MicroRNA-sequencing Analysis of Plasma-derived Exosomes Provides Novel Insights into the Disease Mechanism of Childhood Atopic Dermatitis

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Abstract

Background: Exosomes are nano-size vesicles that plays important roles in transporting cellular modulators including microRNAs (miRNAs), DNA and proteins between cells. Exosomes and their components are proved to implicate a variety of dermatologic disorders in adults. However, the expression pattern of exosomal miRNAs in pediatric atopic dermatitis (AD) patients has not been well studied.

Methods: Plasma samples were drawn from 5 pediatric AD patients and 5 age-matched healthy volunteers. Small RNA sequencing was performed to identify differentially expressed exosomal miRNAs (DEPEMs) between those two groups. Bioinformatics tools including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were used to explore the DEPEMs involved canonical pathways and their relationships with the pathogenesis of AD. Reverse transcription real-time polymerase chain reaction (RT-qPCR) was used for validation and measurement of DEPEM levels.

Results: A total of 40 DEPEMs, including 29 upregulated and 11 downregulated DEPEMs, were identified. Using the miRanda and TargetScan databases, 17,238 target genes of the 40 DEPEMs were predicted. GO and KEGG pathway enrichment analyses suggested that these target genes were involved in multiple functions and pathways associated with AD, such as the skin barrier, T-cell differentiation and keratinocyte proliferation. 10 selected DEPEMS, including 5 downregulated miRNAs (hsa-miR-1343-3p, hsa-miR-301a-5p_R-1, hsa-mir-1273c-p3, hsa-miR-374b-3p and hsa-miR-1908-5p) and 5 upregulated miRNAs (hsa-let-7b-3p_R-1_1ss21CT, hsa-miR-34a-5p, hsa-miR-100-5p_R-1, hsa-miR-27a-5p and hsa-mir-3976-p3_1ss16AT) were verified through RT-qPCR.

Conclusions: This study provides the first genomic profile for understanding the pathogenesis and potential biomarkers for pediatric AD patients. The DEPEMs from our study differs from those from adult AD patients, suggesting children with AD have distinct peripheral exosomal miRNA expression. DEPEMs may serve as promising novel biomarkers or regulators of pediatric AD pathology.

Background

Atopic dermatitis (AD) is one of the most common chronic inflammatory skin diseases of early childhood, affecting up to 15%-30% of children[1]. The etiology of AD is complicated and unclear, and it is currently considered to be related to the interaction of environmental factors, epidermal barrier damage and innate and acquired immune system disorders[2]. Although many genome-wide association studies have been performed to elucidate the genetic factors related to AD, its exact pathogenesis remains largely elusive.

MicroRNAs (miRNAs) are small noncoding RNAs that are 19–25 nucleotides long and highly conserved throughout evolution[3]. At present, miRNAs are considered one of the largest groups of factors that regulate the expression of genes through binding the 3' untranslated regions (UTR) of target mRNAs[4]. MiRNAs play important roles in regulating multiple biological processes, including cell cycle progression,

cell proliferation and differentiation, as well as maintenance of immune homeostasis[5]. MiRNAs are suspected to be involved in the development of AD. Recent studies showed altered expression of miR-143, miR-146a, miR-151a, miR-155, and miR-223 in adult AD patients[6–12]. These miRNAs are closely related with regulations of keratinocyte proliferation/apoptosis, cytokine signaling and nuclear factor (NF)-κB-dependent inflammatory responses, as well as T-cell activities[13–15].

MiRNAs are widely distributed in cells and body fluids including serum, plasma, urine and saliva[16]. Exosomes act as the basic mediator of intracellular communication by transferring proteins and genetic material like mRNA, miRNA and DNA [17]. When exosomes circulate, exosomal miRNAs can be taken up by neighboring cells or distant cells and then regulate recipient cells[18]. Due to the easy acquire of exosomes in peripheral blood and miRNAs' high stability and ability to survive storage[19], it possible to use exosomal miRNAs as biomarkers of diseases.

However, only few studies were focused on the expression of miRNAs in pediatric AD patients and the expression pattern of exosomal miRNAs in pediatric AD patients has not been well studied[20]. We sought to investigate exosomal miRNA expression profiles in pediatric AD patients using miRNA sequencing and discuss the possibility of miRNAs serve as biomarkers of the severity of inflammation in children with AD.

Methods

Patients and sample collection

We recruited 5 pediatric patients of Chinese Han ethnicity with moderate to severe atopic dermatitis who met the HanifinRajka diagnostic criteria in Beijing Children's Hospital from January 2021 to June 2021 and 5 age-matched healthy controls. The study was approved by the Ethics Committee of Beijing Children's Hospital and was conducted according to the principles of the Helsinki Declaration. Disease severity was evaluated by the SCORing Atopic Dermatitis (SCORAD) index. The clinical features of the AD and healthy groups are summarized in Table 1. Blood samples were collected from all participants and centrifuged at 2400 g for 10 minutes at 4°C, and then the supernatants were collected and centrifuged at 800 g for 10 minutes at 4°C. The serum from each sample was transferred into new Eppendorf tubes and stored at -80℃ until used.

Table 1
Clinical features of subjects in the AD and control groups.

Item	AD group(n = 5)	Control group(n = 5)
Age	3.5 (1–10)	3.2 (1–10)
Sex (Female/male)	3/2	3/2
SCORAD	56.4 ±9.97(31–87)	NA

Values are expressed as mean (range) or n. SCORAD, SCORing atopic dermatitis; AD, atopic dermatitis.

Exosome isolation and validation

Exosomes were isolated from the plasma by ultracentrifugation. 250μL of plasma sample was centrifuged at 500 x g for 10 min followed by centrifugation at 200 x g for 20 min at 4℃ to remove cell sediment and debris. The supernatant was then filtered and ultracentrifuged at 100,000 x g for 2 h. The precipitate was collected and resuspended in PBS. After centrifugation at 100,000 x g for 70 min, exosomes were collected and stored at -80℃ until use.

The movement of exosomes were observed through a NanoSight NS300 (Malvern Panalytical, Amesbury, UK) and then analyzed by the nanoparticle tracking analysis (NTA) 3.2 software. A 3-μL exosomes sample (approximately 10^{11} particles/mL) was placed on a formvar/carbon-coated grid and allowed to settle for 2 min, which then were negatively stained with 2% phosphotungstic acid for 1 min. Pictures of exosomes were captured with a Tecnai G2 Spirit BioTwin transmission electron microscope (TEM)[21].

