Original article

CED
Clinical and Experimental Dermatology

Identification and interaction analysis of key genes and microRNAs in atopic dermatitis by bioinformatics analysis

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doi:10.1111/ced.13691

Summary

Background. Atopic dermatitis (AD) is a skin disease that carries a major health burden, but the exact mechanism of the disease is not yet known.

Aim. To identify the key genes and micro (mi)RNAs in AD, and to explore their potential molecular mechanisms.

Methods. From the Gene Expression Omnibus (GEO) database, we downloaded microarray data for GSE32924 (mRNA profile) and GSE31408 (miRNA profile), which were analysed using GEO2R. Functional and pathway enrichment analyses were performed using the DAVID database, and the protein–protein interaction network was constructed with Cytoscape software. In addition, targets of differentially expressed miRNAs (DEMs) were predicted by the online resource miRDB.

Results. In total, 328 differentially expressed genes (DEGs) were identified, including 121 upregulated and 207 downregulated genes. Gene Ontology analyses showed that upregulated genes were significantly enriched in immune responses, while downregulated genes were mainly involved in epidermis development. In addition, we identified three DEMs, all of which were downregulated. Hsa-let-7a-5p may target CCR7, and hsa-miR-26a-5p probably targets HAS3.

Conclusions. We identified lists of DEGs and DEMs in AD. Bioinformatics and interaction analysis may provide new clues for further studies of AD.

Introduction

Atopic dermatitis (AD) is a core challenge for dermatological research around the world, with an estimated prevalence of around 10–20% in developed countries.[1,2] It inflicts a severe psychosocial burden on patients, and increases the risk of food allergy, asthma, allergic rhinitis and other immune-mediated inflammatory diseases.[3] Current research into the mechanisms of AD is mainly focused on skin barrier abnormalities in the epidermis and immune inflammation in the dermis,[4–6] but the pathogenesis of AD is still unclear. It is important to explore the molecular mechanisms involved in AD pathogenesis to improve diagnostic and therapeutic strategies.

Bioinformatics analysis of microarray data has been widely used to identify genetic variations in oncological research, and it is also essential for developing biomarkers in inflammatory disease such as AD.[7] In recent years, a large number of studies on differentially expressed genes (DEGs) in AD have been conducted, and their roles in biological processes, molecular functions and different pathways have been reported.[8–10] However, there are still questions about the molecular pathway interactions between DEGs and micro (mi) RNAs in the pathogenesis of AD. We analysed data generated by microarray technology to explore the interactions between DEGs and miRNAs, and to investigate their potential mechanisms in AD.

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Conflict of interest: the authors declare that they have no conflicts of interest.

Accepted for publication 2 February 2018

Methods

Microarray data

From the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), we downloaded two gene expression profiles, GSE32924 and GSE31408. The array data for GSE32924 consist of an mRNA expression profile of 13 AD samples and 8 normal samples, while those for GSE31408 include miRNA expression profiles for 20 AD samples and 2 normal samples (Table 1). All AD samples were harvested from lesions of AD biopsies when recruited at baseline, while control samples were collected from normal skin biopsies of healthy volunteers.

Identification of differentially expressed genes and microRNAs

GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/) is an interactive online tool to identify DEGs by comparing samples from GEO series.[11] GEO2R was used to screen for mRNAs (DEGs) and miRNAs (differentially expressed miRNAs; DEMs) that were differentially expressed between AD and normal tissue samples. The cut-off criteria were an adjusted *P*-value of < 0.05 and a logFC value of ≥ 2 for DEGs, and $P < 0.05$ and logFC ≥ 1 for DEMs. A heat map of DEG expression (50 upregulated genes and 50 downregulated genes) was developed using HemI1.0.1[12] software (http://hemi.biocuckoo.org/down.php).

Table 1 Characteristics of the two Gene Expression Omnibus datasets included in this analysis.

	GEO no.	
	GSE32924	GSE31408
Year	2011	2011
Country	USA	Sweden
Platform	GPL570*	GPL14149†
Sample size		
AD‡	13	20
Control	8	2
Sample characteristics		
AD severity§	28–97.5	NR
Age (years)	16–81	NR

AD, atopic dermatitis; GEO, Gene Expression Omnibus; NR, not reported. *Affymetrix Human Genome U133 Plus 2.0 Array; †miRCURY LNA microRNA Array, fifth generation; ‡only samples of lesional biopsies from patients at baseline recruitment were included in this analysis; §measured using the SCORing Atopic Dermatitis (SCORAD) questionnaire.

