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Original Article

Bioinformatics analysis of differentially expressed miRNAs in plasma of respiratory distress syndrome

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Abstract: Purpose: This study was aimed to reveal the involvement of miRNAs in RDS by bioinformatics analysis. Methods: The data in this study came from the paper of Kan et al. who analyzed the microRNA (miRNA) expression profile between RDS patients and normal controls. Based on the identified differentially expressed miRNAs, we predicted the target genes, lncRNA and circRNA. Then we performed functional enrichment analysis to analyze the functions of target genes. Results: Hsa-miR-301a and hsa-miR-103-2* showed significantly different expression and had the most predicted target genes. LncRNA of XIST, circRNA of STT3B_hsa_circ_002001, target gene of QKI and enrichment analysis of TGF-β signaling pathway and mTOR signaling pathway may play important roles in the progress of RDS. Conclusions: This research may provide a comprehensive bioinformatics analysis of differentially expressed miRNAs which might be involved in RDS.

Keywords: Bioinformatics, miRNA, respiratory distress syndrome

Introduction

Respiratory distress syndrome (RDS) is a severe type of respiratory disease in neonates, characterized by a lack of pulmonary surfactant (PS). In clinical work, Treatment of this disease has remained difficult, although the assistance of PS and mechanical ventilation has resulted in a remarkable reduction in mortality rate. The key problem to pediatricians is the physiopathologic mechanism of RDS remains unclear.

In our previous report [1], with the assistance of microarrays and subsequent RT-qPCR, we first revealed the involvement of microRNA (miRNA) in RDS by screening miRNAs expression in plasma, and found the abnormal expression of hsa-miR-513a-3p, hsa-miR-103-2*, hsa-miR-130b, hsa-miR-363, hsa-miR-301a, hsa-miR-545, hsa-miR-4284, hsa-miR-3679-3p and hsa-miR-plus-I874* between groups.

In the present study, we took advantage of our previous data to analyze the miRNA expression profile between 20 infants with RDS and 29 infants without RDS. Based on the differentially expressed miRNAs, we predicted the related target genes, lncRNA and circRNA. In addition, we performed functional enrichment analysis to analyze the functions of the target genes. We aimed to further reveal the involvement of miRNAs in the progression of RDS.

Data and methods

Data source

The data in our study came from the study of Kan et al. [1]. They analyzed the miRNA expression profile data of plasma between 20 infants with RDS and 29 infants without RDS (controls) at a gestational age of 28-34 weeks. Finally, 9 differentially expressed miRNAs were identified, 2 up-regulated and 6 down-regulated ones were further selected into this study (**Table 1**). Based on these findings, we try to identify the roles of miRNA associated with RDS.

Target gene prediction

The target genes of miRNAs were predicted by seven published databases, including miRanda (http://microrna.sanger.ac.uk) [2], MirTarget2 (http://nar.oxfordjournals.org/cgi/content/abstract/34/5/1646) [3], PicTar (http://

