European Review for Medical and Pharmacological Sciences 2015; 19: 4275-4284

Bioinformatic analysis of potential candidates for therapy of inflammatory bowel disease

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Abstract. – OBJECTIVE: Inflammatory bowel diseases (IBDs) including ulcerative colitis (UC) and Crohn's disease (CD) increased the risk for developing colorectal cancer. However, there is no effective therapy for IBDs. The aim of this study was to identify potential therapeutic targets for inflammatory bowel disease (IBD) and explore the possible mechanism underlying this disease.

MATERIALS AND METHODS: Gene expression profile GSE6731 was downloaded from Gene Expression Omnibus database, which included 9 UC samples and 19 CD samples. Differentially expressed genes (DEGs) between affected colon tissues and non-affected tissues were identified in UC and CD group. Then, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis of DEGs were performed. Modules in the protein-protein interaction (PPI) network were identified, and significant node genes were selected.

RESULTS: Total 619 DEGs including 285 up-regulated genes and 334 down-regulated genes were identified in UC group and total 1159 DEGs of CD including 585 up-regulated genes and 574 down-regulated genes were selected. Module was selected from PPI network. From the PPI network and module, DEGs of mitogen-activated protein kinase 3 (*MAPK3*), N-myc downstream regulated 1 (*NDRG1*) and major histocompatibility complex, class II, DR alpha (*HLA-DRA*) have high degree.

CONCLUSIONS: *MAPK3*, NDRG1 and *HLA-DRA* may play key roles in the progression and development of IBD. They may be used as specific therapeutic targets in the treatment of IBD. However, further experiments are still needed to confirm our results.

Key Words:
Inflammatory bowel disease, Ulcerative colitis, Crohn's disease, Differentially expressed genes, Protein-protein interaction network, Modules.

Introduction

Inflammatory bowel disease (IBD) belongs to the type of autoimmune diseases, which are the inflammatory conditions of the small colon and intestine[1]. The principal types of IBD are ulcerative colitis (UC) and Crohn's disease (CD)[2]. It is important to note that IBD affect the intestine, oesophagus, mouth, anus and stomach[3-5]. Although CD and UC are different diseases, they may present with common symptoms, such as abdominal pain, rectal bleeding, diarrhea, severe internal muscle spasms, vomiting, and weight loss[6]. There is an increasing trend of patients with IBD and IBD is a risk factor for the development of cancers such as colorectal cancer, endothelial dysfunction and coronary artery disease[7,8]. It is estimated that IBD related deaths will be up to 34,000 in 2010 globally[9]. Therefore, it is essential to develop effective methods for its treatment.

IBD have posed threat to public health, however, CD and UC are not medically curable[10-13]. Until now, the most effective way to treat IBD is surgery, but surgery cannot cure IBD completely[14]. Recently, an increasing number of studies have focused on the treatment for IBD[15]. Antibody against interleukin-12 is proposed to be a promising method for remising Crohn's disease[16]. Probiotics shows therapeutic effects on CD and UC[17]. Besides, it is suggested that ICAM-1 is a potential therapeutic target in the treatment of IBD[18,19]. The epithelial barrier gene extracellular matrix protein 1 (*ECM1*) was found to have an association with UC and it may be helpful in the treatment of UC[20]. Although tremendous efforts have been made to discover the treatment of IBD, the present knowledge seems to be insufficient.

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In this study, we downloaded the IBD related microarray data for further analysis. The differentially expressed genes (DEGs) between affected colon tissues and non-affected tissues of UC and CD patients were identified respectively. The DEG related function and pathways were analyzed and the protein-protein interaction network for the common DEGs in UC and CD patients was constructed. In the present work, we aimed to explore the possible molecular mechanism and the potential therapeutic targets for IBD.