Gene Ontology and pathway enrichment analysis of differentially expressed genes

Gene Ontology (GO) is a commonly used bioinformatics tool that provides comprehensive information on gene function of individual genomic products through ontology. GO consists of three items: molecular functions, biological processes and cellular components.[13,14] The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a set of high-throughput genes and protein pathways.[15] The GO and KEGG analyses can be found in the DAVID Database (https://david.ncifcrf.gov/), which is a bioinformatics database containing functional interpretation of lists of genes.[16] GO and KEGG analyses of DEGs identified in GSE32924 were performed using the DAVID online tools. $P < 0.05$ was set as the cut-off criterion.

Protein–protein interaction network and module analysis

The Search Tool for the Tetrieval of Interacting Genes (STRING) database (http://string-db.org/) is online software that provides comprehensive interactions of lists of proteins and genes.[17] Cytoscape is an open-source tool for network visualization of proteins, genes and other types of biological molecules.[18] DEGs were uploaded to STRING to build a PPI network and then visualized using Cytoscape. The cut-off criteria were a combined score of > 0.4 for a PPI network and a node degree of > 10 for screening hub genes. The Molecular Complex Detection (MCODE) plug-in for Cytoscape was used to screen modules of hub genes from the PPI network.

Prediction of microRNA targets

The target genes for DEMs in GSE31408 were predicted using miRDB (http://mirdb.org/miRDB/), an online database for predicting microRNA targets.[19] The common genes were further analysed by comparing the target genes with DEGs.

Results

Identification of differentially expressed genes in atopic dermatitis

The analysis of the GSE32924 dataset identified 374 DEGs. Of these, 328 were matched to unique genes in AD skin tissue samples, and we compared 121 upregulated genes and 207 downregulated genes. The heat map of DEGs expression is shown in Fig. 1.