Table 1. Differentially expressed miRNAs and target genes

Name	State	Sequence	Fold change	*p*-Value	Chromosome location	Target genes
Hsa-mir-513a-3p	Up	UAAAUUUCACCUUUCUGAGAAGG	2.046	0.0034	chrX: 147213463–147213591 [-]	0
Hsa-mir-103-2*	Up	AGCUUCUUUACAGUGCUGCCUUG	1.319	0.0030	chr20: 3917494-3917571 [+]	687
Hsa-mir-130b	Down	GGCCUGCCCGACACUCUUUCCCUGUUGCACUACUAU-AGGCCGCUGGGAAGCAGUGCAAUGAUGAAAGGGCAUC-GGUCAGGUC	0.769	0.0079	chr22: 21653304-21653385 [+]	767
Hsa-mir-363	Down	UGUUGUCGGGUGGAUCACGAUGCAAU-UUUGAUGAGUAUCAUAGGAGAAAAAUUGCACGGUAUC-CAUCUGUAAACC	0.687	0.0068	chrX: 134169378-134169452 [-]	351
Hsa-mir-301a	Down	ACUGCUAACGAAUGCUCUGACUUUAUUGCACUACUGUA-CUUUACAGCUAGCAGUGCAAUAGUAUUGUCAAAGCAU-CUGAAAGCAGG	0.646	0.0383	chr17: 59151136-59151221 [-]	777
Hsa-mir-545	Down	CCCAGCCUGGCACAUUAGUAGGCCUCAGUAAAU-GUUUAUUAGAUGAAUAAAUGAAUGACUCAUCAGCAAA-CAUUUAUUGUGUGCCUGCUAAAGUGAGCUCCACAGG	0.678	0.0254	chrX: 74287104-74287209 [-]	215
Hsa-mir-4284	Down	GUUCUGUGAGGGGCUCACAUCACCCCAU-CAAAGUGGGGACUCAUGGGGAGAGGGGGUAGUUAG-GAGCUUUGAUAGAGGCGG	0.685	0.0203	chr7: 73711317-73711397 [+]	0
Hsa-mir-3679-3p	Down	CUUCCCCCCAGUAAUCUUCAUC	0.575	0.0125	chr2: 134127125-134127192 [+]	0

pictar.bio.nyu.edu) [4], Probability of Interaction by Target Accessibility (PITA, http://genie.weizmann.ac.il/pubs/mir07) [5], TargetScan (http://targetscan.org) [6], miRecords (http://miRecords.umn.edu/miRecords) [7], Mirwalk (http://mirwalk.uni-hd.de/) [8]. The miRNA-gene pair would be reserved when it was predicted by not less than 4 of the databases above.

Functional enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID, (http://david.niaid.nih.gov) [9] has been developed for systematically mapping a great number of 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 tool. The p-value < 0.05 was considered as threshold.

miRNA-lncRNA regulatory relationship prediction

StarBase v2.0 (http://starbase.sysu.edu.cn/) [10] database is used to systematically identify the RNA-RNA and RNA-protein interaction networks (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/) [11] is a database for annotating and discovering transcriptional regulatory relationships of miRNAs and lncRNAs binding maps from ChIP-Seq data. In our study, all the interaction networks of miRNA-lncRNA were downloaded from starBase v2.0 and ChIPBase, the sub-networks related to the differentially expressed miRNAs were selected.

miRNA-circRNA regulatory relationship prediction

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

Results

Differentially expressed miRNAs and target genes

9 differentially expressed miRNAs were identified through our previous study (Fold change > 1.3 and p-value < 0.05), and 2 up-regulated and 6 down-regulated ones were further selected into this study (**Table 1**). The target genes were reserved when it was predicted by not less than 4 of the databases above. Based on the number of predicted target genes (**Table 1**), we chose one up-regulated miRNAs (Hsa-miR-103-2*) and one down-regulated miRNAs (Hsa-miR-301a) for further study.