Materials and Methods

Affymetrix Microarray data

The gene expression profile data GSE6731 was downloaded from Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). The microarray data were generated based on the platform of GPL8300 ([HG_U95Av2] Affymetrix Human Genome U95 Version 2 Array) and was deposited by Wu et al[21] in GEO database. The gene expression profiling includes 19 CD samples (7 affected colon specimens and 12 adjacent biopsies) and 9 UC samples (5 affected biopsies and 4 unaffected biopsies). The Affymetrix CEL files were downloaded for further analysis.

Identification of Differentially Expressed Genes

The raw data (CEL files) were firstly preprocessed based on the RMA (robust multi-array average) algorithm[22] by using the Affy package[23] in R language. The preprocessing process included background adjustment, quantile normalization, final summarization and log2 transformation. Then, the probes corresponding to multiple genes were deleted and the Coefficient of variation (C.V)[24] was calculated for genes mapped to multiple probes.

$$C.V = \frac{SD}{MN} \times 100\%$$

SD indicates the standard deviation and MN represents the mean value. Genes with C.V > 20% of more than 20% samples were considered to be the missing data and were deleted. For the remaining genes mapped to multiple genes, the mean gene expression value were calculated.

Then, DEGs between affected colon tissues and no-affected tissues were analyzed in UC and CD group respectively by using samr package in R[25]. The multiple testing correction was performed using Beniamini-Hochberg (HB) method[26]. DEGs with false discovery rate (FDR) ≤ 0.05 were considered to be significant.

Hierarchical Clustering Analysis

The DEGs in UC and CD group were subjected to hierarchical clustering analysis with the application of GenePattern (www.broadinstitute.org/cancer/software/gene/pattern)[27], and the results were shown in heat maps. Samples clustered based on the gene expression value can help us determine whether the screening have sample specificity or not.

Gene Ontology and Pathway Enrichment Analysis of DEGs

The Gene Ontology (GO) analysis is a commonly used method for functional studies of large-scale genomic or transcriptomic data[28]. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database[29] contains information of how molecules or genes are networked. Database for annotation visualization and integrated discovery (DAVID)[30] was used to systematically extract biological meaning from large gene or protein lists. GO function and KEGG pathway of DEGs were analyzed using DAVID 6.7 with FDR ≤ 0.05.

PPI Network and Module Analysis

The protein-protein interaction (PPI) pairs were predicted based on the information from Biomolecular Interaction Network Database (BIND)[31], Biological General Repository for Interaction Data sets (BioGRID)[32], Database of Interacting Proteins (DIP)[33], Human Protein Reference Database (HPRD)[34], IntAct[35], Molecular IN Teraction database (MINT)[36], mammalian PPI database of the Munich Information Center on Protein Sequences (MIPS)[37], a PPI database for PDZ-domains (PDZBase)[38] and Reactome[39].

Then the DEGs in both UC and CD group r were mapped to PPI network. Visualizing complex networks and integrating these networks to any type of attribute data were allowed by Cytoscape (http://cytoscape.org/)[40]. The clusterMaker 1.11[41] plugin in Cytoscape and markov cluster (MCL) algorithm[42] were used to screen modules (granularity parameter = 2) of the PPI network.

Results

Identification of Differential Expression Genes

After preprocessing, we obtained the gene expression profiles of 8036 genes in CD group and 7876 genes in UC group. Finally, we obtained 619 DEGs (285 up-regulated genes and 334 down-regulated genes) in UC group and 1159 DEGs (585 up-regulated genes and 574 down-regulated genes) in CD samples. Heat map of DEGs

Hierarchical clustering showed that the DEGs in UC and CD subgroup could distinguish the affected tissues and non-affected tissues clearly (Figure 1).

Gene Ontology and Pathway Enrichment Analysis of DEGs

In UC group, the DEGs were closely related with biological processes such as oxidation reduction, response to organic substance and phosphate metabolic process (Figure 2A). The DEGs in CD group were significantly enriched in biological processes such as intracellular signaling cascade, response to organic substance and regulation of cell proliferation (Figure 2B).

Pathway analysis showed that the DEGs in UC were significantly enriched in complement and coagulation cascades and oxidative phosphorylation (Figure 3A) and the DEGs in CD group were significantly enriched in proteasome and apoptosis pathway (Figure 3B).

