Role of CircRNA in Congenital Diaphragmatic Hernia

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Introduction

Congenital diaphragmatic hernia (CDH) is a devastating congenital anomaly. CDH babies are born with a hole in their diaphragm and abnormal lung development. The abnormal lungs cause high mortality. There is a lack of biomarkers and a poor understanding of the pathogenesis of CDH.[1] Several works of medical literature have shown that circRNAs influence many human diseases, leading to various treatment options but the relationship between CDH and circRNAs is unknown.[2] In the present study, we have analyzed the expression profile of circRNAs from the lung samples collected from control and CDH rats and amniotic fluid samples from survivors and non-survivors to explore the role of CircRNAs in CDH.

[1] Ameis D, Khoshgoo N, Keijzer R. Abnormal lung development in congenital diaphragmatic hernia. Semin Pediatr Surg. 2017 Jun;26(3):123-128. doi: 10.1053/j.sempedsurg.2017.04.011. Epub 2017 Apr 25. PMID: 28641748.

[2]Tang M, Kui L, Lu G, Chen W. Disease-Associated Circular RNAs: From Biology to Computational Identification. Biomed Res Int. 2020 Aug 17;2020:6798590. doi: 10.1155/2020/6798590. PMID: 32908906; PMCID: PMC7450300.

Methods

- CircRNA arrays were carried out on the lung tissue collected from E15 (3 Control and 3 Nitrofen-Treated Rats) and E21 (3 Control and 3 Nitrofen-Treated Rats)
- Amniotic fluid samples were collected from 3 survivors and 3 non-survivors
- The data was quintile standardized and further processed using R's Limma function. The final normalized intensity in the analysis was derived using log2-transformed normalized data.
- Using Benjamini and Hochberg's technique, P-values were adjusted to decrease false discovery rates. CircRNAs with a p-value of less than 0.05 was significant

Results

CDH lungs and amniotic fluid have a unique circular RNA profile compared to control. Similar differentially expressed CircRNAs are present.

Table 1: Downregulated CircRNAs in Rat and Non-Survivor Amniotic Fluid Compared With Survivor

circRNA (Rat)	circRNA (Human)	Chromosome		Gene
rno_circRNA_013451		chr9	exonic	CDK5RAP2
mmu_circRNA_29702		chr3	exonic	GSK3B
rno_circRNA_011534		chr3	exonic	ITPR1
rno_circRNA_010241		chr9	exonic	SPTAN1
rno_circRNA_016796		chr5	exonic	FBXL17
rno_circRNA_013479		chr9	overlapping	KDM4C
rno_circRNA_008767		chr1	overlapping	LRIG2
mmu_circRNA_42344		chr10	exonic	TIAL1
rno_circRNA_006702	hsa_circRNA_017403	chr10	exonic	LARP4B
	hsa_circRNA_104069	chr6	exonic	NUP153
	hsa_circRNA_103748	chr4	exonic	ARHGAP10
	hsa_circRNA_404773	chr10	exonic	CDH23
	hsa_circRNA_069146	chr4	exonic	AFAP1
	hsa_circRNA_405855	chr2	sense	DHX57
	hsa_circRNA_085144	chr8	exonic	PABPC1
	hsa_circRNA_407055	chr8	exonic	NRG1
	hsa_circRNA_102896	chr2	exonic	BMPR2
	hsa_circRNA_403805	chr7	exonic	STARD3NL

Figure 1: Interaction of Rat circRNAs with miRNAs

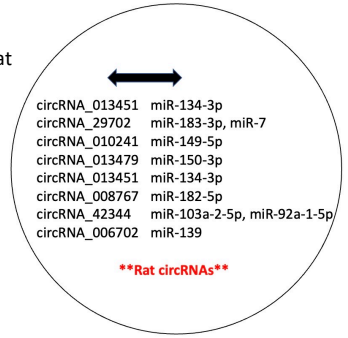


Figure 2: Interaction of Amniotic Fluid circRNAs with miRNAs

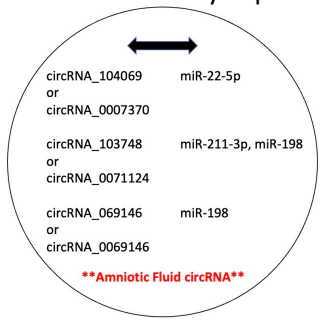
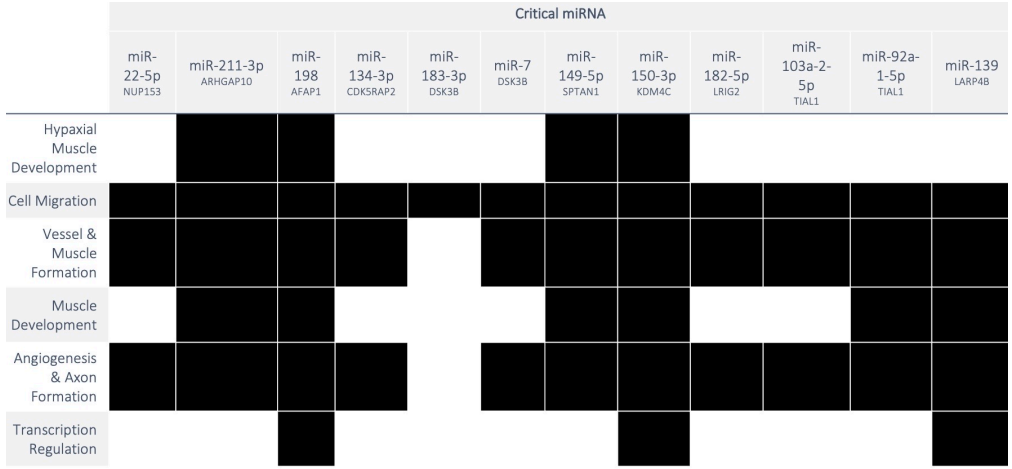


Figure 3: Interaction partners of the miRNAs with the genes involved in Hypaxial Muscle Development (SIX4, EYA2, SIX1, EYA1, RC3H1, PTPN13, EFNB1), Cell Migration (EYA1, GAB1), Vessel & Muscle Formation (MMP2, MMP14), Muscle Development (CDKN1C, NR2F2, MYOD1, MEF2A, MYOG, PBX1), Angiogenesis & Axon Formation (SLIT3, ROBO2, ROBO1), Transcription Regulation (TBX6, CDH7, ZFHX4, GLI2, STK36, GLI3, CTBP2, ELAC2, TGIF1, ZEB1, CTBP1, ZFPM2, GATA4, TBX, SLIT3, ROBO2, ROBO1, IGF1R, NEDD4, COL20A1, WT1)



Conclusion

circRNAs can serve as a biomarker to predict outcomes in CDH. The similar expression profile of the CircRNAs in both CDH lung of rat and the amniotic fluid sample is quite interesting, suggesting that nitrofen-treated rat model could potentially be used as a study model for the development of biomarkers against CDH. Future work includes experimental validation for the CircRNA candidates, RBP analysis interaction, and RNA sequencing analysis for complete transcriptomics analysis.