• Basic Research •

Differentially expressed miRNAs in premature infants with retinopathy-a bioinformatics analysis

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Abstract

• AIM: To reveal the role of miRNAs in retinopathy of prematurity (ROP) by bioinformatics analysis.

• METHODS: The raw data of this study came from the researches of Wang *et al* and Zhao *et al* who analyzed the microRNA (miRNA) expression profile between ROP and controls. Based on the identified differentially expressed miRNAs, the related target genes, lncRNA and circRNA were predicted. Then we performed functional enrichment analysis to further analyze the functions of target genes.

• RESULTS: Hsa-miRNA-128-3p and hsa-miRNA-9-5p showed significantly different expression in both studies. LncRNA of POLDIP2, GAS5, NEFL and UHRF1, circRNA of ZNF280C_hsa_circ_001211 and SIAE_hsa_circ_002083, target gene of QKI showed meaningful differential expression in ROP. Enrichment analysis showed that TGF-β signaling pathway, PI3K-Akt signaling pathway and MAPK signaling pathway might play important roles in the progress of ROP.

• CONCLUSION: This research may provide a comprehensive bioinformatics analysis of differentially expressed miRNAs which are possibly involved in ROP.

• KEYWORDS: bioinformatics; microRNA; retinopathy of prematurity

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INTRODUCTION

Retinopathy of prematurity (ROP) is a potentially preventable neurovascular disorder, which is estimated to make 20 000 infants blind or severely visually impaired each year worldwide[1]. No matter the gestational age at birth, and no matter the ultimate severity of the retinopathy, the onset of ROP seems to start at approximately 32wk gestation[2]. With the maternal age growing, very premature infants gradually increased. Therefore, patients with ROP become common in clinical practice despite developed neonatal care technology and good management of oxygen inhalation. Although the assistance of cryotherapy, laser photocoagulation and anti-vascular endothelial growth factors (VEGF) drugs has resulted in a remarkable reduction in mobility and improved prognosis, treatment of this disease has remained difficult. The key problem for pediatricians is the physiopathologic mechanism of ROP remains unclear yet.

microRNAs (miRNAs) are small non-coding RNAs of 18-26 nucleotides in length that regulate gene expression through sequence-specific base pairing with target miRNAs. miRNA has been reported to be important in various biological processes including vascular angiogenesis, cell growth, embryonic development, cell proliferation, tissue differentiation and apoptosis[3-6]. It is well documented that miRNAs could regulate vascular angiogenesis[7]. And the regulation of VEGF, IGF-2 and HIF-1α by miRNA-126 is associated with retinal vascular angiogenesis under conditions of oxygen-induced retinopathy (OIR)[8], which suggested miRNA's possible role in ROP.

By carefully reviewing the literature in databases (PubMed and Embase), there're only two miRNA profile papers reporting identification of differential miRNAs in ROP newborns. One of them is our previous report[9], with the assistance of microarrays and subsequent reverse transcription-polymerase chain reaction (RT-PCR), we first revealed the involvement of miRNA in ROP by screening miRNAs expression in retinal tissue of mice. In another miRNA article, Zhao *et al*[10] described an initial use of deep sequencing to comprehensively profile miRNAs in the plasma of ROP rats induced by hyperoxia.

Because miRNA sequence is well conservative among species, it gives us a good opportunity to look for common significant miRNAs by using these raw data. In the present study, we took advantage of previous data to analyze the miRNA expression profile between ROP group and control group. Besides, based

Table 1 Differentially expressed miRNAs and target genes

miRNA	Direction	Fold changes	Sequence	Location	Target genes
miR-9-5p/9a-5p	Up	2.09/5.94	ucuuugguuaucuagcuguauga(mmu) ucuuugguuaucuagcuguauga(rno) ucuuugguuaucuagcuguauga(hsa)	chr1: 156420341-156420429[-](hsa)	1101
miR-128-3p/128-3p	Down	2.15/2.54	ucacagugaaccggucucuuu(mmu) ucacagugaaccggucucuuu(rno) ucacagugaaccggucucuuu(hsa)	chr2: 135665397-135665478[+](hsa)	670
miR-377-3p/377-3p	Down	2.00/3.11	aucacacaaaggcaacuuuugu(mmu) ugaaucacacaaaggcaacuuuu(rno) aucacacaaaggcaacuuuugu(hsa)	chr14: 101062050-101062118[+](hsa)	613

on the miRNAs with differential expression, we predicted the relevant target genes, lncRNA and circRNA. In addition, we performed further functional enrichment analysis to analyze the functions of target genes. We aim to explore the involvement of miRNAs in the progression of ROP.

