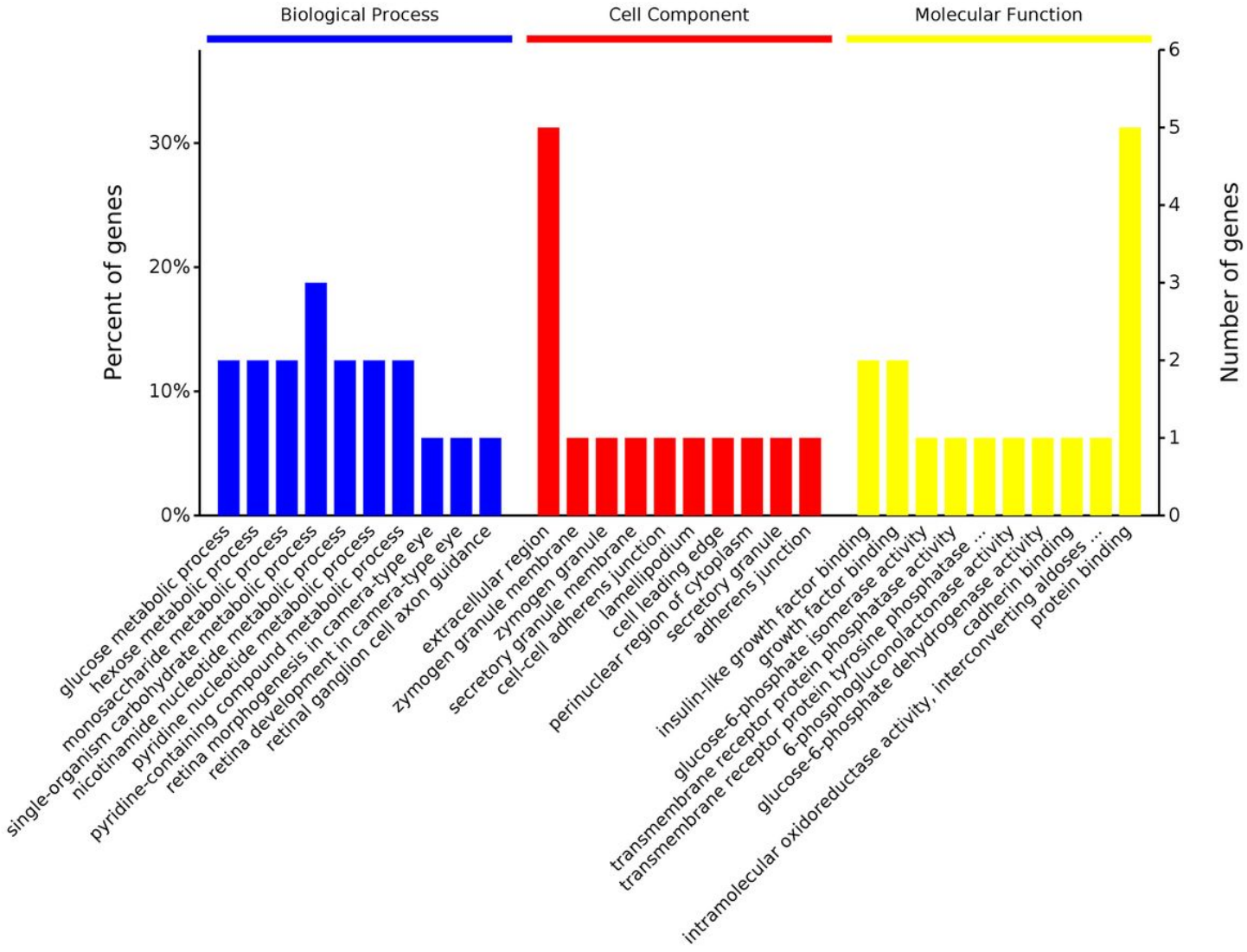


Figure 6

GO (biologic process, cellular component and molecular function) analysis of DEPs in serum. Top 10 significant enrichments of biologic process, cellular component and molecular function were shown in blue, red and yellow respectively. In the same category, the P values were all significant and increased from left to right. Changes in biologic processes were significantly enriched in the glucose metabolic process, hexose metabolic process and monosaccharide metabolic process. Changes in cellular components were mostly enriched in extracellular region. GO analysis results showed that changes in molecular function were mainly enriched in insulin-like growth factor binding and growth factor binding.

