

Background

- Research-focused studies have resulted in beneficial therapies for adults with heart failure (HF), as measured by significant improvement in morbidity and mortality in this population.
- In contrast, though the same therapeutic guidelines developed from adult clinical trials have been applied to children with HF, improvement in clinical outcomes has been minimal, which has resulted in limited survival in this population.
- Our previous studies using pediatric dilated cardiomyopathy (DCM) hearts identified unique age-dependent cellular and molecular characteristics including changes in miRNAs.
- microRNAs (miRNAs) are short single stranded nucleotides that can regulate gene expression.
- Although expression of miRNAs in pediatric dilated cardiomyopathy (DCM) has been evaluated by miRNA array, pathway prediction based on changes in mRNA expression has not been previous analyzed in this population.
- The current study aimed to determine regulation of miRNA expression by miRNA-Seq and, through mRNA-Seq, analyze their putative target genes and altered pathways in pediatric DCM hearts.

Methods

- miRNA expression was determined by miRNA-Seq in pediatric DCM patients [n=10 non-failing (NF), n=20 DCM].
- Expression of a subset of miRNAs was evaluated in adult DCM patients (n=11 NF, n=13 DCM).
- miRNA-mRNA prediction analysis was performed using mRNA-Seq data (n=7 NF, n=7 DCM) from matched samples.
- Functional analysis of putative target genes was performed using PANTHER and ingenuity pathway analysis (IPA)

Results

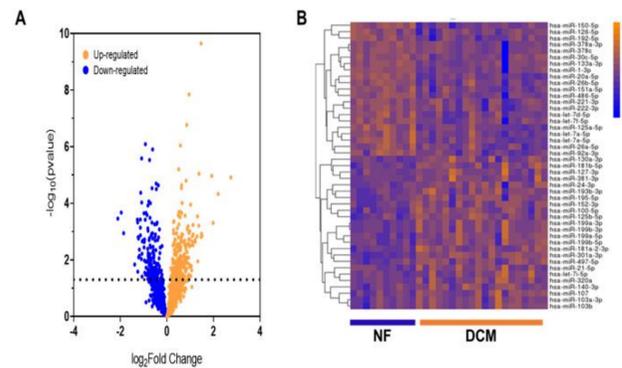


Figure 1. miRNA expression analysis in pediatric DCM patients and NF controls (A) 393 (227 miRNAs up-regulated, 166 miRNAs down-regulated) miRNAs significantly differentially expressed in the heart of pediatric DCM patients (B) 45 miRNAs with high FPKMs differentially expressed in pediatric DCM compared to the non failing control.

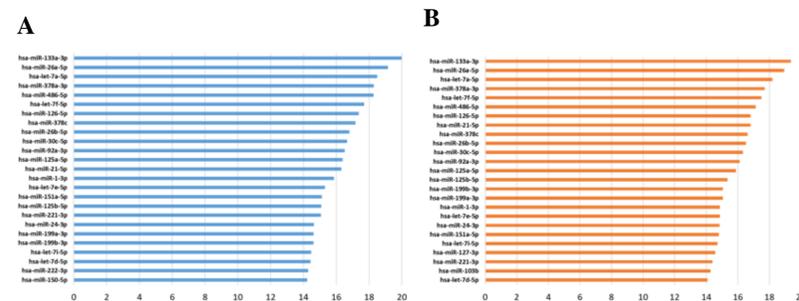


Figure 2. (A) The top 25 most highly expressed miRNAs ranked high to low based on average counts in NF controls (B) The top 25 most highly expressed miRNAs ranked high to low based on average counts in DCM patients.