SUBJECTS AND METHODS

Data Source The data in our study came from the study of Wang *et al*[9] and Zhao *et al*[10]. Wang *et al*[9] analyzed the miRNA profile data of retinal tissue between 40 C57BL/6 neonatal mice with ROP and 40 without ROP (controls) on postnatal day 17. Finally, 67 differentially expressed miRNAs were identified. Zhao *et al*[10] compared miRNAs in neonatal Sprague-Dawley rats. They found 66 differentially expressed plasma miRNAs by deep sequencing technology on postnatal day 14. Based on the above data, we perform a stricter screening standard which is the significant miRNAs are selected from the intersection of the above two articles (Wang *et al*[9] and Zhao *et al*[10]). And fold change must fulfill greater than two in both studies. In addition, miRNA sequence must be well conservative between human and rodent. After screening, only 1 up-regulated and 1 down-regulated miRNAs passed the test, and were further selected into this study (Table 1). Based on these findings, we try to identify the roles of miRNA in ROP.

Target Gene Prediction The target genes of miRNAs were predicted by following seven published databases, including miRanda (http://microrna.sanger.ac.uk)[11], Mir-Target2 (http://nar.oxfordjournals.org/cgi/content/abstract/34/5/1646)[12], PicTar (http://pictar.bio.nyu.edu)[13], Probability of Interaction by Target Accessibility (PITA; http://genie.weizmann.ac.il/pubs/mir07)[14], TargetScan (http://targetscan.org)[15], miRecords (http://miRecords.umn.edu/miRecords)[16], Mirwalk (http://mirwalk.uni-hd.de/)[17]. The miRNA-gene pair would be reserved when it was predicted by no less than four of the databases above.

Functional Enrichment Analysis The Database for Annotation, Visualization and Integrated Discovery (DAVID; http://david.niaid.nih.gov)[18] has been developed for mapping genes to associated pathways and Gene Ontology (GO) terms. We performed GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses to analyze the target genes using the DAVID online database. $P<0.05$ was considered as significance.

miRNA-lncRNA Regulatory Relationship Prediction StarBase v2.0 (http://starbase.sysu.edu.cn/)[19] database is used to identify the RNA-RNA and RNA-protein interaction networks systematically (miRNA-pseudogene, miRNA-lncRNA, miRNA-mRNA, miRNA-circRNA and protein-RNA) from 108 CLIP-Seq (CLASH, PAR-CLIP, iCLIP, HITS-CLIP) generated datasets. ChIPBase (http://deepbase.sysu.edu.cn/chipbase/)[20] is a database for annotating and discovering transcriptional regulatory relationships of miRNAs and lncRNAs binding maps from ChIP-Seq data. All the interaction networks of miRNA-lncRNA in our study were downloaded from starBase v2.0 and ChIPBase. The sub-networks related to the differentially expressed miRNAs were further selected.

miRNA-circRNA Regulatory Relationship Prediction In our study, all the interaction networks of miRNA-circRNA were analyzed and downloaded from starBase v2.0, and then, the sub-networks related to the differentially expressed miRNAs were selected at last.

RESULTS

Differentially Expressed miRNAs and Target Genes Three differentially expressed miRNAs passed the preliminary screening (fold change >2 in both articles), including 1 up-regulated and 2 down-regulated ones (Table 1). However, the sequence of miRNA-377-3p is not conservative among species. So, only miRNA-9-5p (miRNA-9a-5p) and miRNA-128-3p were included into our further study. The target genes were reserved when it was predicted by not less than 4 of the databases above.

Function Enrichment Analysis of hsa-miRNA-9-5p and hsa-miRNA-128-3p Target Genes The target genes of hsa-miRNA-9-5p and hsa-miRNA-128-3p were performed GO enrichment analysis and the result was shown in Table 2. The target genes of hsa-miRNA-9-5p were mainly enriched in negative regulation of transcription from RNA polymerase II promoter. And the target genes of hsa-miRNA-128-3p were mainly enriched in positive regulation of transcription, DNA-templated.