Module Screening from the PPI Network

There were total 253 overlapped DEGs in both UC and CD group. The 253 DEGs were mapped to PPI network and visualized by Cytoscape software. The PPI network with 2016 nodes and 2658 edges was shown in (Figure 4 A). With the application of MCODE plugin of Cytoscape, we obtained 6 modules and the significant module was listed in Figure 4B. Genes of major histocompatibility complex, class II, DR alpha (HLA-DRA) and CD74 molecule, major histocompatibility complex, class II invariant chain (CD74) were included in the module 1 (Figure 4B).

The significant nodes of PPI network with connective degrees ≥ 74 were screened, including mitogen-activated protein kinase 3 (*MAPK3*), v-yes-1 Yamaguchi sarcoma viral related oncogene homolog, and N-myc downstream regulated 1 (*NDRG1*).

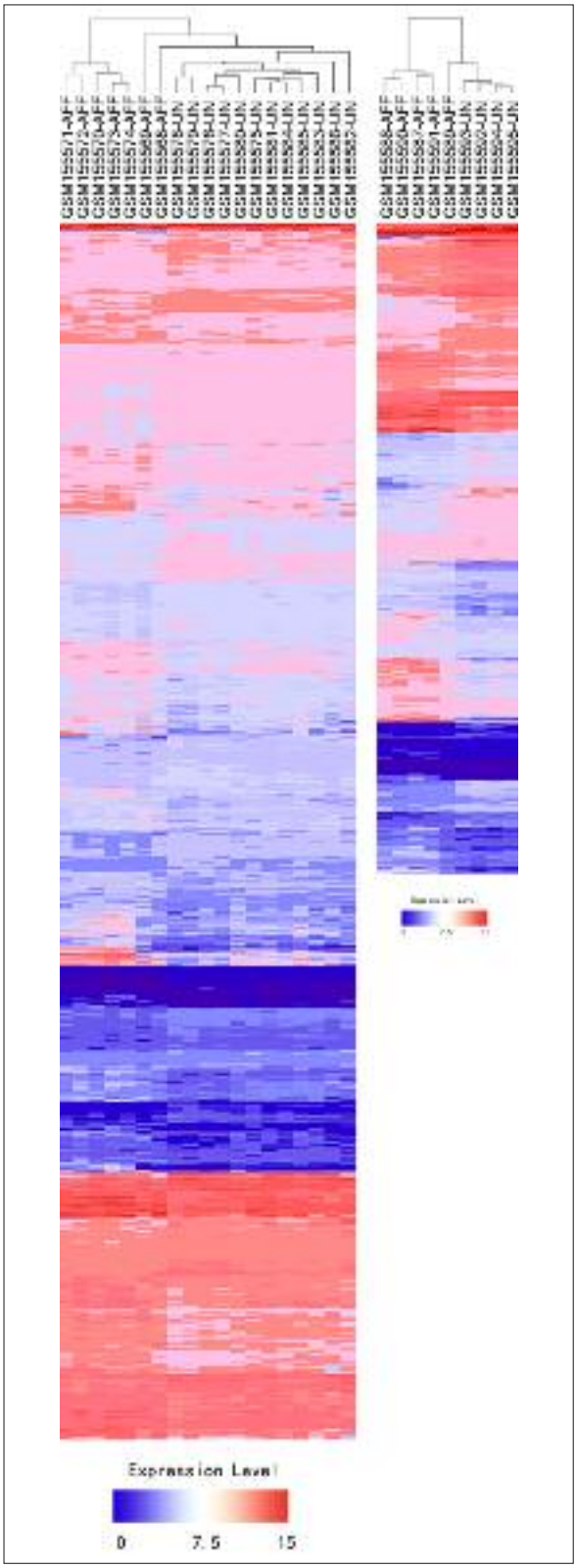

Figure 1. Heatmap of DEGs in UC and CD respectively. ***A,*** Heatmap of DEGs in CD. ***B,*** Heatmap of DEGs in UC. Red colors represent up-regulation; blue colors represent down-regulation.

