Analysis of circulating miRNAs in obese children born small for gestational age

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Background

Children born small for gestational age (SGA) are at increased risk of obesity and metabolic complications, particularly those with a rapid weight gain.
A major problem is the lack of specific molecular biomarkers to use in clinical practice to identify SGA children at higher cardiometabolic risk.
MicroRNAs (miRNAs) are small non-coding regulatory elements, composed of 18-25 nucleotides, that are promising nonivasive biomarkers for detection, classification and prognosis of metabolic diseases.
An association between obesity and altered expression of certain miRNAs has been observed in adults and children.

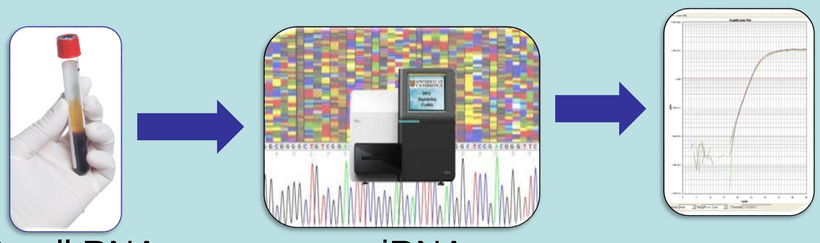
Objectives and hypotheses

This study aimed at investigating the profiles of circulating miRNAs in obese SGA children to identify new molecular biomarkers associated with increased cardiometabolic risk.

Methods

We recruited 4 SGA obese children (BMI-SDS 2.41 ± 0.72, 11.96 ± 1.76 yrs), 4 AGA (appropriate for gestational age) obese children (BMI SDS 2.38 ± 0.57, 13.61 ± 0.5 yrs) and their respective controls matched for sex and age (4 SGA normal weight and 4 AGA normal weight children).
Small RNAs have been extracted by serum by using miRNeasy kit (Qiagen), dosed using Nanodrop (Thermofisher) and Bionalyzer (Agilent) and sequenced using MiSeq platform (Illumina). Raw reads were subjected to quality trimming and adapter removal using a custom perl script. Expression levels were estimated by aligning reads in between 17 bp and 25 bp in size to the complete collection of mature human miRNA (derived from miRBase, version 21) allowing up to 1 mismatch. Read counts were normalized using the trimmed mean normalization, as implemented in the edgeR package. Differential expression analysis was carried out using the latest version of the limma and the edgeR R packages.

Only miRNAs found differentially expressed (FDR <= 0.05) by both methods were considered for downstream analyses. Fold change > 1.5 or < 0.6 were considered for further analyses. The results were validated by RT-qPCR and extended to a wider cohort of subjects. Statistical analyses were performed by Student's t-test.



Results

As shown in Table 1, in the group of obese SGA children, eleven miRNAs were up regulated and five were down regulated, among which five (miR-423-5p, miR-92a-3p, miR486-3p, miR-484, miR-181b-5p) were shared with AGA obese children (blue), suggesting that the differential expression of these miRNAs is correlated with the obesity, independently of SGA condition.
Moreover, eleven miRNAs (miR-486-5p, miR-122-5p, miR-16-5p, miR-532-5p, miR-425-5p, miR-3615, miR-16-2-3p, miR-143-3p, miR-223-3p, miR-23a-3p, miR28-5) appeared specifically correlated with obesity in SGA children (red).

Upregulated	obese SGA	fold change	obese AGA	fold change
miR-423-5p	↑	2,4	↑	1,7
miR-92a-3p	↑	3,8	↑	2,6
miR-486-3p	↑	4,6	↑	2,3
miR-486-5p	↑	4,3		
miR-451a			↑	2,8
miR-25-3p			↑	1,8
miR-15a-5p			↑	2,3
miR-30d-5p			↑	1,9
let-7b-5p			↑	1,8
miR-484	↑	2,5	↑	3,4
miR-660-5p			↑	2
miR-128-3p			↑	2,5
miR-122-5p	↑	4		
miR-16-5p	↑	1,8		
miR-532-5p	↑	2,3		
miR-425-5p	↑	1,9		
miR-3615	↑	3,4		
miR-16-2-3p	↑	3,2		

