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ORIGINAL RESEARCH

SERUM LncRNA SNHG16: A Biomarker for Diagnosing Childhood Obesity and Predicting Its Progression to Metabolic Syndrome

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Purpose: Obesity is a major risk factor for metabolic syndrome (MS) in children. This study explores the expression and clinical significance of long non-coding RNA SNHG16 (SNHG16) in childhood obesity and its complications with MS (obesity-MS).

Patients and Methods: Healthy controls and obese children (categorized as those with simple obesity or obesity-MS) were enrolled. Serum SNHG16 and miR-27a-3p levels were quantified by RT-qPCR. ROC curves evaluated SNHG16's diagnostic value for obesity. Logistic regression analysis identified potential risk factors for the development of obesity-MS. DLR assay and RIP assay confirmed the interaction between SNHG16 and miR-27a-3p. Bioinformatics was used to predict downstream genes of miR-27a-3p and, then GO and KEGG enrichment analysis identified the functions and signaling pathways of these genes.

Results: Serum SNHG16 levels were distinctly upregulated in obese children, especially those with obesity-MS. In contrast, miR-27a-3p expression showed the opposite trend. Additionally, SNHG16 was positively correlated with BMI in obese children. Serum SNHG16 exhibited 81.18% sensitivity and 76.47% specificity in distinguishing controls from obese individuals. Furthermore, serum SNHG16, BMI, HOMA-IR, and TG are potential risk factors for MS in obese children. Mechanistically, SNHG16 directly targets miR-27a-3p, and miR-27a-3p targets 65 genes primarily enriched in insulin response and the MAPK, Ras, and mTOR signaling pathways.

Conclusion: Elevated serum SNHG16 levels may serve as diagnostic biomarkers for obese children and predict obesity-MS. SNHG16 may also contribute to the progression of obesity and MS by targeting miR-27a-3p.

Keywords: SNHG16, obesity, metabolic syndrome, miR-27a-3p, diagnostic

Introduction

Obesity, characterized by abnormal or excessive fat accumulation, impacts 650 million individuals globally,[1] resulting in at least 2.8 million deaths annually from related causes.[2] Robust evidence links childhood obesity to adult overweight risk and a spectrum of complications, including type 1 diabetes, type 2 diabetes, insulin resistance, hypertension, lipid abnormalities, coronary disease, and stroke.[3,4] Global childhood obesity rates have surged by over 50%,[5] leading to a sharp rise in metabolic syndrome (MS) affecting roughly 60% of obese children.[6] MS is a clinical condition featuring hyperglycemia, hypertension, and dyslipidemia, often linked to obesity.[7] Given its serious impact on the further of children, there is an urgent need to identify effective biomarkers and implement early management.

Long noncoding RNAs (LncRNAs) are endogenous RNA molecules longer than 200 bases.[8] Abnormal lncRNA expression and function drive obesity pathogenesis and are emerging biomarkers for therapeutic and comorbidity management. For instance, lncRNA LINK-A,[9] U90926,[10] and MEG3[11] contribute to obesity progression. LncRNA

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small nucleolar RNA host gene 16 (SNHG16, also named ELNAT1, Nbla12061, or Nbla10727) is located on chr17q25.1, consists of four exons, and was initially reported in neuroblastoma. SNHG16 is significantly elevated in patients with diabetes, a common complication of obesity.[12] High glucose conditions elevate SNHG16 levels, which in turn promote dysfunction in retinal microvascular endothelial cells, thereby accelerating the progression of diabetic retinopathy.[13] In patients with type 2 diabetes, SNHG16 is strikingly upregulated in the presence of carotid artery plaques formed due to lipid accumulation and foam cell necrosis.[14] SNHG16 is also markedly elevated in mice subjected to a high-fat dietary regimen, as well as in vascular smooth muscle cells (VSMCs) treated with oxidized low-density lipoprotein.[15] Furthermore, transcriptome analysis of human visceral adipocytes revealed SNHG16 among genes differentially expressed in obesity.[16] However, the expression and clinical significance of SNHG16 in obesity remains unknown.

In this framework, we hypothesize that SNHG16 is abnormally expressed in children obesity. To validate this, we examined SNHG16 levels in the serum of obesity and obesity-MS, assessing its clinical significance.