Function enrichment analysis of hsa-miR-103-2 and hsa-miR-301a target genes*

The target genes of hsa-miR-103-2* and hsa-miR-301a were performed GO enrichment

Table 2. GO analysis of hsa-miR-103-2* and hsa-miR-301a target genes

Hsa-miR-301a-3p (Top10)				Hsa-miR-103-2* (Top10)			
Term	Count	*p*-Value	FDR	Term	Count	*p*-Value	FDR
GO: 0006351 transcription, DNA-dependent	50	1.72E-6	5.8E-4	GO: 0008013 beta-catenin binding	5	8.30E-5	5.6E-3
GO: 0045944 positive regulation of transcription from RNA polymerase II promoter	27	2.60E-6	4.4E-4	GO: 0005515 protein binding	57	1.60E-4	5.6E-3
GO: 0009952 anterior/posterior pattern specification	9	1.37E-5	1.5E-3	GO: 0017048 Rho GTPase binding	3	1.10E-3	2.5E-2
GO: 0000290 deadenylation-dependent decapping of nuclear-transcribed mRNA	3	2.86E-5	2.4E-3	GO: 0042054 histone methyltransferase activity	2	1.44E-3	2.5E-2
GO: 0006897 endocytosis	9	4.19E-5	2.8E-3	GO: 0003682 chromatin binding	8	1.68E-3	2.3E-2
GO: 0051081 nuclear envelope disassembly	2	2.10E-4	1.2E-2	GO: 0003700 sequence-specific DNA binding transcription factor activity	15	1.80E-3	2.0E-2
GO: 0045893 positive regulation of transcription, DNA-dependent	18	2.30E-4	1.1E-2	GO: 0046972 histone acetyltransferase activity (H4-K16 specific)	2	1.85E-3	1.8E-2
GO: 0033962 cytoplasmic mRNA processing body assembly	3	2.30E-4	1.1E-2	GO: 0043996 histone acetyltransferase activity (H4-K8 specific)	2	1.85E-3	1.8E-2
GO: 0008610 lipid biosynthetic process	3	3.20E-4	1.2E-2	GO: 0043995 histone acetyltransferase activity (H4-K5 specific)	2	1.85E-3	1.8E-2
GO: 0006355 regulation of transcription, DNA-dependent	33	3.60E-4	1.2E-2	GO: 0008270 zinc ion binding	26	2.07E-3	1.4E-2

Data were arranged as *p*-value.

Table 3. Pathway enrichment analysis of hsa-miR-103-2* and hsa-miR-301a target genes

Hsa-miR-301a-3p (Top10)				Hsa-miR-103-2* (Top10)			
Term	Count	*p*-Value	FDR	Term	Count	*p*-Value	FDR
TGF-beta signaling pathway	17	1.36E-8	2.1E-6	Ubiquitin mediated proteolysis	48	3.79E-13	8.5E-11
Endocytosis	21	5.16E-5	4.1E-3	Hippo signaling pathway	38	3.59E-6	4.0E-4
Circadian rhythm	7	1.30E-4	7.0E-3	Circadian rhythm	13	1.07E-5	8.0E-4
RNA degradation	10	4.50E-4	1.8E-2	Cell cycle	31	2.08E-5	1.2E-3
mTOR signaling pathway	9	5.40E-4	1.7E-2	Pathways in cancer	62	4.86E-5	2.2E-3
HTLV-I infection	22	8.20E-4	2.1E-2	mTOR signaling pathway	18	9.14E-5	3.4E-3
Phosphatidylinositol signaling system	10	1.06E-3	2.4E-2	Prostate cancer	23	1.4E-4	4.3E-3
Pathways in cancer	25	1.29E-3	2.5E-2	Oocyte meiosis	26	2.5E-4	7.0E-3
Axon guidance	13	2.04E-3	3.6E-2	HTLV-I infection	50	2.6E-4	6.4E-3
Prostate cancer	10	2.63E-3	4.1E-2	RNA degradation	19	3.1E-4	7.0E-3

Data were arranged as *p*-value.

Table 4. lncRNAs and target genes targeted by hsa-miR-103-2* and hsa-miR-301

Hsa-miR-301a-3p			Hsa-miR-103-2*		
LncRNA name	Target sites	Commen target genes	LncRNA name	Target sites	Commen target genes
CTA-204B4.6	5	QKI; TNRC6	*CTA-204B4.6*	4	QKI; ZC3H7B
XIST	3	TNRC6	*XIST*	1	ZC3H7B
AC084219.4	1	/	*AC084219.4*	1	ZC3H7B
RP11-361F15.2	1	/	*RP11-361F15.2*	1	/
LINC00116	1	TNRC6	RP11-102F4.3	1	/
MIR17HG	2	/	SENP3-EIF4A1	1	QKI; ZC3H7B; SFRS1
RP11-656D10.3	1	/	NEAT1	1	ZC3H7B; QKI; SFRS1
RP11-197P3.5	1	/	RP6-24A23.7	1	QKI; ZC3H7B
MAP3K14	1	/	AD000090.2	1	ZC3H7B; SFRS1
RP11-290D2.4	1	/	PTCHD3P1	2	ZC3H7B
RP11-363E7.4	1	/	DCP1A	1	ZC3H7B
CTB-92J24.2	1	/	RP11-473I1.10	1	ZC3H7B
RP11-84C13.1	1	/	RP3-486L4.3	1	ZC3H7B
HOXD-AS1	1	/	FGD5-AS1	1	ZC3H7B
RP1-178F10.3	1	/	RP11-446H18.3	1	/
PRKCQ-AS1	1	/	RP11-923I11.1	1	/
CTC-281B15.1	1	/	LINC00662	2	/
LINC00667	1	/	CTC-273B12.6	1	/
CTD-2369P2.2	1	/	H19	2	/
			AC016747.3	1	/
			RP11-383J24.5	1	/
			RP11-21N3.1	1	/
			LINC00152	1	/
			C14orf169	1	/
			TTC28-AS1	1	/