Specific exosomal markers including CD9 and CD81 were validated through Western blot analysis. The protein concentration of the exosome samples was measured using a Qubit 4.0 protein assay kit and was adjusted to 10 μg/20 μL. 10 μg of protein was drawn from each sample for electrophoresis using a 12% polyacrylamide gel, which were later transferred to a polyvinylidene fluoride membrane (PVDF, Millipore) by a Trans-Blot Turbo Transfer System (Bio–Rad). After blocking the membrane in 5% non-fat powdered milk (dissolved in TBST) for 30 min at room temperature, primary antibodies (rabbit monoclonal anti-human CD9 antibody and rabbit monoclonal anti-human CD81 antibody, Abcam) were added to incubate the membrane overnight at 4°C, followed by incubating it in secondary antibody (horseradish peroxidase-labeled goat anti-rabbit antibody, Abcam) for 2 hours at room temperature. Images were captured on an Amersham Imager 600 (GE Healthcare Life Sciences)[21].

RNA extraction and small RNA library preparation

Total RNA was extracted from plasma exosomes using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). After purification, the exosomal RNA samples were sent to Saidite Co. for small RNA libraries construction, sequencing and further analysis. Complementary cDNAs of small RNAs were created by reverse transcription polymerase chain reaction (RT-PCR). After excision and purification of the

productions, libraries were quantified and validated. Small RNA sequencing was performed on an Illumina HiSeq 2500 instrument (Illumina, Inc.)[22].

Data analysis

Raw reads were compared with an in-house database (ACGT101-miR, LC Sciences, Houston, Texas, USA) to remove the adapter dimers, junk, low complexity, common RNA families (rRNA, tRNA, snRNA, snoRNA) and repeats. 18 ~ 26 nucleotides unique sequences were mapped by Basic Local Alignment Search Tool (BLAST) to identify known miRNAs and novel 3' and 5' derived miRNAs[23]. The known miRNAs are known as the unique sequences mapping to specific species mature miRNAs in hairpin arms, while sequences mapping to the other arm were categorized as novel 5' or 3' derived miRNA candidates. The unmapped sequences were BLASTed against the specific genomes, and the hairpin RNA structures containing sequences were predicated from the flanking 80 nt sequences using RNAfold software (http://rna.tbi.univie.ac. at/cgi-bin/RNAfold.cgi)[24]. Differentially expressed exosomal miRNAs (DEPEMs) based on normalized deep-sequencing counts was analyzed by using Fisher's exact test, Chi-squared 2X2 test, Chi-squared nXn test, Student's t test, or ANOVA based on the experimental design. The significance threshold was set to 0.01 and 0.05 for each test. To predict the genes targeted by the most abundant miRNAs, two computational target prediction algorithms (TargetScan, v5.0 and Miranda, v3.3a) were used to identify miRNA binding sites. Finally, the data predicted by both algorithms were combined, and the overlaps were identified. Gene Ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotation of the most abundant miRNAs and miRNA targets was also performed[25].

DEPEMs validation through RT–qPCR

DEPEMs with potential function in AD's etiology and development trough GO and KEGG analysis were further verified by RT-qPCR. TaqMan® microRNA reverse transcription kit was used for cDNA synthesis. A total of 10 ng RNA from each sample was mixed with 1 μl of dNTPs, 5 U of MultiScribe reverse transcriptase, 2 μl of 10× RT buffer, 0.5 μl RNase inhibitor with Megaplex™ PreAmp Primers (Applied Biosystems), Human Pool. The reaction mixture was incubated at 16°C for 30 minutes, 42°C for 60 minutes, and 85°C for 5 minutes. Quantitative PCR (qPCR) was performed with a miRNA-specific probe mix and cDNA template. The reaction mixture was incubated at 94°C for 5 minutes, followed by 94°C for 10 seconds, 60°C for 20 seconds, and 72°C for 30 seconds. Relative quantification and calculations were performed with the comparative threshold (Ct) cycle method (2-△△CT)[26].

Statistical analysis

Statistical analysis was performed using SPSS version 12.0 statistical software (Chicago, IL, USA), and the data were visualized using Cytoscape 3.6.1 and GraphPad Prism 5.0 software. The quantitative data are displayed as the mean ± standard deviation (SD) and were compared by two-tailed unpaired Student's t test. P values < 0.05 indicated a statistically significant difference.

Results

Isolation and characterization of AD-exosomes

The particle size of the AD-exosomes defined by NTA ranged from 50–150 nm, and the average particle size was approximately 84.36 nm (Figure. 1a). Then TEM confirmed that the AD exosomes were spherical in shape and revealed the presence of clear lipid bilayer structures (Figure. 1b). To further characterize the exosomes, Western blotting showed the expression of exosomal markers including CD9 and CD81 were indeed enriched in our isolated exosomes, as well as the negative marker calnexin was down regulated (Figure. 1c). Full-length blots/gels are presented in Supplementary Fig. 1.

Differential expression analysis of plasma exosomal miRNAs in AD

A total of 40 DEPEMs (P value < 0.05, fold change (FC) ratio (|log2FC| ≥ 1)) were identified when the two groups were compared. Among them, 29 miRNAs were upregulated (72.5%), and 11 miRNAs (27.5%) were downregulated. A heatmap and volcano map of the DEPEMs were used to visualize global miRNA expression changes in AD patients and controls (Figure. 2a, b).

Functional enrichment and annotation

Analyses with the TargetScan (v5.0) and miRanda (v3.3a) databases identified 17,238 target genes associated with the DEPEMs. We divided the GO terms associated with these genes into three categories: molecular function, biological process and cellular component (Figure. 3a). GO enrichment analysis revealed that these target genes were functionally enriched in signal transduction, cell differentiation, protein binding, and metal ion binding. The DEPEMs were also enriched in several clusters related to AD pathogenesis, including “keratinocyte proliferation” (GO: 0043616, P = 3.58E-02), “apoptotic process” (GO: 0006915, P = 4.87E-06), “T-cell differentiation” (GO: 0030217, P = 1.17E-03), “autophagy” (GO: GO: 0061436, P = 7.69E-02), “positive regulation of keratinocyte migration” (GO: 0051549, P = 2.64E-02) and “establishment of skin barrier” (GO: 0061436, P = 7.69E-02) (Fig. 4a).

KEGG pathway analysis revealed that these target genes were significantly enriched in cellular processes, signal transduction, genetic information processing, and metabolism, including amino acid and lipid metabolism (Figure. 3b). We further found that the DEPEMs were enriched in multiple cytokine signaling pathways associated with inflammation and adaptive immunity that are known to play important roles in AD pathogenesis, including the “PI3K-Akt signaling pathway” (ko04151, P = 2.50E-11), “MAPK signaling pathway” (ko04010, P = 2.03E-08), “EGFR tyrosine kinase inhibitor resistance” (ko01521, 6.79E-07), “IL-17 signaling pathway” (ko04657, P = 1.43E-01), “Th1 and Th2 cell differentiation” (ko04658, 1.00E + 00) and “leukocyte transendothelial migration” (ko04670, P = 9.84E-04) (Figure. 4b).