Materials and Methods

Participants

The study received ethics approval from Sihui People's Hospital and adhered to the Helsinki Declaration. Patients and their families provided informed consent. Eighty-five obese children attending Sihui People's Hospital from June 2020 to September 2023 were included. Inclusion criteria for obese children were: a) aged 7–18 years; 2) meeting the age- and sex-specific BMI cutoffs recommended by the Working Group on Obesity in China (WGOC), with obesity defined as a body mass index (BMI) greater than or equal to 95% of the total body mass.[17] In addition, children with obesity were categorized into the simple obesity group and obesity with MS group based on the presence of MS. Obese children were diagnosed with MS if they had two or more of the following:[18] a) fasting blood glucose (FBG) $\geq$ 5.6 mmol/l or type 2 diabetes mellitus; b) blood pressure $\geq$ 130/80 mmHg; c) triglycerides (TG) $\geq$ 1.7 mmol/l; d) high-density lipoprotein cholesterol (HDL-C) < 1.03 mmol/l. Children with a) cardiac insufficiency; b) hepatic and renal dysfunction; c) hypothyroidism; d) secondary obesity or obesity syndromes from other causes; e) acute and chronic inflammatory conditions; and f) autoimmune disorders were excluded. In addition, 68 healthy children from our medical check-up center, matched for gender and age with the obese group, were included as a control group. All control children exhibited normal weight and height, underwent assessment to confirm normal metabolic function, and showed no signs of overt pathology.

Collection of Clinical Baseline Characteristics and Biochemical Indicators

Clinical baseline characteristics such as height, weight, age, gender, and waist circumference (WC) were recorded. 6 mL venous blood was collected from fasting children, of which 3 mL was centrifuged at 12,000 rpm/min for 5 min, and the upper serum was collected and stored at-80°C for lncRNA and miRNA levels. The remaining 3 mL of venous blood was analyzed for fasting blood glucose (FBG), fasting insulin (FINS), triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) using a Siemens ADVIA2400 automatic biochemical analyzer. Body mass index (BMI) was evaluated as weight (kg)/height (m^2). The homeostasis model assessment-insulin resistance (HOMA-IR) was evaluated as FBG (mmol/l) × FINS (mU/l)/22.5. The blood pressure of the subject was measured twice at 30-s intervals over 10 min of sitting still, and the mean values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken.

RNA Extraction and Real-Time Quantitative Reverse Transcription PCR (RT-qPCR)

TRizol LS reagent and RNAeasy Plus micro Kit were used to extract RNA from serum, and the concentration and purity of RNA were subsequently determined by a 260/280 spectrophotometric assay. PrimeScript RT Kit (for LncRNA) and Mir-X miRNA first-Strand Synthesis kit (for miRNA) were used to reverse transcribe 2 μg of RNA into complementary cDNA. Then, the TB Green Premix Ex Taq kit and Mir-X miRNA RT-qPCR TB Green Kit were mixed with the primers, cDNA, and ddH_2O to perform an amplification reaction. GAPDH and U6 were employed as internal references for SNHG16 and miR-27a-3p, respectively, and the expression of RNAs was quantified by the $2^{-\Delta\Delta Ct}$ method.

Bioinformatics Analysis

LncBook2 and DIANA databases identified potential downstream target miRNAs of SNHG16. ENCORI, Targetscan, miRDB, miRWalk, and StarBase databases identified the mRNA targets of miR-27a-3p. Venn analyzed the overlapping target mRNAs from 5 databases, then input the overlapping mRNAs into the STRING database, and selected the "Homo sapiens" to construct a protein-protein interaction (PPI) network to explore the interactions between these overlapping target genes. Top hub genes were identified by exploring the degree of connectivity within the PPI network using Cytoscape. Meanwhile, overlapping target genes were inputted into the macrobiotics analysis platform to explore the GO categories in which they were enriched, including biological process (BP), molecular function (MF), and cellular component (CC), however, KEGG suggested that overlapping target biological pathways of enrichment.

