Circulating Exosomal miRNAs Profiles in Children's Nonalcoholic Steatohepatitis

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Abstract

Background: The incidence of non-alcoholic fatty liver disease (NAFLD) in children increased rapidly paralleled with the global burden of obesity and diabetes. Although most patients are nonalcoholic fatty liver (NAFL), once progress to nonalcoholic steatohepatitis (NASH), the risk of liver fibrosis and cirrhosis increase significantly. However, the pathogenesis of NAFLD, especially how NAFL progress to NASH is still unclear. Exosomal miRNAs have attracted attention to provide further insights into the pathogenesis of NASH, and it may also serve as biomarkers of NASH.

Methods: Children diagnosed as NASH (n=20) and age matched health control (n=20) were enrolled in this study. They were randomly divided into test set (3 NASH/3Controls) and validation set (17 NASH/17Controls). Circulating exosomes were isolated from both sets according to the protocol of the miRCURY Exosome Serum/Plasma Kit. For the test set, Illumina HiSeqTM 2500 was performed to analyze the differential expression of exosomal miRNAs between the two groups; bioinformatics analysis was applied to identify the molecular signature differences. The differentially expressed miRNAs were further validated in the validation set. ANOVA analysis was used to compare the clinical parameters between the NASH and control group. Spearman correlation analysis was used to investigate the association between differential miRNAs and indicators of body fat, inflammation, glucose and lipid metabolism.