We further assessed the results of enrichment analysis of target genes and sorted out the miRNAs and mRNAs related to the GO terms establishment of skin barrier, T-cell differentiation and keratinocyte proliferation, which are involved in AD pathogenesis. The network diagram showed that these miRNAs and mRNAs that are important in the pathogenesis of AD form a large regulatory network and interact

with each other to participate in the occurrence and development of AD (Figure. 5). We also selected the KEGG terms Th1 and Th2 cell differentiation, leukocyte transendothelial migration, IL-17 signaling pathway and B-cell receptor signaling pathway (Figure. 6).

Validation of the miRNA-Seq results with RT-qPCR

RT-qPCR analysis of 10 dysregulated miRNAs, including 5 downregulated miRNAs and 5 upregulated miRNAs was carried out. We observed significant increases in hsa-let-7b-3p_R-1_1ss21CT, hsa-miR-34a-5p, hsa-miR-27a-5p and hsa-mir-3976-p3_1ss16AT expression, as well as significant decreases in hsa-miR-1343-3p, hsa-miR-301a-5p_R-1, hsa-mir-1273c-p3, hsa-miR-374b-3p and hsa-miR-1908-5p expression, which is similar to the miRNA-seq data (Figure. 7). We also drew a network diagram including the 10 miRNAs, which showed the relationship of the upregulated and downregulated miRNAs with mRNAs (Figure. 8).

Discussion

AD is a chronic, complex and relapsing skin disorder characterized by significant dry and itchy skin[27]. The prevalence of AD worldwide has been reported to be nearly 30% and 3% in children and adults, respectively[28]. It is currently believed that AD results from the interaction of multiple factors, including genetic, environmental, immune and psychological factors[29]. However, AD is a systematic and multisite skin-involved disease, while the previous studies concerning miRNA analysis mainly focused on intracellular miRNA expression in blood or lesions, and the expression levels of circulating extracellular miRNAs in remains largely unknown. In this study, we conducted miRNA-seq analysis of plasma exosomes from children with moderate to severe AD. Our study revealed significant changes in plasma exosomal miRNA expression, which may provide insights into pediatric AD pathogenesis and potential biomarkers for diagnosis and treatment.

Exosomes are thought to have vital roles in intercellular communication and have been proven to be involved in the pathogenesis of numerous diseases[30]. They exist in almost all body fluids, including blood, saliva, and cerebrospinal fluid but are more stable in plasma. In this study, we isolated exosomes from the plasma of patients with moderate to severe AD. TEM and western blotting have shown that circulating exosomes are present in AD patients. The contents of exosomes include miRNAs, which are small (19–25 nt) noncoding RNAs that act as important posttranscriptional regulators of gene expression and bind to the 30 untranslated region of their target mRNAs[31]. Exosomes can transfer miRNAs for intercellular communication and regulate target gene expression and the function in the recipient cell[32]. Most studies focus on miRNA changes in skin lesions or blood in adult AD. However, the patterns of miRNAs in childhood AD, especially in circulating exosomes, are rarely studied. Therefore, the expression levels of exosomal miRNAs were compared between pediatric patients with AD and control patients.

AD is closely related to keratinocyte proliferation, apoptotic processes, T-cell differentiation, autophagy, keratinocyte migration and inflammation etc. and our results suggested likewise. 40 miRNAs were significantly differentially expressed between circulating exosomes from children with AD and those from

healthy controls. Analysis with the TargetScan and miRanda databases identified 17,238 target genes associated with the DEPEMs and GO enrichment analysis indicated that the DEPEMs were significantly enriched in signal transduction, cell differentiation, protein binding, metal ion binding, etc. Interactions between the imbalance of T-helper cells, abnormal proliferation of keratinocytes and skin barrier dysfunction have been proven to be responsible for the pathophysiology of AD[33].Our results showed that the DEPEMs were enriched in keratinocyte proliferation, apoptotic processes, T-cell differentiation, autophagy, positive regulation of keratinocyte migration, and establishment of the skin barrier, which are related to the pathogenesis of AD. Apoptosis and autophagy are new research hotspots related to AD. Recent evidence suggests that impaired autophagy is involved in inflammation and disturbance of keratinocyte differentiation, which indicates that dysfunctional autophagy is linked to AD[34]. Further, KEGG pathway analysis showed that the target genes of the DEPEMs were significantly enriched in the PI3K-Akt signaling pathway, EGFR tyrosine kinase inhibitor resistance, B-cell receptor signaling pathway, IL-17 signaling pathway, Th1 and Th2 cell differentiation and leukocyte transendothelial migration. These results suggest that plasma exosomal miRNAs might regulate inflammatory signaling pathways, resulting in the development of AD. IL-17 has been reported to reduce the expression of filaggrin protein (FLG) and involucrin, which contribute to skin barrier dysfunction and the development of AD[35]. In addition multiple DEPEMs and their target genes involved in the IL-17 signaling pathway, such as the downregulated miRNA hsa-miR-6852-5p, may target IL-17RA and IL-17D. It was observed that the GO term and KEGG pathways in which the target genes of the DEPEMs were enriched overlapped and interacted, which proves that AD is definitely not the result of a single factor. The DEPEMs involved in these pathways may be potential regulators of AD.

We selected ten DEPEMs, including 5 upregulated miRNAs (hsa-let-7b-3p_R-1_1ss21CT, hsa-miR-34a-5p, hsa-miR-100-5p_R-1, hsa-miR-27a-5p and hsa-mir-3976-p3_1ss16AT) and 5 downregulated miRNAs (hsa-miR-1343-3p, hsa-miR-301a-5p_R-1, hsa-mir-1273c-p3, hsa-miR-374b-3p and hsa-miR-1908-5p) whose target genes have been reported to be involved in the pathogenesis of AD. For example, hsa-miR-301a-5p_R-1 targets filaggrin protein 2 (FLG2), which is related to dysfunction of the skin barrier in AD lesions[36]. hsa-let-7b-3p_R-1_1ss21CT and hsa-miR-1908-5p target IL-4R, which may be a therapeutic target for AD[37]. In addition, five miRNAs are predicted to bind with genes that have been confirmed to be involved in AD. Such target genes and miRNAs include TLR4 (hsa-miR-1343-3p and hsa-miR-1273c-p3), CXCL16 (hsa-miR-100-5p_R-1 and hsa-miR-3976-p3_1ss16AT target cxcl16) and CCL13 (hsa-miR-374b-3p). Furthermore, the expression of hsa-miR-27a-5p was experimentally confirmed to be attenuated in classical Hodgkin lymphoma via silencing of B-cell-specific transcription factors[38]. We predicted that hsa-miR-27a-5p may bind SERPINB7, which can lead to impairment of the integrity of the stratum corneum structure of the skin barrier in AD[39]. Nine out of ten DEPEMs (except hsa-miR-100-5p_R-1) were confirmed to be significantly differentially expressed between plasma exosomes from children with AD and those from healthy controls using RT–qPCR, suggesting a potential role for these miRNAs as regulators or biomarkers of pediatric AD.