analysis and the result was shown in **Table 2**. The target genes of hsa-miR-103-2* were mainly enriched in regulation of transcription. And the target genes of hsa-miR-301a were mainly enriched in protein binding, histone acetyltransferase and chromatin binding.

Pathway enrichment analysis of hsa-miR-103-2 and hsa-miR-301a target genes*

The pathways enriched by the target genes of hsa-miR-103-2* and has-miR-301a were shown in **Table 3**. The target genes of hsa-

Table 5. CircRNAs interaction with hsa-miR-103-2* and hsa-miR-301a

Hsa-miR-301a-3p		Hsa-miR-103-2*	
CircRNA name	Target sites	CircRNA name	Target sites
STT3B_hsa_circ_002001	1	NFX1_hsa_circ_001612	1
DNAJC16_hsa_circ_000541	1	PDHX_hsa_circ_001223	1
USP48_hsa_circ_001786	1	GMPS_hsa_circ_001595	1
MBOAT2_hsa_circ_002141	1	PRKAA1_hsa_circ_000955	2
CNIH4_hsa_circ_002125	1	YTHDF1_hsa_circ_000663	1
GDI2_hsa_circ_001837	1	GMPS_hsa_circ_001957	1
SUPT16H_hsa_circ_000586	1	BACH1_hsa_circ_000666	1
MRPS35_hsa_circ_001042	1	PCDH9_hsa_circ_001561	1
MBOAT2_hsa_circ_000689	1	CFL1_hsa_circ_001842	1
		LMTK2_hsa_circ_002138	1
		ETFA_hsa_circ_000858	2
		ERC1_hsa_circ_000133	1
		GMPS_hsa_circ_001860	1
		APOBEC3C_hsa_circ_001120	1
		ANKIB1_hsa_circ_001193	2
		NFATC3_hsa_circ_002060	1
		NFATC3_hsa_circ_000216	1
		TUBA1B_hsa_circ_002179	2
		NUP88_hsa_circ_001080	1
		TUBGCP3_hsa_circ_001373	1
		TSPAN3_hsa_circ_001995	1
		ISY1_hsa_circ_001859	2

miR-103-2* were mainly enriched in Ubiquitin mediated proteolysis pathway, Hippo signaling pathway, mTOR signaling pathway. And the target genes of hsa-miR-301a were mainly enriched in TGF-β signaling pathway, mTOR signaling pathway and RNA degradation pathway.

lncRNAs targeted by hsa-miR-103-2 and hsa-miR-301a*

From the predicted lncRNAs of hsa-miR-103-2* and hsa-miR-301a, we found that some lncRNAs were regulated by both miRNAs, such as CTA-204B4.6, XIST, AC084219.4, RP11-361F15.2. In addition, some common target genes regulated by both lncRNA and miRNA were found, such as QKI and TNRC6 in hsa-miR-301a, such as QKI, ZC3H7B and SFRS1 in hsa-miR-103-2*. Shown in **Table 4**.

circRNAs interaction with hsa-miR-103-2 and hsa-miR-301a*

As for the predicted circRNAs of hsa-miR-103-2* and hsa-miR-301a, we finally found 22 and 9 candidates from StarBase including STT3B_hsa_circ_002001, NFX1_hsa_circ_001612 and etc. Shown in **Table 5**.