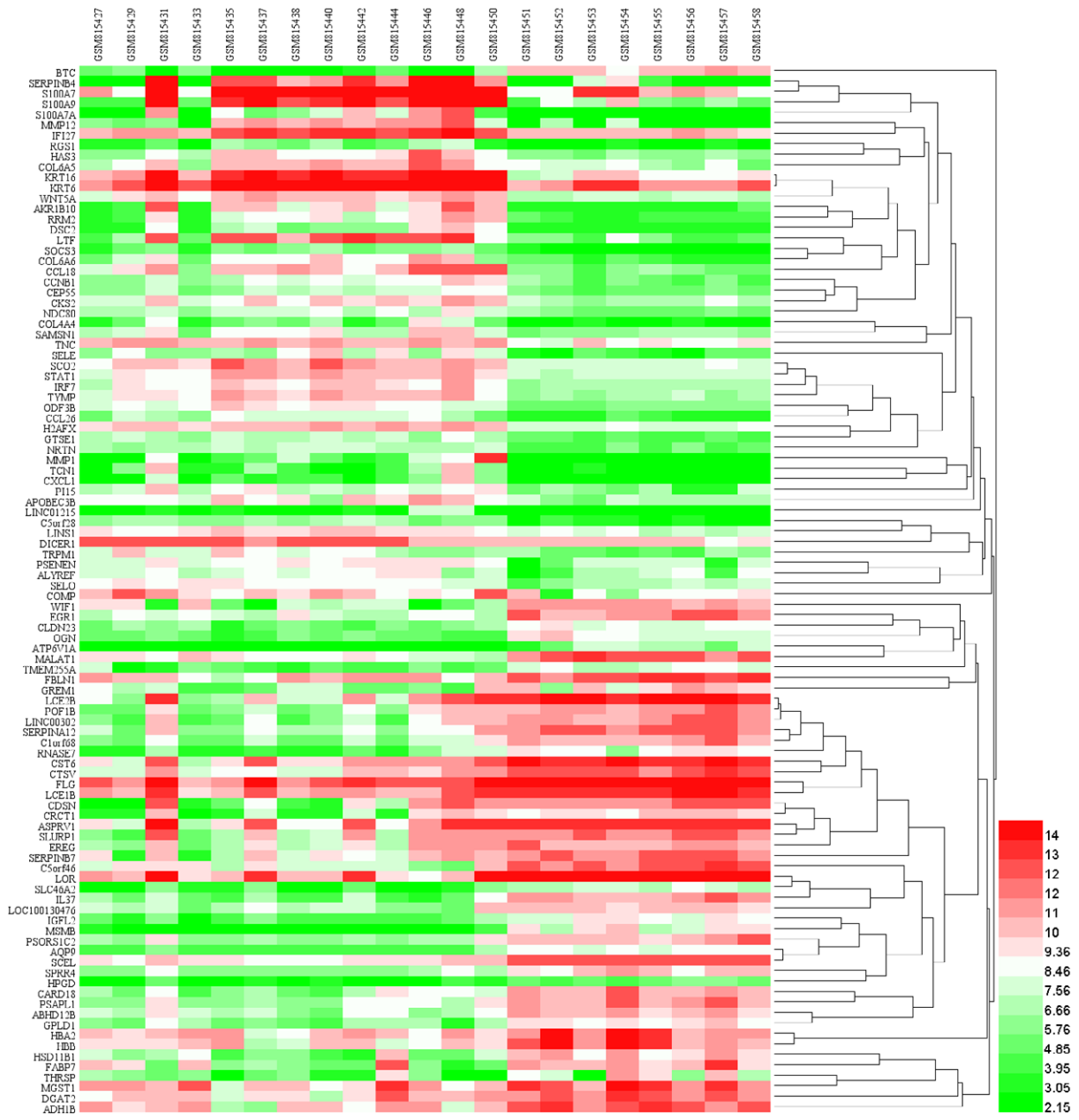

Figure 1 Heatmap of top 50 upregulated genes and top 50 downregulated genes. The 13 Gene Expression Omnibus (GEO) samples (which are designated GEO sample; GSM) in the left columns were collected from lesional biopsies of patients with atopic dermatitis, while the 8 GSM samples in the right columns were taken from healthy skin tissue of healthy volunteers.

Functional and pathway enrichment analyses

DAVID was used to perform GO and KEGG analyses to explore the function and pathways of the328 identified DEGs. GO term enrichment analysis showed that in the biological processes category, upregulated genes were significantly enriched in immune responses, whereas downregulated genes were mainly involved in skin development and epidermal cell differentiation (Table 2). In addition, cellular component analysis revealed that most of the upregulated genes were

Table 2 Functional enrichment analysis of DEGs in atopic dermatitis.

Category	Term	Involved in	*n**	%	*P*
Upregulated					
GOTERM_BP_FAT	GO:0006955	Immune response	43	36.1	< 0.001
GOTERM_BP_FAT	GO:0045087	Innate immune response	33	27.7	< 0.001
GOTERM_BP_FAT	GO:0034097	Response to cytokine	28	23.5	< 0.001
GOTERM_BP_FAT	GO:0006952	Defence response	37	31.1	< 0.001
GOTERM_BP_FAT	GO:0019221	Cytokine-mediated signalling pathway	21	17.6	< 0.001
GOTERM_CC_FAT	GO:0000793	Condensed chromosome	10	8.4	< 0.001
GOTERM_CC_FAT	GO:0098687	Chromosomal region	12	10.1	< 0.001
GOTERM_CC_FAT	GO:0000794	Condensed nuclear chromosome	7	5.9	< 0.001
GOTERM_CC_FAT	GO:0005819	Spindle	11	9.2	< 0.001
GOTERM_CC_FAT	GO:0000775	Chromosome, centromeric region	9	7.6	< 0.001
GOTERM_MF_FAT	GO:0048020	CCR chemokine receptor binding	6	5.0	< 0.001
GOTERM_MF_FAT	GO:0042379	Chemokine receptor binding	6	5.0	< 0.001
GOTERM_MF_FAT	GO:0005102	Receptor binding	23	19.3	< 0.001
GOTERM_MF_FAT	GO:0015631	Tubulin binding	9	7.6	< 0.001
GOTERM_MF_FAT	GO:0001664	G-protein coupled receptor binding	8	6.7	0.001
Downregulated					
GOTERM_BP_FAT	GO:0008544	Epidermis development	24	11.8	< 0.001
GOTERM_BP_FAT	GO:0043588	Skin development	20	9.9	< 0.001
GOTERM_BP_FAT	GO:0030855	Epithelial cell differentiation	28	13.8	< 0.001
GOTERM_BP_FAT	GO:0030216	Keratinocyte differentiation	14	6.9	< 0.001
GOTERM_BP_FAT	GO:0009913	Epidermal cell differentiation	16	7.9	< 0.001
GOTERM_CC_FAT	GO:0001533	Cornified envelope	10	4.9	< 0.001
GOTERM_CC_FAT	GO:0005576	Extracellular region	82	40.4	< 0.001
GOTERM_CC_FAT	GO:0044421	Extracellular region part	67	33.0	< 0.001
GOTERM_CC_FAT	GO:0005615	Extracellular space	32	15.8	0.001
GOTERM_CC_FAT	GO:0005911	Cell–cell junctions	18	8.9	< 0.01
GOTERM_MF_FAT	GO:0050839	Cell adhesion molecule binding	15	7.4	< 0.001
GOTERM_MF_FAT	GO:0098641	Cadherin binding involved in cell–cell adhesion	11	5.4	0.001
GOTERM_MF_FAT	GO:0098632	Protein binding involved in cell–cell adhesion	11	5.4	< 0.01
GOTERM_MF_FAT	GO:0098631	Protein binding involved in cell adhesion	11	5.4	< 0.01
GOTERM_MF_FAT	GO:0045296	Cadherin binding	11	5.4	< 0.01