AD frequently starts at an early age and seriously affects the physical and mental health of children. Unfortunately, only few studies have focused on the distinct genetic etiology of pediatric AD patients.

Evaluation of miRNAs in adult AD patients showed elevated expression of miR-146a, miR-10b, miR-10a, miR-10a*, miR-216, miR-921-1, miR-454, and miR-29b-1 and downregulation of miR-99a, miR34c-5p and miR-30a expression[12].By comparison with functional miRNAs in adult AD (Table 2), our results suggest that the expression patterns of exosomal miRNAs in pediatric AD patients are different from those in adult AD patients and that these miRNAs may be regulators and biomarkers of AD in children.

Table 2
MiRNAs and their associated mRNAs in adult AD in previously published reports compared to pediatric AD of this research

	MiRNA name	Possible target mRNA in AD	Functional mechanism	Up/down regulated in AD	Log2 (fold change)	P value
Vaher et al.2019[6]	MiR-10a-5p	hyaluronan synthase 3 (HAS3)	Impairment of keratinocyte proliferation and migration	up	NA	NA
Gu et al. 2017[7]	MiR-29b	Bcl-2-like protein 2(BCL2L2)	Promoting interferon (IFN)-γ-induced keratinocyte apoptosis	up	NA	NA
Rebane et al. 2014[8]	MiR-146a	IRAK1, CARD1, CCL5	Suppressing NF-κB-dependent inflammatory reaction and type-2-cell-mediated immune responses	up	NA	NA
Yang et al. 2017[9]	MiR-124	RELA	Regulation of inflammatory responses in keratinocytes	down	NA	NA
Sonkoly et al. 2010[10]	MiR-155	CTLA-4, PKIα	Promoting proliferation in T helper cells	up	NA	NA
Chen et al. 2018[11]	MiR-151a	IL12RB2	Down-regulated IL-12 pathway	up	NA	NA
Jia et al. 2018[12]	MiR-223	Not specified	Affecting histamine-N-methyltransferase	up	NA	NA
Our research	miR-1343-3p	TLR4	Not specified	down	-2.72	2.44E-03
	miR-301a-5p_R-1	FLG2	Not specified	down	-2.73	6.34E-03
	miR-1273c-p3	TLR4	Not specified	down	-2.03	6.89E-03
	miR-let-7b-3p_R-1_1ss21CT	IL-4R	Not specified	up	1.56	7.40E-03
	miR-374b-3p	CCL13	Not specified	down	-2.45	7.55E-03

	MiRNA name	Possible target mRNA in AD	Functional mechanism	Up/down regulated in AD	Log2 (fold change)	P value
	miR-34a-5p	TMEM79, NOD1	Not specified	up	2.51	1.06E-02
	miR-1908-5p	IL-4R	Not specified	down	-1.24	4.07E-02
	miR-100-5p_R-1	CXCL16	Not specified	up	1.07	4.11E-02
	miR-27a-5p	SERPINB7	Not specified	up	1.19	4.75E-02
	miR-3976-p3_1ss16AT	CXCL16	Not specified	up	2.24	4.88E-02

Hsa-miR-34a-5p was the most significantly upregulated miRNA in our study. Hsa-miR-34a-5p has been reported to be expressed in blood mononuclear cells[40], neurons[41], T cells[42], and several cancer cell lines[43]. It has been shown to regulate T-cell differentiation and plasticity by targeting histone gene expression and histone modification[42] to play a critical role as a tumor suppressor via regulation of the p53 signaling pathway to mediate cell proliferation, invasion, and apoptosis[44] and to be implicated in large B-cell lymphoma[44] and lung fibroblasts[45]. Hsa-mir-34a-5p has also been proven to be a T-cell activation regulator through the WNT, Ras-ERK and NF-κB pathways[40, 46]. However, its role in skin diseases has not yet been studied. There were numerous target genes of hsa-miR-34a-5p, including NOD1, TMEM79, TLR4 and CXCL16. These genes are associated with T cell immune response, which has also been suggested to play vital roles in the pathogenesis of AD. Therefore, we bring up the hypotheses that hsa-miR-34a-5p may regulate inflammatory response of T cells and subsequently involve in the pathophysiology of AD and may serve as a promising novel regulator or biomarker for pediatric AD.

We provide a comprehensive analysis of exosomal miRNA expression and the genomic profile for understanding the pathogenesis in pediatric AD patients. Hsa-miR-34a-5p and other differentially expressed exosomal miRNAs are potential biomarkers in AD patients of young age regarding their essential roles in regulating immune system which may possibly influence the unset or progression of the disease. Future studies are required to uncover the roles of hsa-miR-34a-5p as well as other epigenetic factors in the pathogenesis in pediatric AD.

Abbreviations

miRNA: microRNA; AD: atopic dermatitis; DEPEM: differentially expressed exosomal miRNAs; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; RT-qPCR: reverse transcription real-time polymerase chain reaction; SCORAD: SCORing Atopic Dermatitis; NTA: nanoparticle tracking analysis; TEM: transmission electron microscope; BLAST: Basic Local Alignment Search Tool.

Declarations

Ethics approval and Consent to participate: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Beijing Children's hospital, Capital Medical University, China. Informed consent was obtained from all subjects' legal guardian(s) for study participation.

Consent for Publication: Informed written consent was obtained from all subjects' legal guardians for publication of this report and any accompanying images.

Availability of data and material: The datasets generated and/or analysed during the current study are available in the GEO repository, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE205773, accession number GSE205773.