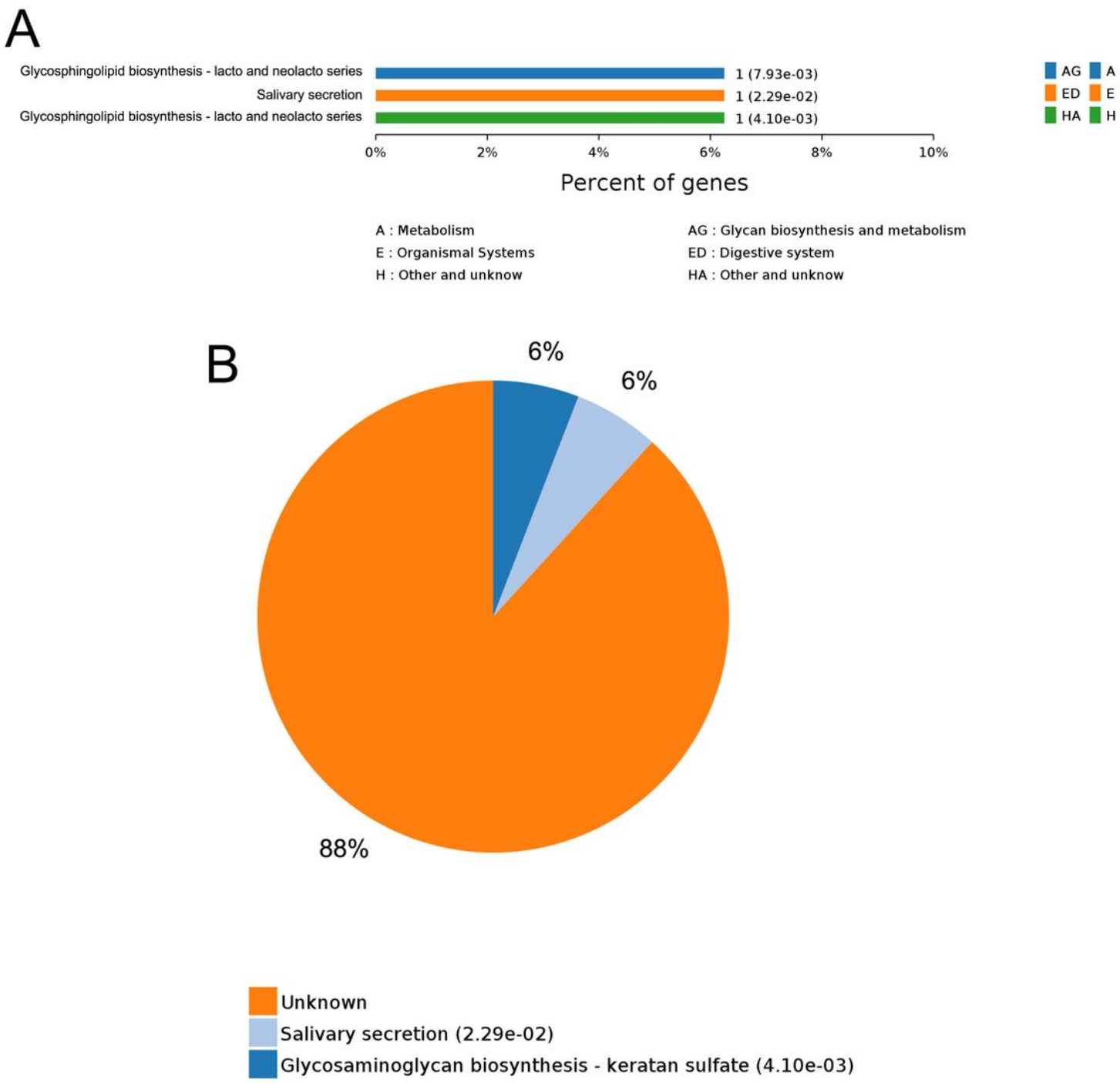


Figure 7

The KEGG enrichment analysis of DEPs in serum. (A) KEGG pathway enrichment categories. The abscissa was the percentage of genes enriched, and the ordinate was different enrichment categories in different colors. The number after each column is the number of proteins in the category. (B) KEGG pathways of DEPs in tears in pie chart.