Table 2 GO analysis of hsa-miRNA-9-5p and hsa-miRNA-128-3p target genes

miRNA-9-5p	Count	*P*	Benjamini	miRNA-128-3p	Count	*P*	Benjamini
Negative regulation of transcription from RNA polymerase II promoter	39	4.0E-5	8.6E-2	Positive regulation of transcription, DNA-templated	22	2.6E-5	4.8E-2
Regulation of membrane potential	11	2.4E-4	2.3E-1	Positive regulation of transcription from RNA polymerase II promoter	35	1.4E-4	1.2E-1
Cell adhesion	18	7.8E-4	4.4E-1	Multicellular organism growth	11	1.5E-4	9.1E-2
Positive regulation of nuclear-transcribed mRNA poly(A) tail shortening	5	1.6E-3	6.0E-1	Axon guidance	12	1.9E-4	8.5E-2
Proteasome-mediated ubiquitin-dependent protein catabolic process	14	4.3E-3	8.6E-1	Transcription, DNA-templated	26	4.4E-4	1.5E-1
Positive regulation of proteasomal ubiquitin-dependent protein catabolic process	9	4.4E-3	8.0E-1	Heart development	12	6.3E-4	1.8E-1
Regulation of synaptic vesicle exocytosis	4	6.0E-3	8.5E-1	Peptidyl-serine phosphorylation	12	7.5E-4	1.8E-1
Negative regulation of epidermal growth factor-activated receptor activity	4	8.7E-3	9.1E-1	Negative regulation of transcription from RNA polymerase II promoter	27	8.1E-4	1.7E-1
Cellular response to starvation	7	8.8E-3	8.9E-1	Positive regulation of ubiquitin-protein transferase activity	5	1.0E-3	1.9E-1
Positive regulation of transcription from RNA polymerase II promoter	40	8.9E-3	8.6E-1	Positive regulation of angiogenesis	10	1.5E-3	2.5E-1

Table 3 Pathway enrichment analysis of hsa-miRNA-9-5p and hsa-miRNA-128-3p target genes

miRNA-9-5p	Count	*P*	Benjamini	miRNA-128-3p	Count	*P*	Benjamini
Thyroid hormone signaling pathway	15	8.3E-4	1.7E-1	Signaling pathways regulating pluripotency of stem cells	15	1.5E-4	3.1E-2
Transcriptional misregulation in cancer	18	9.9E-4	1.1E-1	Pathways in cancer	26	9.5E-4	9.7E-2
Protein digestion and absorption	12	1.9E-3	1.3E-1	Transcriptional misregulation in cancer	15	1.3E-3	8.8E-2
Pathways in cancer	32	3.2E-3	1.6E-1	FoxO signaling pathway	12	3.3E-3	1.6E-1
Endocytosis	23	3.5E-3	1.5E-1	AMPK signaling pathway	11	5.5E-3	2.1E-1
Neurotrophin signaling pathway	14	3.9E-3	1.4E-1	RNA degradation	8	9.9E-3	3.0E-1
Ras signaling pathway	20	7.2E-3	2.1E-1	mRNA surveillance pathway	9	1.0E-2	2.8E-1
Glycosaminoglycan biosynthesis-chondroitin sulfate / dermatan sulfate	5	1.3E-2	3.1E-1	GABAergic synapse	8	1.7E-2	3.7E-1
Ubiquitin mediated proteolysis	14	1.3E-2	2.8E-1	TGF-beta signaling pathway	8	1.9E-2	3.7E-1
miRNAs in cancer	14	1.4E-2	2.7E-1	MAPK signaling pathway	16	2.1E-2	3.7E-1
Vasopressin-regulated water reabsorption	7	1.5E-2	2.6E-1	ErbB signaling pathway	8	2.1E-2	3.4E-1
MAPK signaling pathway	20	2.8E-2	4.1E-1	Rap1 signaling pathway	14	2.2E-2	3.3E-1
AMPK signaling pathway	12	2.9E-2	4.0E-1	MicroRNAs in cancer	16	2.3E-2	3.2E-1
Renal cell carcinoma	8	3.3E-2	4.2E-1	PI3K-Akt signaling pathway	19	2.9E-2	3.6E-1
Focal adhesion	17	3.6E-2	4.2E-1	GnRH signaling pathway	8	3.5E-2	3.9E-1
Dorso-ventral axis formation	5	3.7E-2	4.1E-1	Estrogen signaling pathway	8	3.5E-2	3.9E-1
Regulation of actin cytoskeleton	17	4.0E-2	4.2E-1	Ubiquitin mediated proteolysis	10	3.8E-2	4.1E-1
Insulin secretion	9	4.7E-2	4.5E-1	Neurotrophin signaling pathway	9	4.7E-2	4.5E-1

Pathway Enrichment Analysis of hsa-miRNA-9-5p and hsa-miRNA-128-3p Target Genes The signaling pathways enriched by the target genes of hsa-miRNA-9-5p and hsa-miRNA-128-3p were shown in Table 3. The target genes of hsa-miRNA-9-5p were enriched in Ras signaling pathway, cancer pathway, MAPK signaling pathway. And the target genes of hsa-miRNA-128-3p were mainly enriched in TGF-β signaling pathway and PI3K-Akt signaling pathway.