Dual Luciferase Reporter (DLR) Assay

SNHG16 sequences in the presence of the miR-27a-3p binding site were inserted into the pmirGLO dual luciferase vector to construct the SNHG16 wild-type (SNHG16-WT) plasmid, whereas insertion of SNHG16 sequence with mutations in the binding site into the vector constructed the SNHG16 mutant (SNHG-MT) plasmid. Commonly used tool cells 293T were inoculated into 48-well plates and cultured in a medium containing 10% FBS. After overnight, miR-27a-3p mimic, miR-27a-3p inhibitor, and miR NC were mixed with SNHG16-WT and SNHG16-MT, respectively, and the transfection reagent lipofectamine 3000 was added. Changes in dual luciferase were detected 48 h after transfection under the Dual-luciferase reporter kit.

RNA Immunoprecipitation (RIP) Assay

The target binding of SNHG16 to miR-27a-3p was validated using the RNA immunoprecipitation (RIP) kit. RIP lysis buffer was added to the 293T cells, followed by 10% cell extract as input, and the rest of the lysate was treated with argonaute 2 (Ago2) or negative control IgG antibody magnetic beads. After overnight incubation at 4°C, proteins were removed by shaking at 55°C for 30 min using proteinase K. Input was used as a positive control while IgG was used as a negative control, RNA was purified and normalized to the Input group, and RT-qPCR was used to examine the enrichment of SNHG16 and miR-27a-3p.

Statistical Analysis

Data were entered into SPSS 23.0 and GraphPad Prism 9.0 were used for data analysis and visualization. Measurement data were expressed as mean ± SD, and differences between groups were assessed by the Student's *T* test and ANOVA and post Tukey's test. Count data were analyzed using the Chi-square experience. Correlations were analyzed using Pearson coefficients analysis. MS risk factors were analyzed using logistic regression analysis. $P < 0.05$ was considered a statistically significant difference.

Results

Comparison of Clinical Baseline Characteristics of Subjects

Obese children had significantly higher levels of BMI, WC, FBG, HOMA-IR, TG, TC, LDL-C, SBP, and DBP, and lower levels of HDL-C compared to controls ($P < 0.05$, Table 1). Furthermore, no statistically significant disparities were observed between the two groups of children regarding age and gender ($P > 0.05$, Table 1).

Elevated Serum SNHG16 is a Diagnostic Biomarker for Obese Children

Obese children exhibited a 1.9-fold increase in serum SNHG16 levels compared to controls, as determined by RT-qPCR ($P < 0.001$, Figure 1A). Interestingly, Pearson coefficient analysis revealed that serum SNHG16 exhibited a significant positive correlation with BMI in obese children ($r = 0.698$, $P < 0.001$, Figure 1B). Furthermore, obese children were categorized into low and high SNHG16 groups using a mean cutoff of 1.93±0.79. Table 2 indicated that obese children with upregulated SNHG16 expression exhibit significantly increased levels of BMI, WC, as well as clinical indicators such as FBG, HOMA-IR, TG, LDL-

Table 1 Clinical Baseline Characteristics of Subjects

Indicators	Controls (n = 68)	Obesity (n = 85)	P value
Age (y)	9.21 ±2.05	9.05 ± 1.95	0.652
Gender (M/F)	33/35	42/43	0.914
BMI (kg/m^2)	18.37 ± 1.29	28.41 ± 1.83	0.000
WC (cm)	67.97 ± 10.23	82.74 ± 16.93	0.000
FBG (mmol/L)	4.40 ± 1.11	5.43 ± 1.36	0.000
HOMA-IR	1.76 ± 0.49	3.64 ± 1.47	0.000
TG (mmol/L)	1.12 ± 0.20	1.53 ± 0.35	0.000
TC (mmol/L)	3.59 ± 0.66	4.21 ± 0.66	0.000
HDL-C (mmol/L)	1.26 ± 0.29	1.12 ± 0.27	0.003
LDL-C (mmol/L)	1.74 ± 0.18	2.35 ± 0.58	0.000
SBP (mmHg)	107.34 ± 11.69	122.32 ± 11.79	0.000
DBP (mmHg)	64.71 ± 9.92	74.82 ± 12.42	0.000

Abbreviations: BMI, body mass index; FBG, fasting blood glucose; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment-insulin resistance index; WC, waist circumference; Y, years; F, female; M, male.