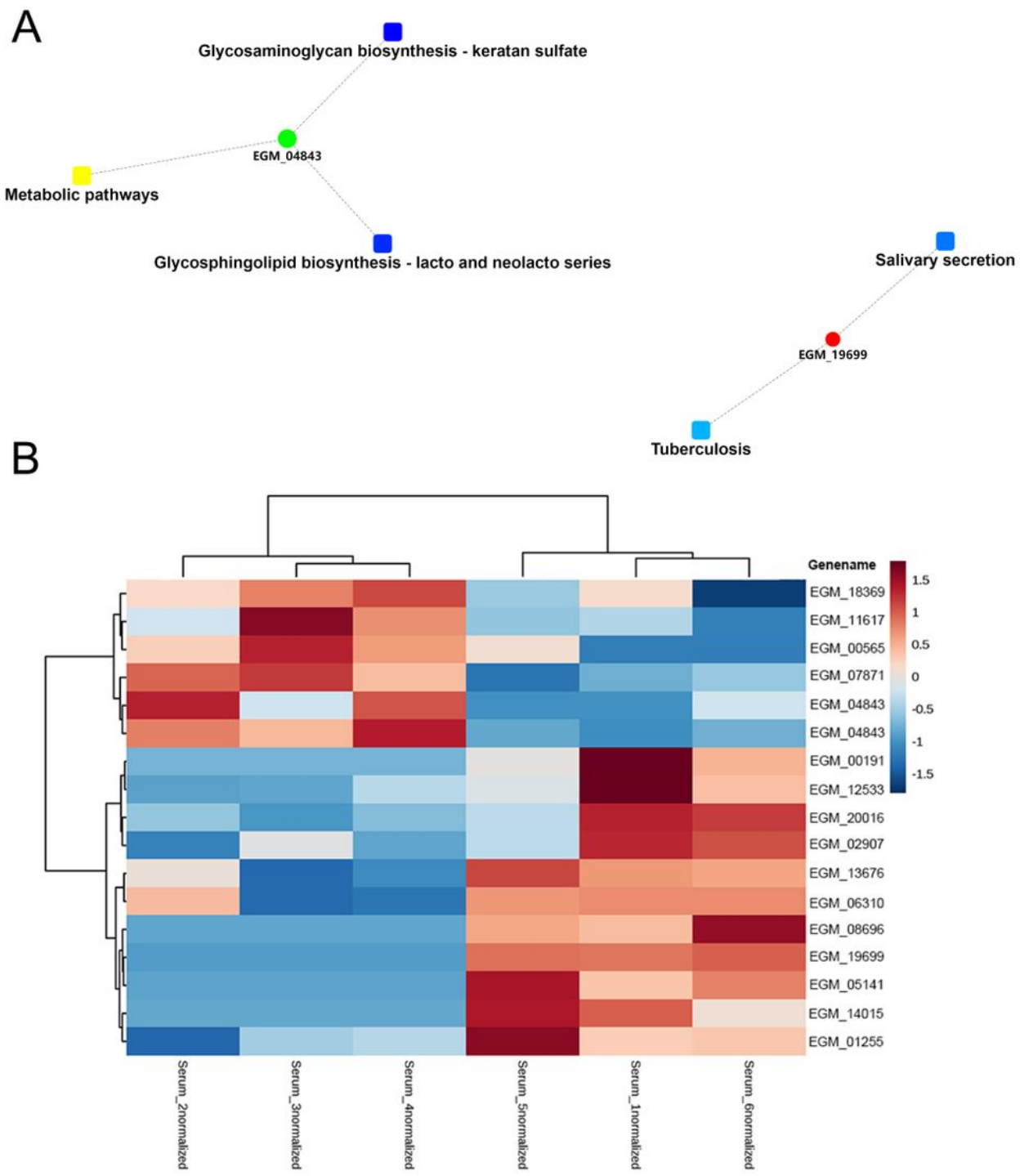


Figure 8

Functional annotations and hierarchical clustering of DEPs in serum. (A) The PPI network of DEPs in serum was constructed. (B) Hierarchical partitioning of DEPs in serum was set up visually. It represents the level of DEPs in serum across 6 comparable samples with high expression samples marked in red and low in blue.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfigure2.jpg
- Supplementaryfigure3.jpg
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