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

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Figures

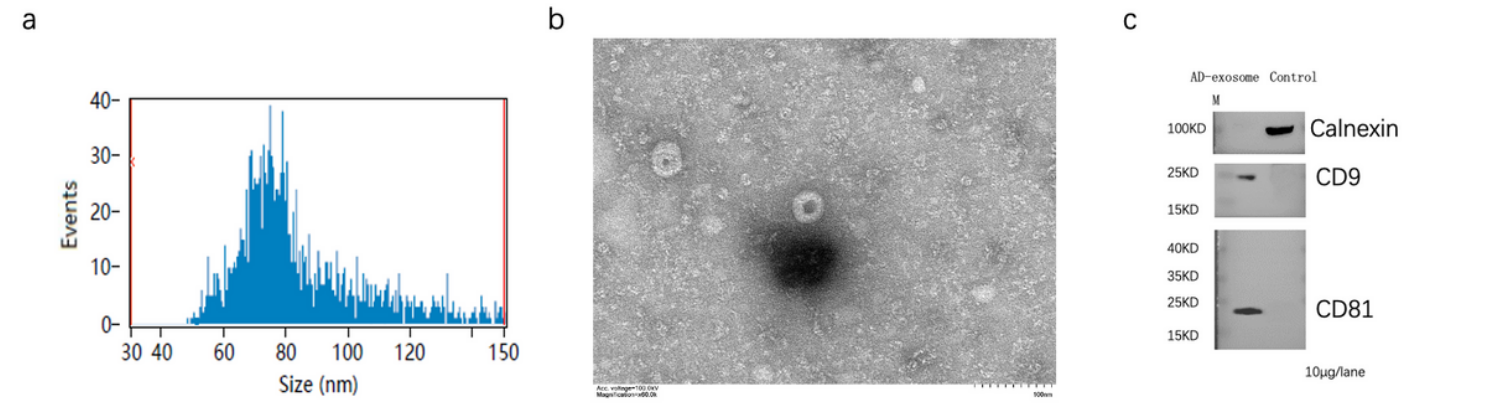

Figure 1

(a)Particle concentration and size distribution of AD-exosomes measured by nanoparticle tracking analysis (NTA); (b) TEM image of AD-exosomes. Scale bar: 100 nm; (c) AD-exosomes were analyzed by western blots for the presence of exosomal markers CD9, and CD81 and the negative markers Calnexin.

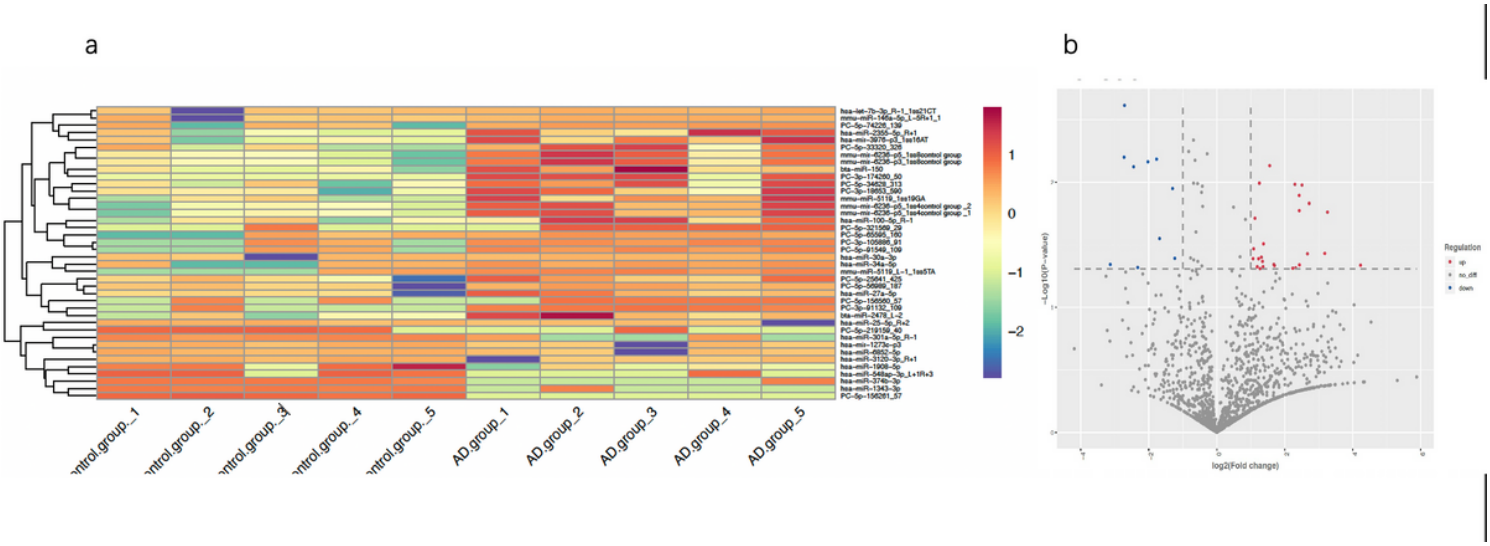

Figure 2

(a) Hierarchical clustering of differentially expressed exosomal miRNA (DEPEMS) between the AD (n=5) and control groups (n=5). The colour scale represents high (red) to medium (yellow) to low (blue) relative expression. (b) Valcano plot displayed significantly upregulated DEPEMS (red), significantly downregulated DEPEMS (blue).

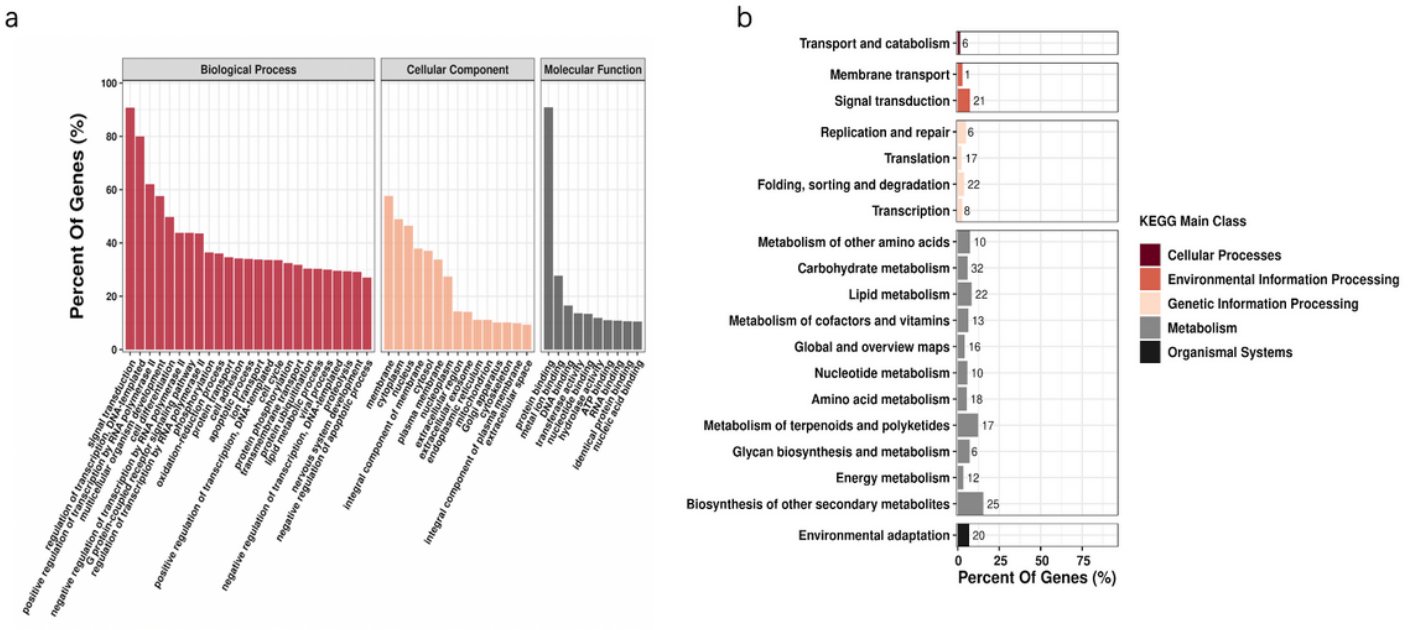

Figure 3

Functional enrichment and annotation for DEPEMs. (a)GO analysis of DEPEMs in molecular function, biological process and cellular component;(b)KEGG pathway enrichment of DEPEMs in cellular processes, signal transduction, genetic information processing, and metabolism, including amino acid and lipid metabolism.