Table I. The KEGG pathway of significantly differentially expressed gene in UC and CD.

Subtype	Term	Count	FDR
UC	hsa00650: Butanoate metabolism	12	1.60E-05
	hsa05012: Parkinson's disease	22	8.45E-05
	hsa00071: Fatty acid metabolism	12	9.52E-05
	hsa00190: Oxidative phosphorylation	22	0.000109
	hsa00280: Valine, leucine and isoleucine degradation	12	0.000258
	hsa05010: Alzheimer's disease	24	0.000399
	hsa05016: Huntington's disease	24	0.001909
	hsa00620: Pyruvate metabolism	10	0.003503
	hsa00072: Synthesis and degradation of ketone bodies	5	0.011888
	hsa00020: Citrate cycle (TCA cycle)	8	0.016261
	hsa04610: Complement and coagulation cascades	12	0.017288
	hsa00010: Glycolysis/Gluconeogenesis	11	0.019964
CD	hsa03050: Proteasome	16	0.000291
	hsa00071: Fatty acid metabolism	14	0.000825
	hsa04062: Chemokine signaling pathway	35	0.003025
	hsa04612: Antigen processing and presentation	18	0.029413
	hsa04210: Apoptosis	18	0.04889

Discussion

IBD has affected the quality of people's lives worldwide[43]. Moreover, IBD is a risk factor for the development of colorectal cancer, however, there is no effective method in the treatment of IBD until now[10-13]. Thus, the potential use of therapeutic targets appears to be the most promising area of research. In this work, we used bioinformatic approach to predict the potential therapeutic targets for IBD. We have identified 619 DEGs including 285 up-regulated genes and 334 down-regulated genes in UC group and total 1159 DEGs including 585 up-regulated genes and 574 down-regulated genes in CD group were selected. By constructing PPI network and module screening, we found key genes including *MAPK3*, *NDRG1* and *HLA-DRA*.

MAPK3 is a member of the MAP kinase family[44]. MAP kinases act in a signaling cascade that regulates many cellular processes such as differentiation, proliferation, adhesion, survival and cell cycle progression through the regulation of transcription, translation, cytoskeletal rearrangements[45]. Studies[46] also showed that MAPK played a role in the regulation and initiation of mitosis, meiosis and postmitotic functions in differentiated cells. Dysregulation of MAPK kinase pathways associated with diseases such as cancer, neurodegeneration and inflammation[47]. Santini et al[48] showed that many genes involved in cell adhesion, cell cycle control and DNA repair can be methylated in colon cancer. Furthermore, Lengauer et al[49] showed that dysregulation of cell cycle was related to colorectal cancer. Baba et al[50] showed that MAPK3 was related to colorectal cancer. In our study, MAPK3 was the overlapping DEG in UC and CD, and it has high degree in the PPI network. In a word, all these above suggested that MAPK3 might be a therapeutic target in IBD.

NDRG1 is a cytoplasmic protein involved in hormone responses, stress responses, differentiation and cell growth[51]. NDRG1 plays an important role in p53-mediated caspase apoptosis and activation[52]. Chua et al[53] showed that the expression of NDRG1 might be a prognostic indicator for some cancers. It was reported[54,55] that colon cancer is related to apoptosis, differentiation and cell growth. Furthermore, studies[56,57] also showed that NDRG1 was an indicator of poor prognosis in colon cancer. In our study, NDRG1 was the overlapping DEG in UC and CD group, and it has high degree in the PPI network. Thus, NDRG1 may be a therapeutic target for IBD.