LncRNAs Targeted by hsa-miRNA-9-5p and hsa-miRNA-128-3p From the predicted lncRNAs of hsa-miRNA-9-5p and hsa-miRNA-128-3p, we found that some lncRNAs were regulated by both miRNAs, such as RP6-24A23.7. In addition, some common target genes regulated by both lncRNA and miRNA were found, such as IGF2BP1, QKI and TNRC6 in hsa-miRNA-9-5p; such as QKI, UPF1 and SFRS1 in hsa-miRNA-128-3p (Table 4).

Table 4 LncRNAs and target genes targeted by hsa-miRNA-9-5p and hsa-miRNA-128-3p

miRNA-9-5p	Target sites	Common target genes	miRNA-128-3p	Target sites	Common target genes
lnc RNA			lnc RNA		
HNRNPU-AS1	1	IGF2BP1;QKI;TNRC6; LIN28B; LIN28; SFRS1	CTD-2319I12.1	1	SFRS1; UPF1
RP11-793H13.8	1	IGF2BP1; LIN28B; LIN28; C17ORF85; SFRS1	POLDIP2	1	FXR2; LIN28; UPF1
RP11-299M14.2	1	/	OIP5-AS1	1	FXR2; LIN28; SFRS1; UPF1
AC093642.3	1	/	RP4-717I23.3	3	UPF1
RP11-170L3.8	1	IGF2BP1; TNRC6; LIN28B; LIN28; SFRS1	SNHG16	1	QKI; FXR2; LIN28; C17ORF85; SFRS1; UPF1
XIST	3	TNRC6; LIN28; C17ORF85; IGF2BP2; SFRS1;LIN28B	RP5-1120P11.1	1	UPF1
SENP3-EIF4A1	1	IGF2BP2;QKI; LIN28B; LIN28; SFRS1	RP11-325K4.3	1	/
PTCHD3P1	1	LIN28B	AC084219.4	1	UPF1
AC005076.5	1	LIN28B	AC083843.1	1	UPF1
AC007246.3	1	/	TP73-AS1	1	QKI; UPF1
UHRF1	1	IGF2BP2; LIN28; SFRS1	ZNRD1-AS1	1	UPF1
PCBP1-AS1	1	LIN28B; LIN28; SFRS1	MIR497HG	3	/
RP6-24A23.7	1	IGF2BP2;QKI; TNRC6; LIN28B	GAS5	1	UPF1; FXR2; LIN28; C17ORF85; SFRS1
ZNRD1-AS1	2	LIN28B	AC068610.3	1	/
LINC00665	1	LIN28B; LIN28	RP11-421L21.3	3	UPF1
RP11-519M16.1	1	/	KCNQ1OT1	1	UPF1
RP11-511P7.2	1	/	RP11-158K1.3	1	UPF1
MAP3K14	1	/	DCP1A	1	FXR2; LIN28; SFRS1; UPF1
RP1-37E16.12	1	IGF2BP2; LIN28B; LIN28; C17ORF85; SFRS1	ZNF252P-AS1	1	/
CTB-25J19.9	1	IGF2BP2; LIN28B	CTC-444N24.11	1	QKI; FXR2; UPF1
RP11-473I1.10	1	IGF2BP2; TNRC6; LIN28B; LIN28	RP11-334J6.4	1	LIN28
RP13-507I23.1	1	/	SNORD62A	1	UPF1
HCG18	1	LIN28B; IGF2BP2; LIN28	XXbac-B461K10.4	1	UPF1
TUG1	1	TNRC6; SFRS1; IGF2BP2; LIN28B; LIN28	LINC00339	1	UPF1
RP11-96D1.10	1	IGF2BP2; TNRC6; LIN28B; LIN28	CTB-89H12.4	1	FXR2; LIN28; SFRS1; UPF1
AL163636.6	1	LIN28B	hsa-mir-210	1	/
SNHG7	1	IGF2BP2; SFRS1; LIN28B; LIN28	RP11-151N17.2	1	UPF1
RP11-64K12.2	1	IGF2BP2; LIN28B	RP6-24A23.7	2	QKI; FXR2
MAGI1-IT1	1	/	RP1-178F10.3	1	FXR2; LIN28; UPF1
RP11-622K12.1	1	IGF2BP2; LIN28B; LIN28	CTA-204B4.6	2	QKI; FXR2; UPF1
ZNF252P-AS1	1	/	AC004696.2	1	/
NEFL	3	IGF2BP2; TNRC6; LIN28B; LIN28; SFRS1	AC090587.2	1	UPF1
NEAT1	1	TNRC6;QKI; SFRS1; IGF2BP2; LIN28B	LA16c-306E5.2	1	FXR2; SFRS1; UPF1
RP11-658F2.8	1	IGF2BP2	PVT1	1	LIN28; SFRS1; UPF1
AC017048.3	1	IGF2BP2	RP11-24B19.3	1	FXR2; UPF1
			RP5-1043L13.1	1	/
			HCP5	1	UPF1