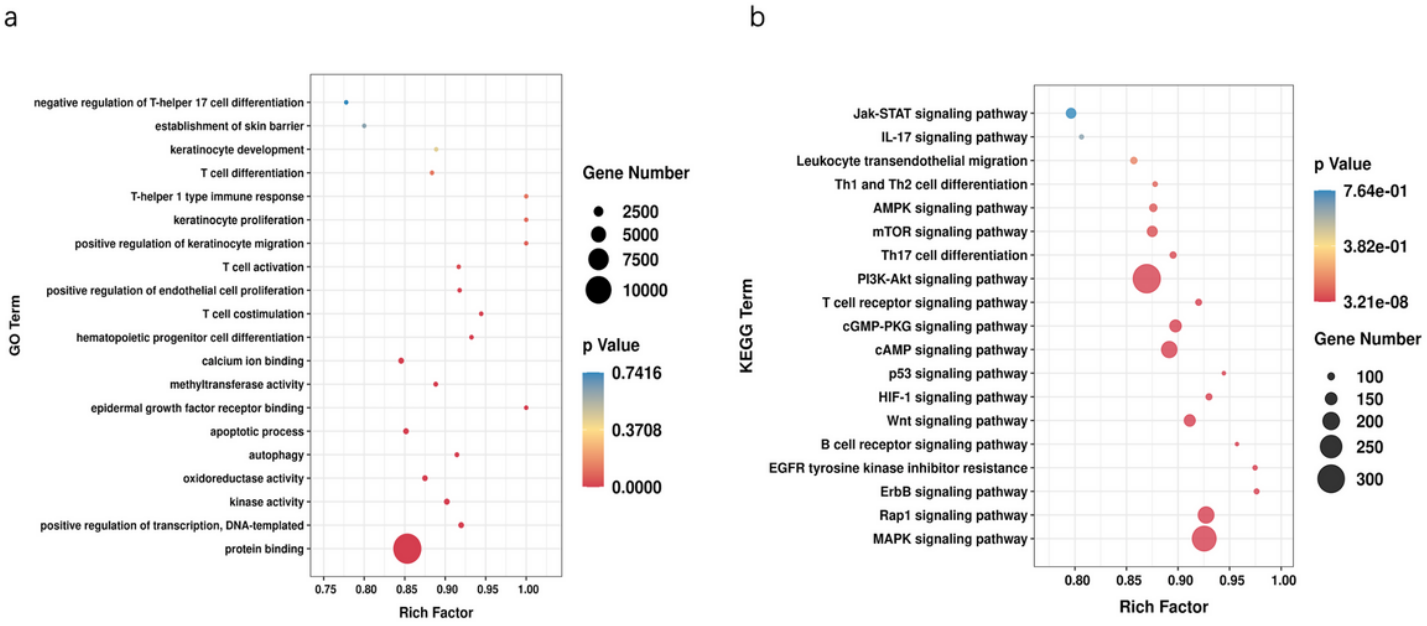

Figure 4

Scatter plots of GO (a) and KEGG (b) pathway enrichment analyses each showing 20/19 identified pathways for target genes of DEPMs (larger circles represent a larger number of genes) which related with AD. The colour scale represents low (blue) to high (red) relative expression. DEPEMs: differentially expressed exosomal plasma miRNAs.