HLA-DRA is a member of the HLA class II alpha chain paralogues[58]. HLA-DRA was demonstrated to play a key role in the immune system through presenting peptides originated from extracellular proteins[59]. It is reported that HLA-DRA play a role in various diseases such as Graham Little-Piccardi-Lasseur syndrome, and allergic encephalomyelitis[60]. Studies showed that IBD belonged to the type of autoimmune diseases and it was related to the imbalance of immune system[1]. Furthermore, studies showed that the abnormal change of HLA

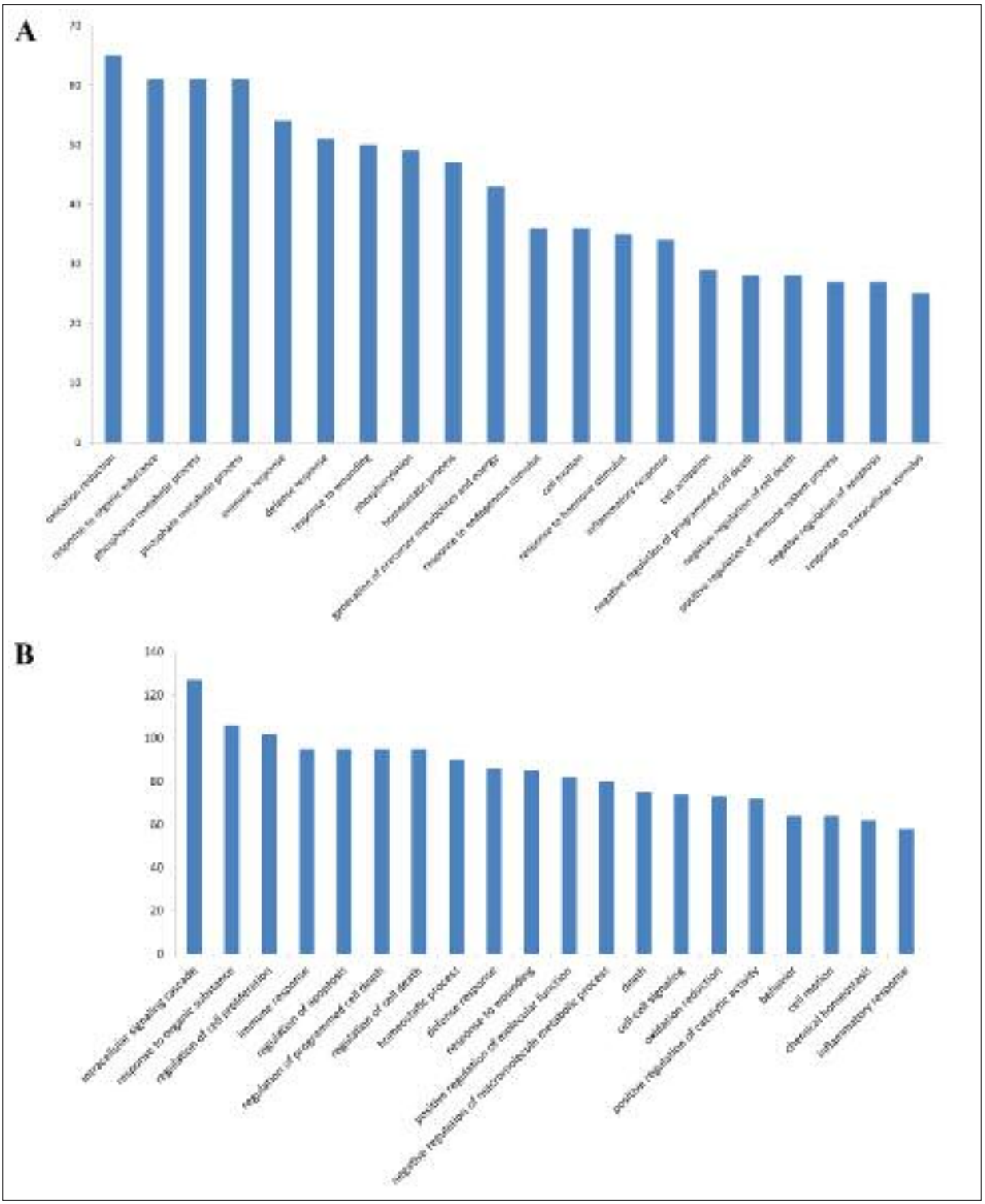

Figure 2. The Gene Ontology analysis of significantly differentially expressed gene in UC and CD. ***A,*** The Gene Ontology analysis of significantly differentially expressed gene in UC. ***B,*** The Gene Ontology analysis of significantly differentially expressed gene in CD.