*Number of enriched genes in each term. If there were more than five terms enriched in this category, the top five terms based on *P* value were chosen.

located in the chromosomal region, while the downregulated genes were mainly distributed in the extracellular space (Table 2). Moreover, according to the results of molecular function analysis, upregulated genes were mainly associated with receptor binding, while downregulated genes were involved in cell adhesion and molecular binding (Table 2). Furthermore, KEGG pathway analysis showed that most of the upregulated genes participated in extracellular matrix–receptor interactions and chemokine signalling pathways, whereas downregulated genes mainly took part in leucocyte trans-endothelial migration (Table 3).

Protein–protein interaction network construction and analysis of modules

In total, 218 nodes and 749 edges were mapped in the PPI network of identified DEGs (Fig. 2a). The 10 highest-scoring nodes were screened as hub genes: *CDC20, CCNB1, BUB1, NDC80, AURKA, HMMR, TTK, KIF2C, FOXM1* and *RRM2*. A significant module containing 22 nodes and 221 edges was generated by MCODE. In addition to the 10 genes listed above, other nodes in the module were *CKS2, KIF18B, KIF14, DEPDC1, HELLS, GTSE1, NCAPG, FANCI, KIF4A, KIF20A, DLGAP5* and *NEK2* (Fig. 2b). All genes in the module were upregulated.

MicroRNA–differentially expressed gene pairs

After analysing the GSE31408 dataset, three DEMs that were differentially regulated between the AD and the normal tissue samples were identified. All three miRNAs were downregulated. The miRDB database was used to predict target genes of the identified DEMs (Table 4). By comparing the target genes with DEGs

Table 3 Pathway enrichment analysis of differentially expressed genes in atopic dermatitis.

Category		Term	Count*	%	*P*
Upregulated					
KEGG_PATHWAY	hsa04062	Chemokine signalling pathway	7	5.9	< 0.01
KEGG_PATHWAY	hsa04380	Osteoclast differentiation	6	5.0	< 0.01
KEGG_PATHWAY	hsa04512	ECM–receptor interaction	5	4.2	< 0.01
KEGG_PATHWAY	hsa05166	HTLV-1 infection	7	5.9	0.02
KEGG_PATHWAY	hsa05200	Pathways in cancer	8	6.7	< 0.05
Downregulated					
KEGG_PATHWAY	hsa04670	Leucocyte transendothelial migration	7	3.4	< 0.01
KEGG_PATHWAY	hsa04722	Neurotrophin signalling pathway	5	2.5	0.05
KEGG_PATHWAY	hsa04071	Sphingolipid signalling pathway	5	2.5	0.05
KEGG_PATHWAY	hsa04530	Tight junction	5	2.5	0.07
KEGG_PATHWAY	hsa00071	Fatty acid degradation	3	1.5	0.09

ECM, extracellular matrix; HTLV, human T-cell lymphotropic virus. *Count: the number of enriched genes in each term. If there were more than five terms enriched in this category, the top five terms based on *P* value were chosen.

from GSE32924, we screened genes with an opposing expression trend for further analysis. Hsa-let-7a probably targets *RRM2* and *CCR7*, while miR-26a potentially targets *HAS3, DEPDC1B, NAMPT, DENND1B, ADAM19* and *DEPDC1*, and miR-143 may target *DENND1B*.