C, and SBP ($P < 0.05$). Furthermore, SNHG16 showed an AUC of 0.857 (95% CI: 0.799–0.915) at a cut-off of 1.265, with 81.18% sensitivity and 76.47% specificity in differentiating obese from healthy children (Figure 1C).

SNHG16 Levels Indicate a Risk for Metabolic Syndrome in Children with Obesity

To investigate the influence of SNHG16 on MS among obese children, we categorized them into two groups: those with simple obesity and those with obesity with MS (obesity-MS). As illustrated in Table 3, children with obesity-MS have notably higher BMI and WC, as well as higher levels of FBG, HOMA-IR, LDL-C, TG, and SBP, compared with simple obese children ($P < 0.05$). Interestingly, serum SNHG16 levels were markedly increased in children with simple obesity as well as those with obesity-MS compared to controls, with the latter group exhibiting the highest levels ($P < 0.001$, Figure 2A). Furthermore, we categorized the study participants by age and gender. The Supplementary Figure 1 illustrates that the distribution of control children, those with simple obesity, and those with obesity-MS did not differ significantly across various sex and age strata. Notably, SNHG16 expression was markedly elevated in obese children

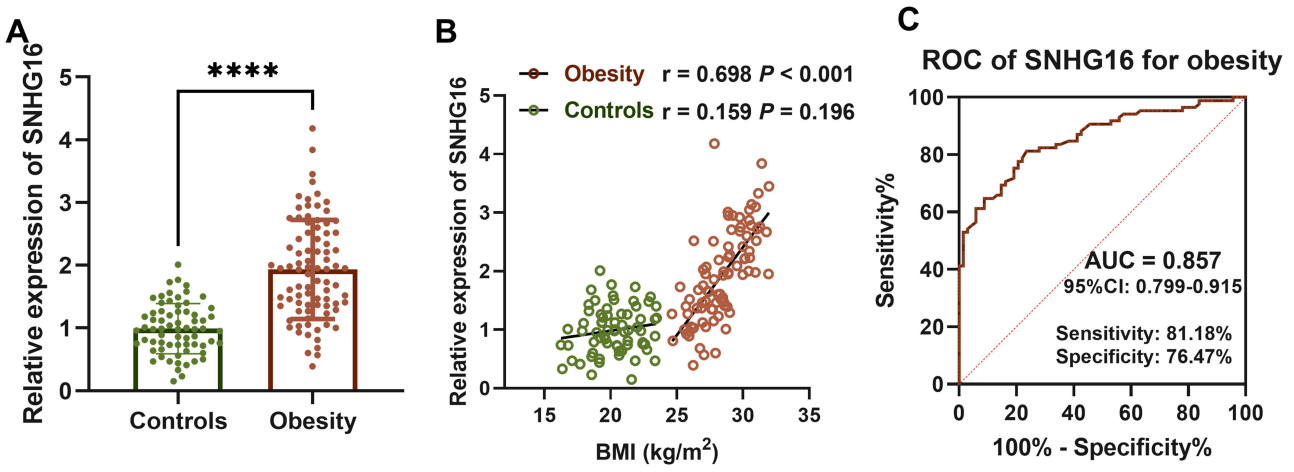

Figure 1 SNHG16 expression in the serum of obese children and its diagnostic significance. (**A**) RT-qPCR was performed to examine the serum expression of SNHG16 in healthy and obese children. (**B**) Pearson coefficient analysis of the correlation between BMI and serum SNHG16 levels in healthy and obese children. (**C**) ROC curves were used to analyze the diagnostic significance of SNHG16 in obese children. **** P < 0.0001 vs Controls.