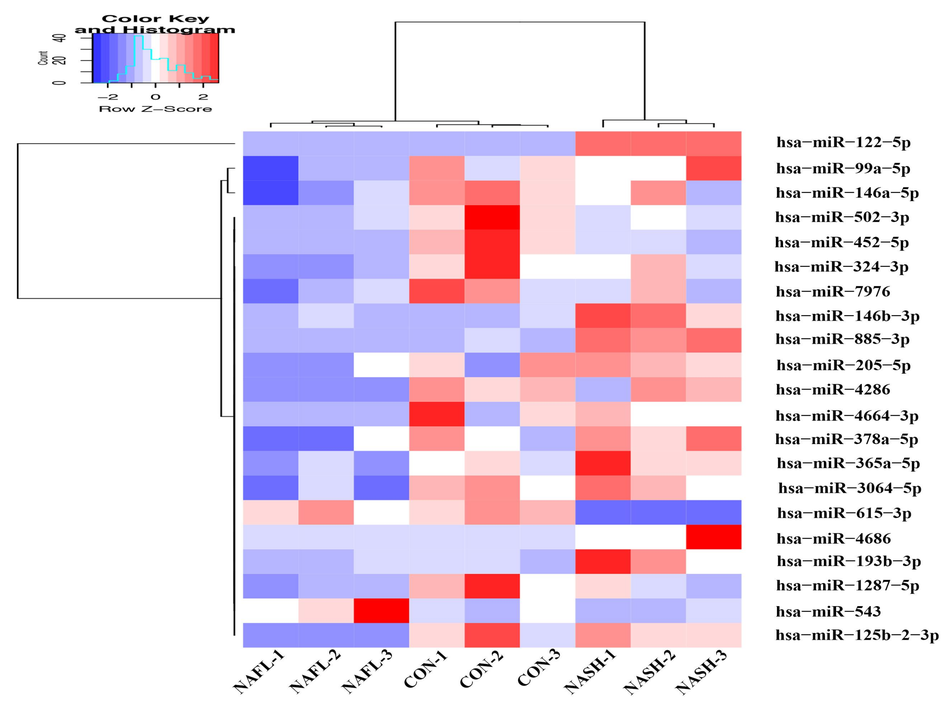

Figure 1. The differential expressed exosomal miRNAs

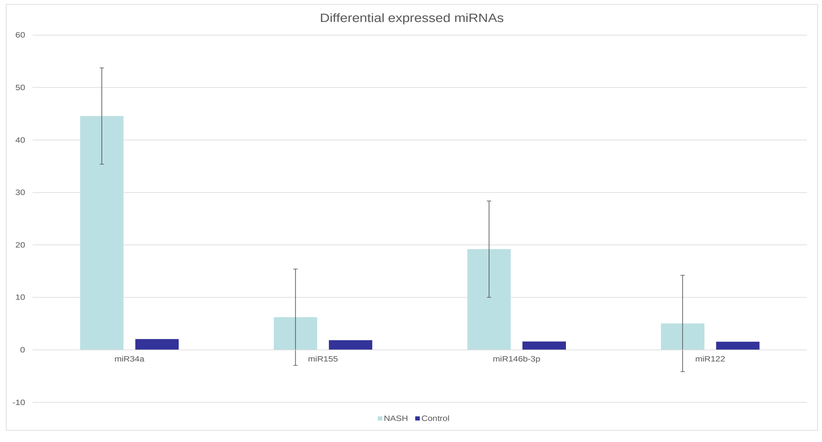

Figure 2. Clinical validation of the target miRNAs

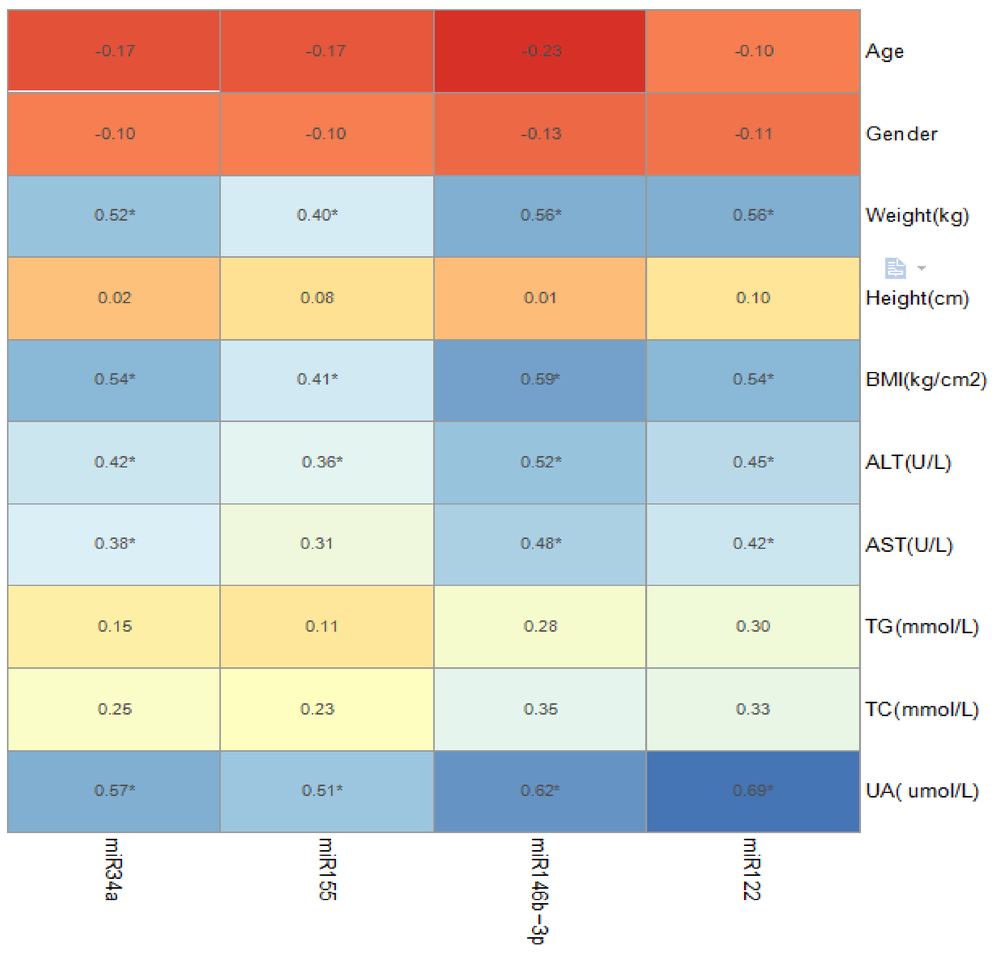

Figure 3. Correlation analysis between the target miRNAs and clinical parameters

Results: Exosomes were validated by NTA and flow cytometry (CD81 and CD63). With Illumina HiSeqTM 2500, 42 miRNAs were differentially expressed (| log2(fold change) | ≥1, P<0.05) in the test set. GO annotation and Pathway analysis revealed that the differential miRNAs were involved in lipid metabolism, insulin signaling, apoptosis and inflammation pathway. Among which, miRNA122, miRNA34a, miRNA155 and miRNA146b-3p were up-regulated significantly in NASH group than the control Group (P＜0.05). These 4 miRNAs were positively correlated with BMI (r,0.41-0.59), ALT (r, 0.36-0.52), AST (r, 0.31-0.48) and Uric Acid (UA, r, 0.51-0.69) (P＜0.05). While there is no relationship between the miRNAs with triglyceride and cholesterol.

Conclusions: Circulating exosomal miRNA122, miRNA34a, miRNA155 and miRNA146b-3p were up-regulated in NASH group, and positively correlated with serum transaminase and UA. So the exosome derived miRNAs may involve in the pathogenesis of NASH and can be used as a potential biomarker for diagnosis of children NASH.

Acknowledgement

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