Discussion

There are many uncertainties about the pathogenesis of AD, and study of the biomarkers and mechanisms of AD remains necessary as the global prevalence of the disease is on the rise. In this study, 328 DEGs were screened, comprising 121 upregulated genes and 207 downregulated genes. Upregulated genes were involved in immune response, while downregulated genes were mainly involved in skin development and epidermal cell differentiation. Functional enrichment analysis of DEGs provided a comprehensive overview of the two major and confluent pathophysiological mechanisms of dysregulation in AD: skin barrier dysfunction and immune inflammation. These are two closely interacting features in AD, although it is unclear which is the key driver of the disease.

There is growing evidence that miRNAs play an important role in the pathogenesis of AD. In our study, we identified three DEMs, all of which were downregulated. By comparing target genes with DEGs, we identified potential targets of these DEMs.

The first DEM, hsa-let-7a-5p, potentially targets the chemokine receptor CCR7. It has been reported that CCR7 is overexpressed in T cells and dendritic cells (DCs) in AD lesions.[20] CCR7 can control the migratiion of memory T cells in inflamed tissues, as well as stimulate DCs to maturation,[21] and it may play an important role in the activation and polarization of T cells in the pathogenesis of AD.[20] It was reported that CCR7 in breast cancer cells could be suppressed by the miRNA let-7a,[22] but the role of let-7a in the regulation of CCR7 in AD pathogenesis still needs further study.

The second DEM, hsa-miR-26a-5p, probably regulates the hyaluronan synthase 3 (*HAS3*) gene. *HAS3* is a member of the *NODC/HAS* gene family. It is involved in the synthesis of hyaluronic acid, a major component of the extracellular matrix. Malaisse *et al.* reported an increase in HAS3 in AD lesions compared with healthy skin and nonlesional AD skin.[23] Proinflammatory cytokines, such as interleukin (IL)-4, IL-13 and interferon-γ,[24] which can upregulate HAS3, may result in reduced hyaluronic acid synthesis.[23] However, the specific regulation of HAS3 in proliferation and differentiation of keratinocytes has not yet been characterized.

The third DEM, miR-26a, was also found to be downregulated. Decreased expression of miR-26a was previously detected in IL-22-producing T cells, which play an important role in allergic diseases.[25] Variations of miR-26a and its targeted gene *HAS3* might play a significant role in the abnormality of the skin barrier in patients with AD.

Conclusion

Our study attempted to identify DEGs using bioinformatics analyses and to find potential miRNAs to predict the regulatory mechanisms of these genes. In this work, 328 DEGs and 3 DEMs were screened. Upregulated genes were mainly involved in the immune response, while downregulated genes were involved in epidermis development. Given their interactions with