Discussion

Though clinical advancements in neonatal techniques have improved the survival rate of preterm infants, the incidence of RDS didn't show a downward trend [12, 13]. Up to now, it has been suggested that RDS is a complex network disease which's characterized by immature lung development and lack of PS. In the past several years, the let-7 family and miR-17-92 cluster have been demonstrated for the most times to be important in mammals' lung development [14-17]. Johnson *et al* [7] showed that let-7 is highly expressed in normal lung tissue, and can repress cell proliferation pathways. Ventura *et al* [19] demonstrated that mice deficient in the miR-17-92 cluster exhibit lung hypoplasia defects, characterized by smaller, hypoplastic lungs. In our preliminary study [19], several novel differentially expressed miRNAs were also identified during normal lung development in rats, including miR-103 and miR-301a, which were differentially expressed in our RDS patients [1].

As for hsa-miR-103-2*, which received less investigation, was generally reported in response to hypoxia [20] and is also involved in nervous system [21]. About its related lncRNA, Tantai J once reported the expression of XIST in serum as an effective screening for non-small cell lung cancer [22]. While the results of Hatakeyama S et al strongly suggest that the predicted target gene SFRS is responsible for fucosylated carbohydrate-dependent lung metastasis of epithelial cancers.

However, it's worth noting that the ubiquitin mediated proteolysis pathway which passed enrichment analysis of hsa-miR-103 was found

the key pathway involved in acute lung injury in mouse model [23]. Similarly, Chung C et al showed that the mammalian hippo kinases play crucial roles in surfactant homeostasis and coordination of peripheral lung differentiation through regulation of Foxa2 rather than of YAP, which also suggested the potential effect of this pathway in lung development [24, 25].

Hsa-miR-301a is a newly identified miRNA, of which the majority of associated studies have focused on cancer. Lu *et al* demonstrated that miR-301a down-regulates nuclear factor-κB repressing factor (NKRF) in cancer cells [26]. This is considered to interact with specific negative regulatory elements to mediate the transcriptional activity of NF-κB, which regulates the expression of three NF-κB-responsive genes, IL-8, IFN-b, and nitric oxide synthase 2A. And IL-8 is a cytokine with high levels of expression in the lung tissues of patients of model animals with RDS [27, 28]. Enrichment analysis of hsa-miR-301a was located in TGF-β signaling pathway and mTOR signaling pathway.

For mTOR signaling pathway, Ikeda H *et al* proved that aberrant activation of the Akt-mTOR pathway in lung epithelium plays a causal role in the pathogenesis of infant RDS, presumably through down-regulation of HIF-2-dependent VEGF expression in the lung. TGF-β signaling pathway is another prestigious pathway in the lung disease including RDS, Lung cancer and pulmonary fibrosis. Under normal circumstances, TGF-β inhibition enhanced airway branching by acting on Smad2/3/4. In addition, many important pathways related to lung development diseases such as Notch signaling is also related to TGF-β signaling pathway [29]. In the case of lung injury, report from Wesselkamper SC showed that genes that decreased the most after nickel exposure play important roles in lung fluid absorption or surfactant and phospholipid synthesis, and genes that increased the most were involved in TGF-β signaling. MAPPFinder analysis further established TGF-β signaling to be significantly altered. TGF-β-inducible genes involved in the regulation of extracellular matrix function and fibrinolysis were significantly increased after nickel exposure, treatment with TGF-β1 dose-dependently repressed Sftpb promoter activity *in vitro*. These data suggest that TGF-β acts as a central mediator of acute lung injury through the alteration of several different molecular pathways [30].

In conclusion, our data provided a comprehensive bioinformatics analysis of differentially expressed miRNAs which might be involved in RDS. Hsa-miR-301a and hsa-miR-103-2* showed significantly different expression and had the most predicted target genes. LncRNA of XIST, circRNA of STT3B_hsa_circ_002001, target gene of QKI and enrichment analysis of TGF-β signaling pathway and mTOR signaling pathway may play important roles in the progress of RDS. However, further genetic and experimental studies with larger sample size are still needed to confirm our results.

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Disclosure of conflict of interest

None.

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