circRNAs Interaction with hsa-miRNA-9-5p and hsa-miRNA-128-3p As for the predicted circRNAs of hsa-miRNA-9-5p and hsa-miRNA-128-3p, we finally found 35 and 27 candidates from StarBase including ZNF280C_hsa_circ_001211, SIAE_hsa_circ_002083 and *etc* (Table 5).

DISCUSSION

ROP, which afflicts premature infants often, is characterized by abnormal retinal vasculature. Onset of ROP occurs when the neurosensory retina is quite immature. Its pathogenesis has not been very clear yet. But the effect of oxygen toxicity

Table 5 circRNAs interaction with hsa-miRNA-9-5p and has-miRNA-128-3p

miRNA-9-5p	Target sites	miRNA-128-3p	Target sites
circ RNA		circRNA	
ZNF280C_hsa_circ_001211	1	SIAE_hsa_circ_002083	1
ACBD6_hsa_circ_000547	1	PHIP_hsa_circ_001319	1
DYM_hsa_circ_001852	1	SLC25A3_hsa_circ_001372	1
MRPS15_hsa_circ_001377	1	ATP7A_hsa_circ_001615	1
WDR48_hsa_circ_000939	1	UBQLN1_hsa_circ_000998	1
GPBP1L1_hsa_circ_001477	1	EIF3B_hsa_circ_001977	1
C2orf29_hsa_circ_000665	1	PHF20_hsa_circ_001403	1
SENP6_hsa_circ_000436	1	EEF2_hsa_circ_001399	1
FAT1_hsa_circ_000713	1	ELP3_hsa_circ_000758	1
NR3C1_hsa_circ_000420	1	AFF1_hsa_circ_000009	1
SPECC1_hsa_circ_000013	1	WDR48_hsa_circ_000939	1
LMNB2_hsa_circ_001499	1	ANKRD17_hsa_circ_001160	1
NOLC1_hsa_circ_000099	1	TCONS_l2_00022231_hsa_circ_000724	1
ING5_hsa_circ_000922	1	FAM120A_hsa_circ_001209	1
EIF2AK3_hsa_circ_001128	1	PLCL2_hsa_circ_001828	1
MYB_hsa_circ_001864	1	TUBA1B_hsa_circ_002179	1
WDR48_hsa_circ_001515	1	FAT1_hsa_circ_000713	1
LNPEP_hsa_circ_000735	1	MRPS35_hsa_circ_001042	1
NFATC1_hsa_circ_001267	1	PSMD1_hsa_circ_001736	1
NFATC3_hsa_circ_002176	1	ZNF780B_hsa_circ_002015	1
MGA_hsa_circ_001756	1	RBM5_hsa_circ_001647	1
TNFRSF21_hsa_circ_001713	1	NEK9_hsa_circ_000182	1
SEC11A_hsa_circ_002047	1	CTBP1_hsa_circ_001895	1
PCDH9_hsa_circ_001561	1	EEF2_hsa_circ_000254	1
MND1_hsa_circ_001152	1	C18orf1_hsa_circ_001090	1
ARHGAP35_hsa_circ_001101	1	ANKRD44_hsa_circ_001698	1
ZNF264_hsa_circ_001634	1	VASP_hsa_circ_000899	1
PIKFYVE_hsa_circ_002114	1		
CSDE1_hsa_circ_001457	1		
ISY1_hsa_circ_001859	1		
ZNF394_hsa_circ_002103	1		
CDK13_hsa_circ_001778	1		
PRRC2B_hsa_circ_000761	1		
PIKFYVE_hsa_circ_001734	1		
SNHG7_hsa_circ_001978	1		

on the retina on the immature vasculature has been reported in two important stages: 1) vasoconstrictive stage: this occurs during exposure to hyperoxia and there is also suppression of the normal anterior ward vascularization of the retina. This mechanism of vasoconstrictive and obliterative effect of oxygen is often seen in the developing retinal vessels; 2) vasoproliferative stage: it involves dilatation and tortuosity of the existing larger vessels with neovascularization and proliferation of new vessels into the vitreous[21]. Consequently, the irreversible vascularization is the core mechanism of ROP.