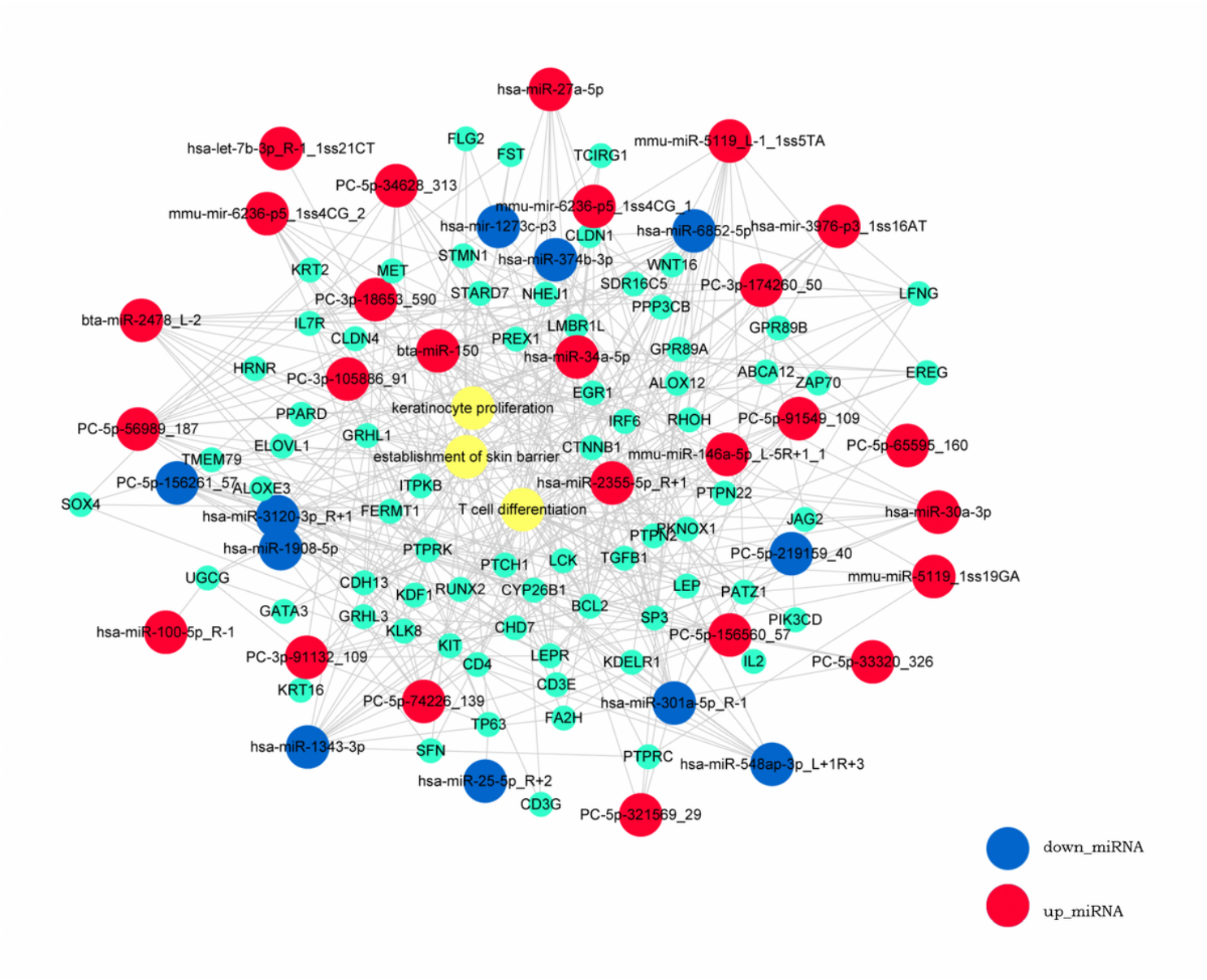

Figure 5

The network diagram showed the miRNAs and mRNAs involved in GO terms: establishment of skin barrier, T-cell differentiation and keratinocyte proliferation, form a large regulatory network and interact with each other to participate in the occurrence and development of pediatric AD. The color in blue represent down-regulated and the color in red represent up-regulated.

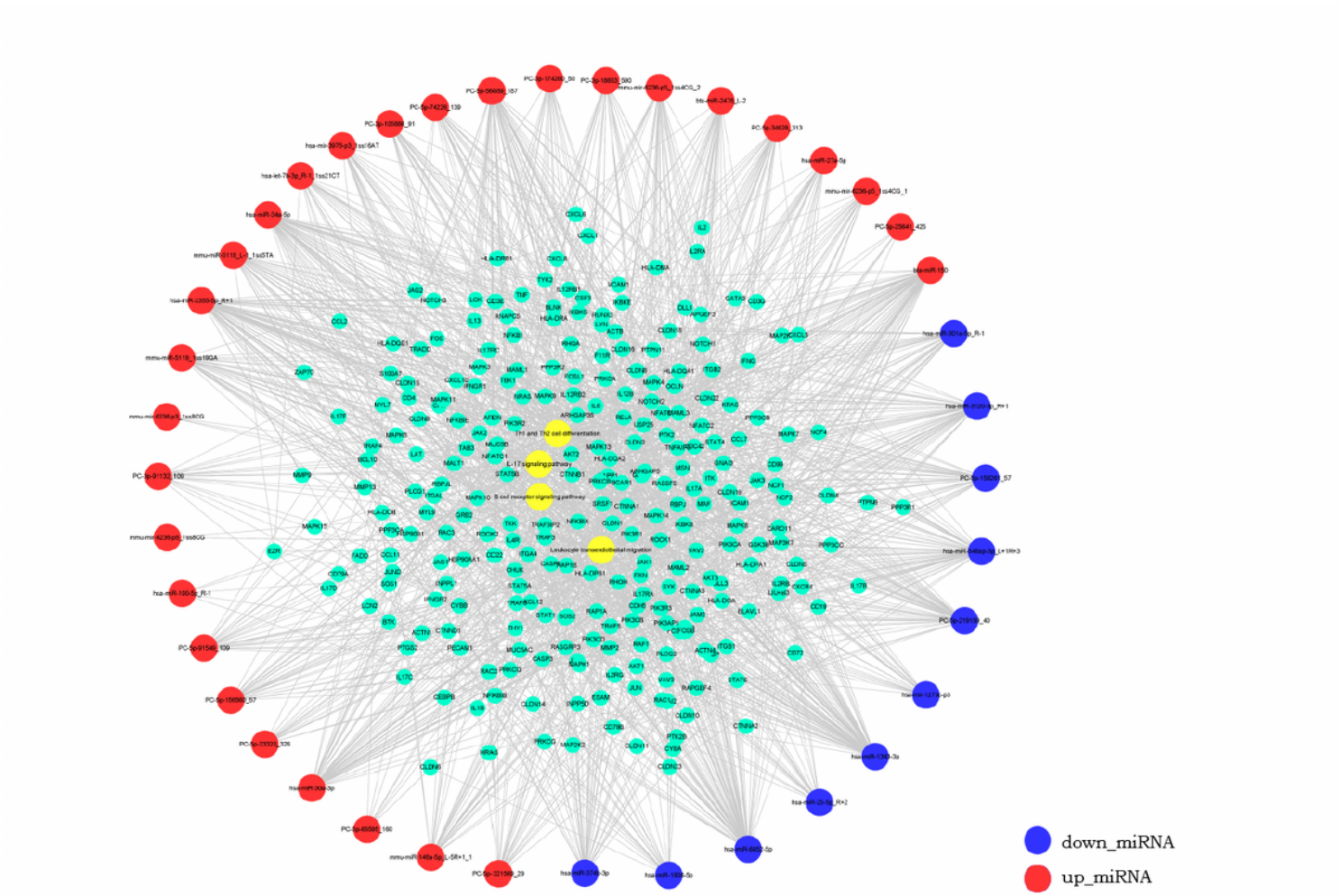

Figure 6

The interaction of miRNAs and mRNAs involved in Th1 and Th2 cell differentiation, leukocyte transendothelial migration, IL-17 signaling pathway and B-cell receptor signaling pathway show the interaction in pediatric AD.