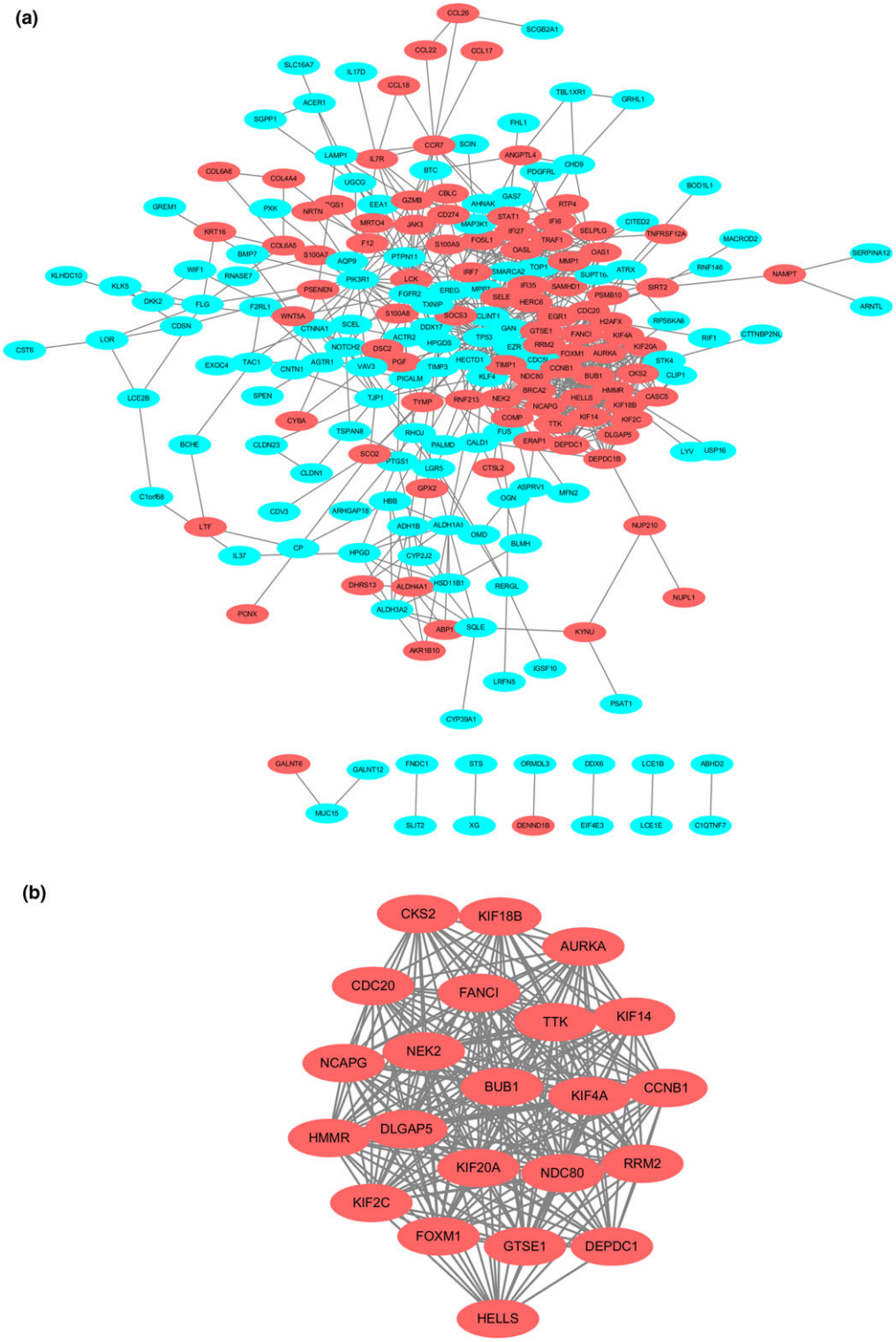

Figure 2 (a) Using the Search Tool for the retrieval of Interacting Genes (STRING) database online database, 328 differentially expressed genes (DEGs) (upregulated genes are displayed in red and downregulated genes in blue) were filtered into the DEGs protein–protein interaction (PPI) network complex. The areas in the highlighted circles were statistically significant modules. (b) The significant module consisted of 22 nodes and 221 edges, and all genes in this module were upregulated.

Table 4 Differentially expressed microRNAs in atopic dermatis tissue and their potential target genes.

			Target genes	
miRNA	*P*	logFC	Upregulated	Downregulated
hsa-let-7a-5p	0.001	−1.009	*RRM2*, *CCR7*	*GAN*, *GAS7*, *LOR*, *CLDN1*, *NXT2*, *MAP3K1*, *EEA1*
hsa-miR-26a-5p	< 0.01	−1.083	*HAS3*, *DEPDC1B*, *NAMPT*, *DENND1B*, *ADAM19*, *DEPDC1*	*C1S*, *PALMD*, *ABHD2*, *CTSV*, *POF1B*, *RPS6KA6*, *CTTNBP2NL*, *MAP3K1*
hsa-miR-143–3p	< 0.01	−1.014	*DENND1B*	*BLMH*, *PTPN11*, *SLC16A7*, *ABHD2*, *TMEM170B*, *ATP6V1A*

FC, fold change.

CCR7 and HAS3, respectively, the role of let-7a and miR-26a in AD may provide new clues for future research into the mechanisms of AD. We plan to validate these predicted results obtained from bioinformatics analysis in further experimental studies of these genes in the future.

Acknowledgements

This work was supported by Jiangsu Province Hospital of Chinese Medicine Foundation (grant no. Y17003) to HL.

What's already known about this topic?

- Research into the mechanisms of AD has mainly focused on skin barrier abnormalities and immune inflammation.
- There are many uncertainties as to the mechanism of AD.

What does this study add?

- Upregulated genes in AD were significantly enriched in the immune response, while downregulated genes were mainly involved in epidermis development.
- The DEMs hsa-let-7a-5p and hsa-miR-26a-5p potentially target CCR7 and HAS3, respectively.

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