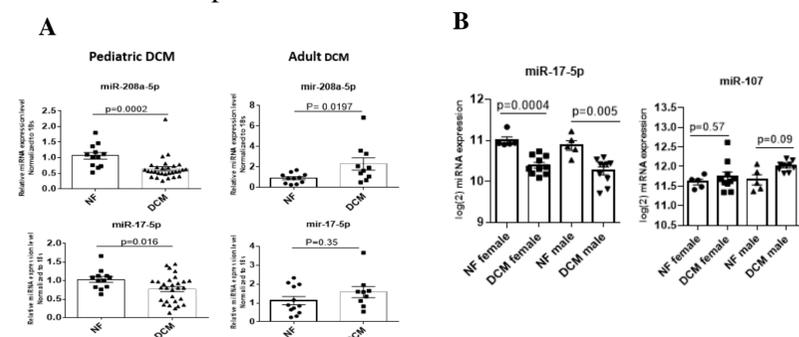


Figure 3. (A) miRNAs uniquely regulated in pediatric DCM compared to adult DCM patients (B) miRNA expression affected by sex differences in pediatric DCM patients.

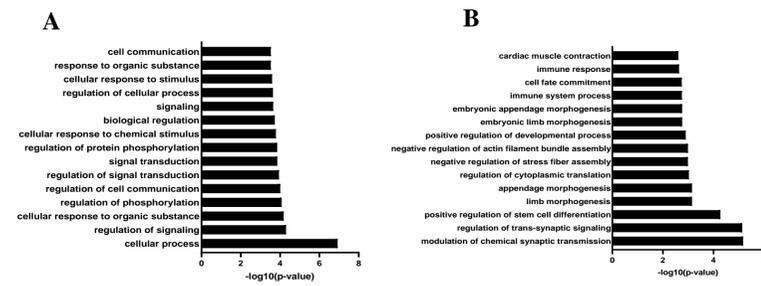


Figure 4. Functional analysis of dysregulated putative target genes using PANTHER (A) Top 15 biological processes associated with the down-regulated putative target genes (B) Top 15 biological processes associated with the up-regulated putative target genes.

Canonical Pathways	-log(p-value)
STAT3 Pathway	6.66
Synaptogenesis Signaling Pathway	6.6
ErbB Signaling	6.3
Cardiac Hypertrophy Signaling (Enhanced)	6.29
ERK5 Signaling	5.86
PI3K/AKT Signaling	5.8
Acute Myeloid Leukemia Signaling	5.74
HGF Signaling	5.47
FAK Signaling	5.44
ErbB2-ErbB3 Signaling	5.41

Table 1. IPA enriched top canonical pathways related to the mRNAs changed by RNA-Seq that are predicted targets of significantly differentially regulated miRNAs in the heart of pediatric DCM patients.

Categories	-log(p-value)
Cardiac Necrosis/Cell Death	5.28
Cardiac Enlargement	4.60
Cardiac Proliferation	4.22
Cardiac Dilatation	4.14
Cardiac Arrhythmia	3.43
Congenital Heart Anomaly	2.75
Cardiac Dysfunction	2.74
Cardiac Arterioopathy	2.27
Cardiac Stenosis	2.19
Heart Failure	1.83
Congestive Cardiac Failure	1.76
Cardiac Damage	1.75
Cardiac Hypoplasia	1.61
Cardiac Inflammation	1.61
Cardiac Infarction	1.34

Table 2. IPA enriched top tox function related to the mRNAs changed by RNA-Seq that are predicted targets of significantly differentially regulated miRNAs in the heart of pediatric DCM patients.

Summary

- Several miRNAs are altered in pediatric DCM patients compared to NF control.
- Age- and sex-specific regulation of miRNAs has been identified in pediatric DCM patients.
- The putative target genes of dysregulated miRNAs are related to biological processes such as cardiac muscle contraction, positive regulation of stem cell differentiation and cellular process and are involved in pathways related to cardiac toxicity.
- Therefore, further investigations of the implication of dysregulated miRNAs in the hearts of children with DCM may help lead to identification of potential age-specific miRNA-based therapy.

Conclusions

- Our results demonstrated a unique age-dependent regulation of miRNAs and their putative target genes which may contribute to distinctive phenotypic characteristics of DCM in children.

Impact

- This is the first study to compare miRNA expression in the heart of pediatric dilated cardiomyopathy patients to age-matched healthy controls by RNA-sequencing
- Expression of a subset of miRNAs is uniquely dysregulated in children
- Using mRNA-seq and miRNA-seq from matched samples, target prediction was performed.
- This study underscore the importance of pediatric-focused studies.

Acknowledgements

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