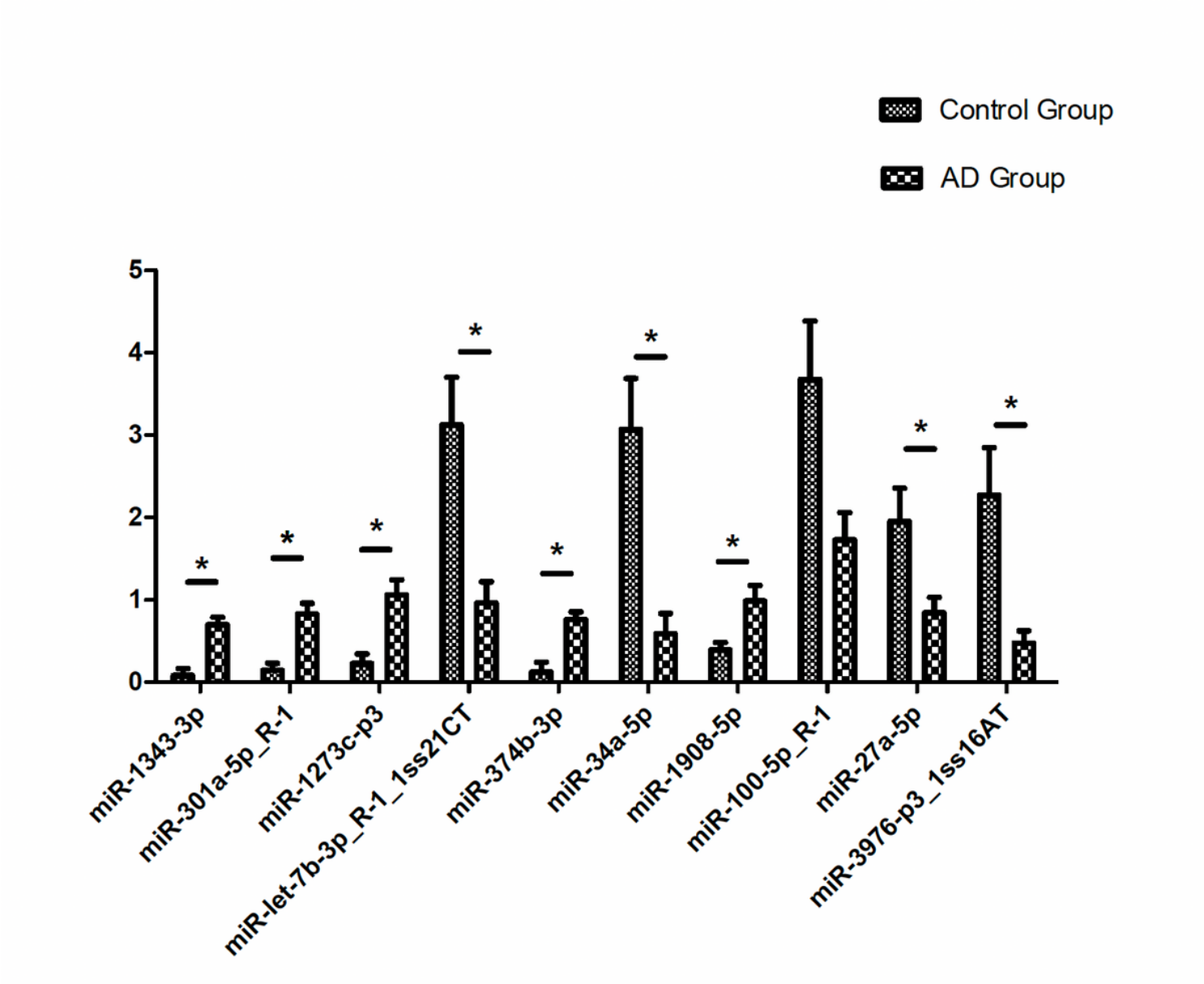

Figure 7

Verification of 10 DEPEMS in 5 pediatric AD patients and 5 controls by RT-qPCR. *P<0.05.

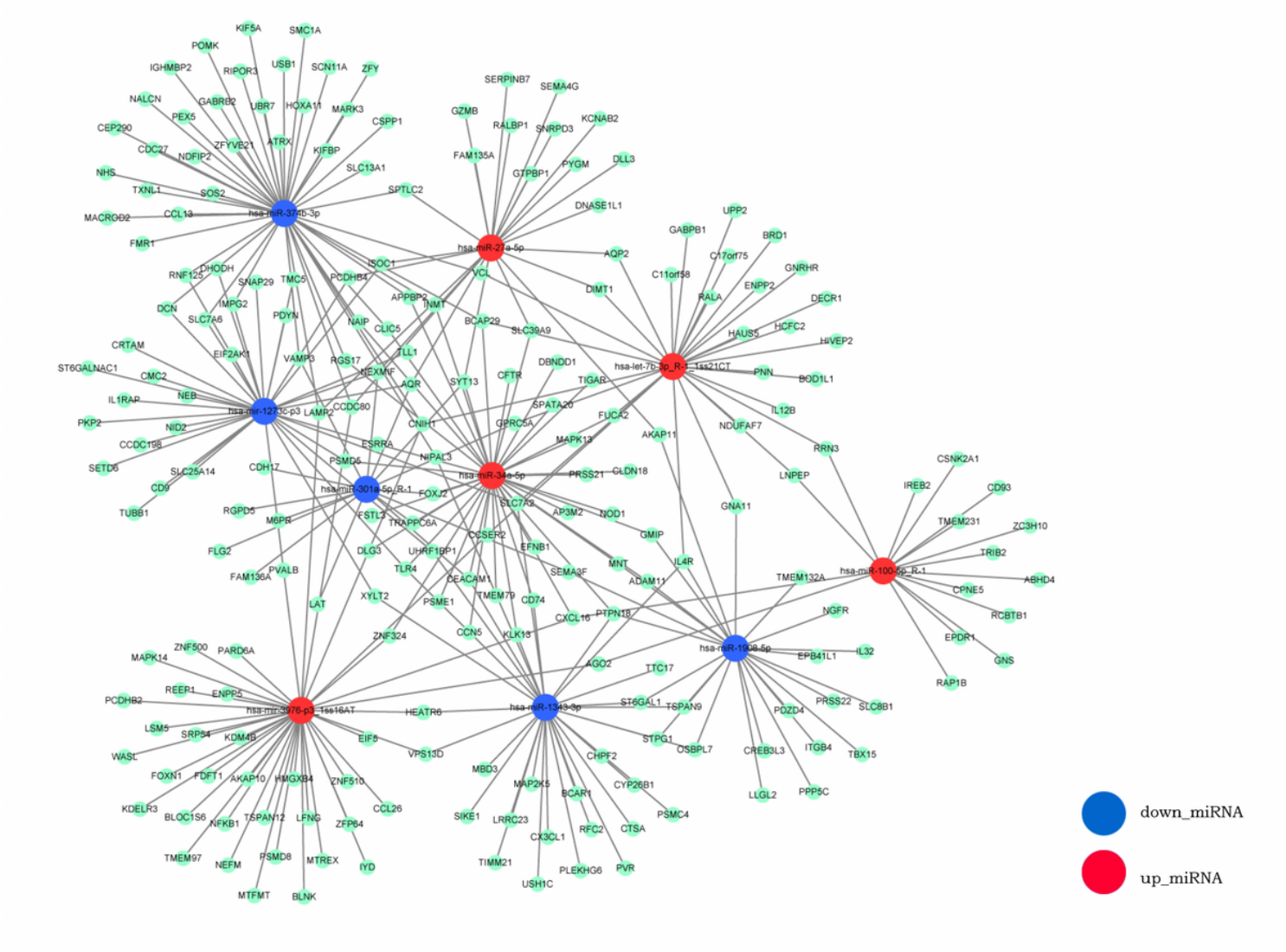

Figure 8

The miRNA–mRNA network showed the relationship of the 5 upregulated and 5 downregulated miRNAs with mRNAs.

Supplementary Files

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