In the past several years, few reports have shown that miRNA expression is associated with ocular physiologic and pathological processes and displays tissue- and spatiotemporal-specific expression[22-23]. By our further analysis, miRNA-128-3p and miRNA-9-5p are the only up and down regulated miRNAs in ROP among reported profile studies[9-10]. As for miRNA-128-3p, which received less investigation, was generally reported in hepatocellular carcinoma[24] and also involved in ischemia-induced brain injury[25]. About its related lncRNA, POLDIP2 is a molecule that has been shown to be involved in cell migration of vascular cells and angiogenesis[26]. It's also involved in a variety of processes of organ fibrosis lesions[27]. Another lncRNA-GAS5 seems play an important role in angiogenesis and migration, in colorectal cancer it acts as tumor suppressor by inhibiting IL-10 and VEGF expression[28]. Mechanistically, GAS5 regulated endothelial cells and vascular

smooth muscle cells function through β-catenin signaling[29]. So, though obvious evidences are missing, it also suggested the possible roles of miRNA-128-3p and its lncRNA in the aspect of angiogenesis in ROP.

It's worthy noting that PI3K-Akt signaling pathway and TGF-β signaling pathway passed enrichment analysis of hsa-miRNA-128-3p. Di *et al*[30] found CCN1/Cyr61-PI3K/AKT signaling promotes retinal neovascularization in oxygen-induced retinopathy. TGF-β signaling is also essential for the differentiation of retinal pericytes during vascular development of the retina. Kimsa *et al*[31] once found treatment of proliferative vitreoretinopathy is associated with processes regulated by TGF-β, such as inflammation, proliferation, epithelial-mesenchymal transition, and fibrosis.

The sequence of miRNA-9-5p is conserved among species and less reported in the previous references. Inspite of the abundant expression of the brain-specific miRNA (miRNA-9-5p/3p) in both proliferating and postmitotic neurons, most functional studies have focused on their role in neuronal development. Sim *et al*[32] found miRNA-9-5p has an important role in hippocampal synaptic plasticity and memory. In addition, miRNA-9-5p dysregulation is a hallmark of many neurodegenerative disorders, including those involving the retina. It has been described differentially expressed in the RHO-P347S mouse retina, a model for a common form of inherited blindness[33].

As for the lncRNA of miRNA-9-5p, NEFL and UHRF1 have been studied the most widely. Kim *et al*[34] found that mRNA levels of previously described ganglion cell markers, *e.g.* NEFL was highly enriched in the isolated retinal ganglion cell layer. Liu *et al*[35] found NEFL is correlated with human corneal fibroblasts in ocular inflammatory diseases. These data provide the first evidence that NEFL and UHRF1 function are required for retina development and perfection. Enrichment analysis of miRNA-9-5p was mainly located in MAPK signaling pathway. For MAPK signaling pathway, the reactive oxygen species-dependent phosphorylation of EGFR/MAPK is an important signaling pathway for Auranofin-induced inhibition of retinal pigment epithelium cell survival, and may have the potential for treatment of abnormal survival of retinal pigment epithelia cells in proliferative vitreoretinopathy[36].

In conclusion, our data provided a bioinformatics analysis of differentially expressed miRNAs which are involved in ROP. hsa-miRNA-128-3p and hsa-miRNA-9-5p showed significantly differential expression between ROP group and control group. LncRNA of POLDIP2, GAS5, NEFL and UHRF1, circRNA of ZNF280C_hsa_circ_001211 and SIAE_hsa_circ_002083, target gene of QKI and enrichment analysis of TGF-β signaling pathway, PI3K-Akt signaling pathway and MAPK signaling pathway may play important roles in the progress of ROP. Here, we want to remind readers to pay attention to the physiological and pathological differences between animal model and human. Therefore, further genetic and experimental studies with larger sample size are still needed to confirm these results.

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