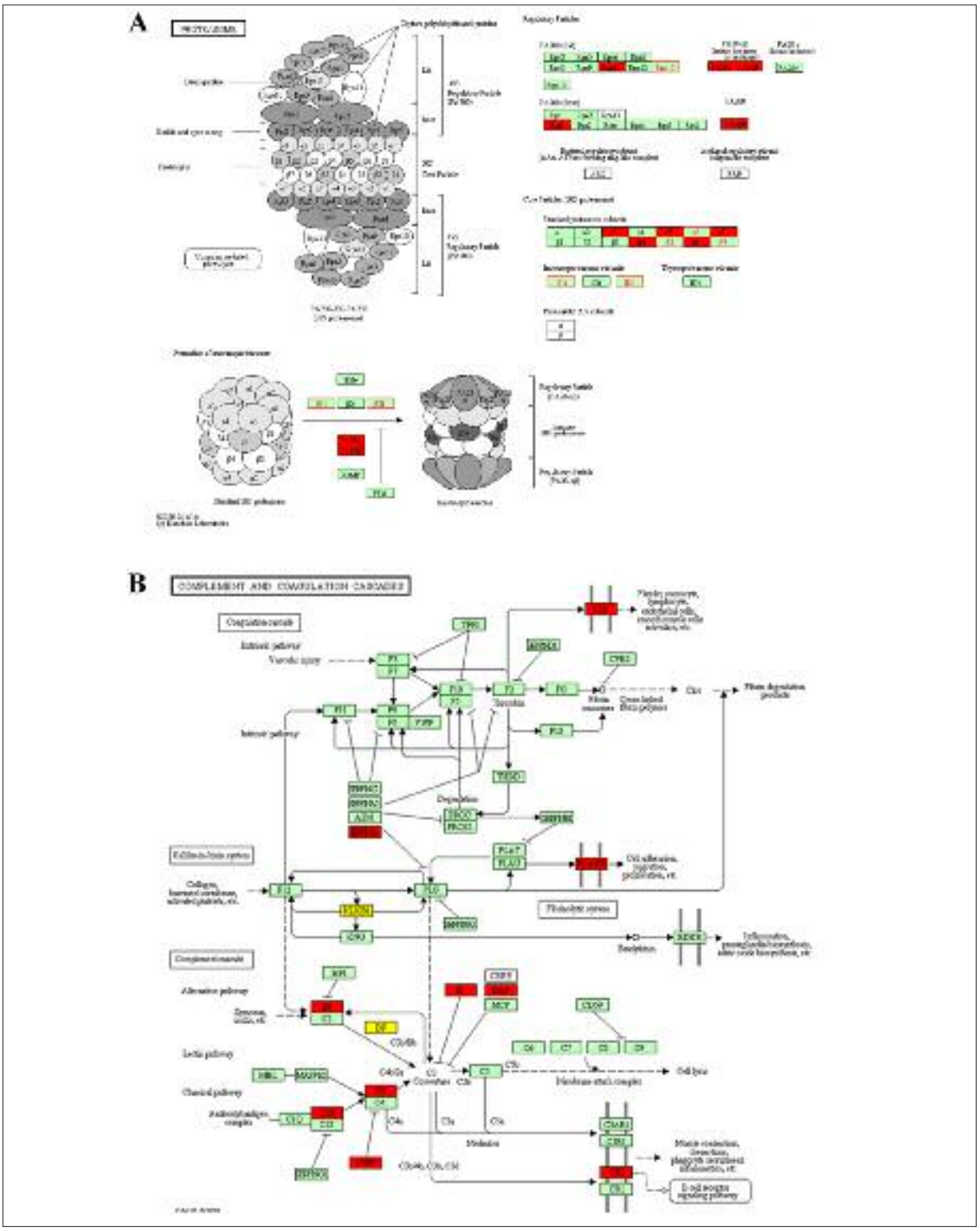

Figure 3. A, The representative KEGG pathways of significantly differentially expressed genes in UC and CD. DEGs in CD were demonstrated in proteasome pathway and cell apoptosis pathway. ***B,*** DEGs in UC were demonstrated in complement system. The red stands indicated the up-regulated genes, and the yellow stands indicated the down-regulated genes. The green stands indicated the genes or enzymes which belong to a specific species and have detailed information about them. The colorlessness stands indicated the substance which doesn't have detailed information. The font color with red stands for the important genes or enzymes in KEGG pathways.

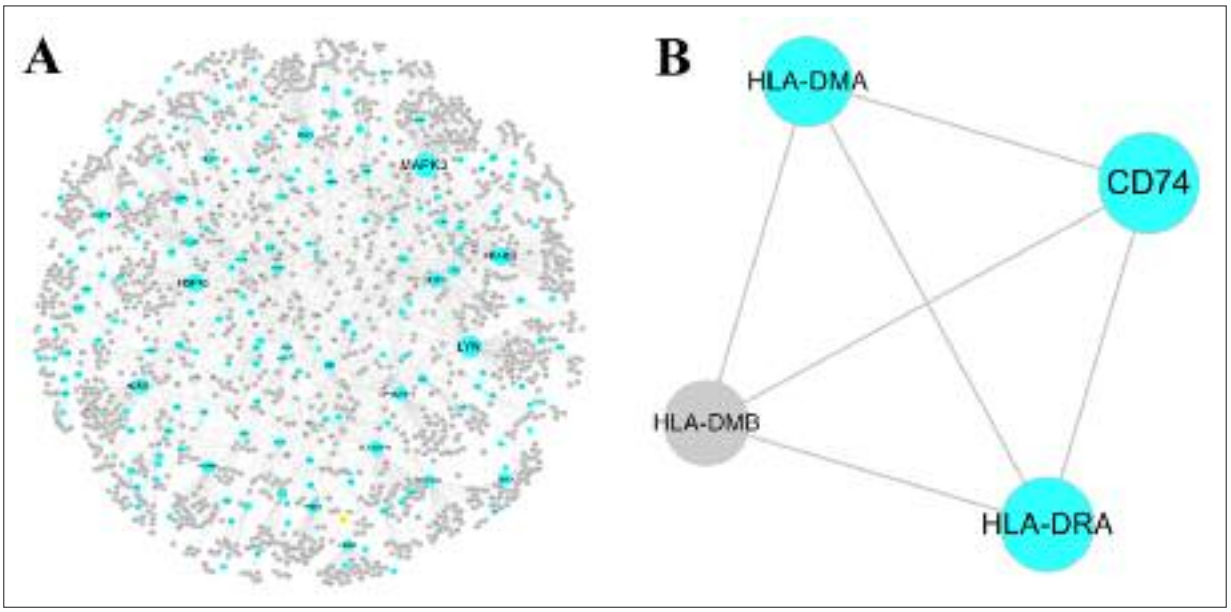

Figure 4. The protein-protein interaction (PPI) network analysis of the differentially expressed genes (DEGs). ***A,*** PPI network. ***B,*** Module. Orange and blue nodes represent products of up-and down-regulated DEGs, respectively. Blue nodes represent the overlapping DEGs, the grey nodes represent the normal genes.

was involved in IBD, which indicated the close relationship between HLA and IBD[61,62]. In our study, HLA-DRA was the overlapping DEG in UC and CD group, and it has high degree in the PPI network. In a word, combined with the studies above, HLA-DRA may be a therapeutic target in IBD.

Conclusions

The *MAPK3*, *NDRG1* and *HLA-DRA* may play key roles in the progression and development of IBD. They may be used as specific therapeutic targets in the treatment of IBD. However, further experiments are still needed to confirm our findings.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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