Downregulated	obese SGA	fold change	obese AGA	fold change
miR-181b-5p	↓	0,6	↓	0,6
miR-143-3p	↓	0,3		
miR-223-3p	↓	0,2		
miR-23a-3p	↓	0,3		
miR-28-5	↓	0,3		

Table 1

By using mirTarBase (miRNA-target interactions database) we search experimentally validated mRNA targets of the deregulated miRNAs in SGA obese children. A functional analysis of these genes in DAVID database showed a significant statistical enrichment in "regulation of cell proliferation" and "regulation of metabolic processes" (Figure 1).

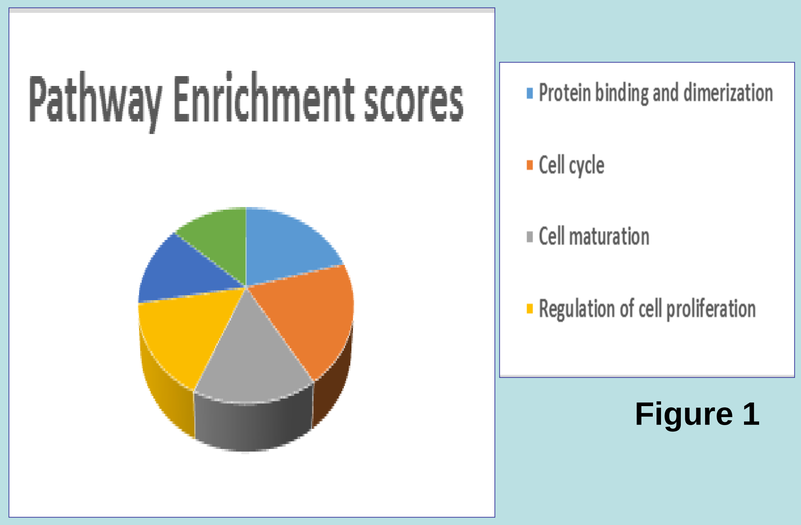


Figure 1

We validated the possibility of using miR-181b-5p as a new biomarker for obesity. Serum miR-181b-5p levels were determined by RT-qPCR in a wider cohort of subjects (11 obese AGA and 9 normal weight AGA; 11 obese SGA and 9 normal weight SGA). Consistent with NGS (Next Generation Sequencing) results, we found that miR-181b-5p is down regulated in SGA and AGA obese children with respect to normal weight children (p-value <0.05) (Figure 2).

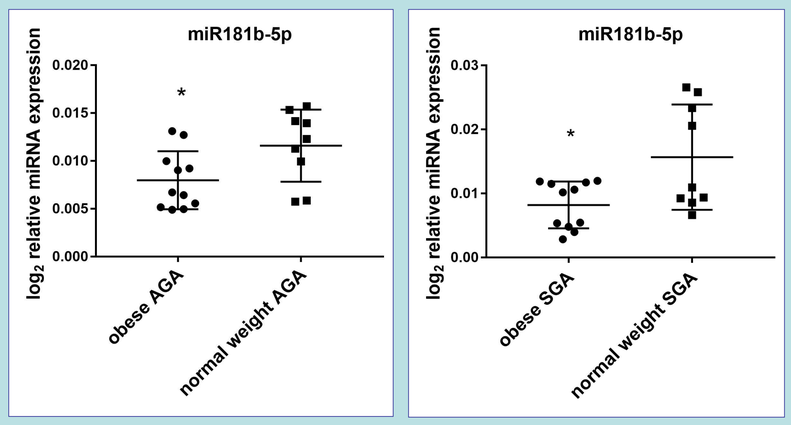


Figure 2

Conclusions

We performed a deep sequencing of serum small RNAs in SGA and AGA obese children compared to SGA and AGA normal weight children. We identified a set of novel miRNAs correlated with the obesity both in SGA and AGA children. Moreover, SGA children show a specific profile of serum miRNAs which may be associated with higher cardiometabolic risk.

The authors disclose any conflict of interest

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