Table 2 Relationship Between Expression Levels of SNHG16 Levels and Clinicopathological Features of Children with Obesity

Parameters	Cases No. (n = 85)	SNHG16 Expression		P value
		Low (n = 41)	High (n = 44)	
Age (years)				0.388
<9	46	20	26	
≥9	39	21	18	
Gender				0.281
Female	42	23	18	
Male	43	19	25	
BMI				0.000
<28.41	42	35	7	
≥28.41	43	6	37	
WC (cm)				0.004
<82.74	50	31	19	
≥82.74	35	10	25	
FBG (mmol/L)				0.030
<5.43	39	24	15	
≥5.43	46	17	29	
HOMA-IR				0.015
<3.64	50	30	20	
≥3.64	35	11	24	
TG (mmol/L)				0.017
<1.53	38	24	14	
≥1.53	47	17	30	
TC (mmol/L)				0.664
<4.21	38	17	21	
≥4.21	47	24	23	
HDL-C (mmol/L)				0.051
<1.12	38	23	15	
≥1.12	47	18	29	
LDL-C (mmol/L)				0.049
<2.35	48	28	20	
≥2.35	37	13	24	
SBP (mmHg)				0.030
<122.32	39	24	15	
≥122.32	46	17	29	
DBP (mmHg)				0.275
<74.82	37	15	22	
≥74.82	48	26	22	

Abbreviations: BMI, body mass index; FBG, fasting blood glucose; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment-insulin resistance index; WC, waist circumference; No, number.

compared to controls, irrespective of gender or age. Moreover, children with obesity-MS exhibited higher SNHG16 expression than those with simple obesity, with this difference being statistically significant across multiple subgroups.

A binary logistic regression analysis, incorporating clinical baseline characteristics and SNHG16 levels, identified BMI, HOMA-IR, TG, and SNHG16 as independent risk factors for the development of MS in obese children (Table 4). In addition, the ROC curve revealed that SNHG16 had 87.10% sensitivity and 71.15% specificity to predict the occurrence of MS in obese children when the cut-off value was 1.185 (Figure 2B).

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Table 3 Clinical Baseline Comparison Between Obese Children and Those with Obesity with Metabolic Syndrome

Indicators	Simple Obesity (n = 54)	Obesity with MS (n = 31)	P value
Age (y)	9.06 ± 1.92	9.23 ± 1.91	0.695
Gender (M/F)	29/13	25/18	0.206
BMI (kg/m^2)	27.62 ± 1.46	29.80 ± 1.58	0.000
WC (cm)	75.49 ± 11.89	95.38 ± 17.13	0.000
FBG (mmol/L)	5.21 ± 1.32	5.82 ± 1.35	0.046
HOMA-IR	2.99 ± 0.96	4.78 ± 1.53	0.000
TG (mmol/L)	1.41 ± 0.32	1.73 ± 0.29	0.000
TC (mmol/L)	4.15 ± 0.65	4.30 ± 0.66	0.342
HDL-C (mmol/L)	1.16 ± 0.30	1.05 ± 0.18	0.063
LDL-C (mmol/L)	2.23 ± 0.50	2.56 ± 0.65	0.011
SBP (mmHg)	119.86 ± 11.96	126.61 ± 10.32	0.010
DBP (mmHg)	74.13 ± 11.19	76.01 ± 14.44	0.505

Abbreviations: BMI, body mass index; WC, Waist circumference; FBG, fasting blood glucose; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment-insulin resistance index; Y, years; F, female; M, male; MS, metabolic syndrome.

MiR-27a-3p is a Direct Target miRNA of SNHG16

To further investigate the impact of SNHG16 on children's obesity and MS, we explore its downstream miRNAs. Both lncBook2 and DIANA databases identified putative binding sequences of SNHG16 to miR-27a-3p (Figure 3A). The DLR assay illustrated that miR-27a-3p mimic suppressed luciferase activity in SNHG16-WT, while its inhibitor enhanced it. However, neither the mimic nor the inhibitor exerted any influence on the luciferase activity of the SNHG16-MT construct ($P > 0.05$, Figure 3B). Furthermore, both SNHG16 and miR-27a-3p were enriched on the anti-Ago2 antibody compared to the anti-IgG ($P < 0.05$, Figure 3C). More intriguingly, serum miR-27a-3p levels were markedly decreased in obese children compared to healthy controls, with even lower expression observed in those with obesity-MS ($P < 0.05$, Figure 3D). In both children with simple obesity and those with obesity-MS, serum miR-27a-3p levels exhibited a significant negative correlation with SNHG16 levels (r = −0.790 and r = −0.644, $P < 0.001$, Figure 3E and F).

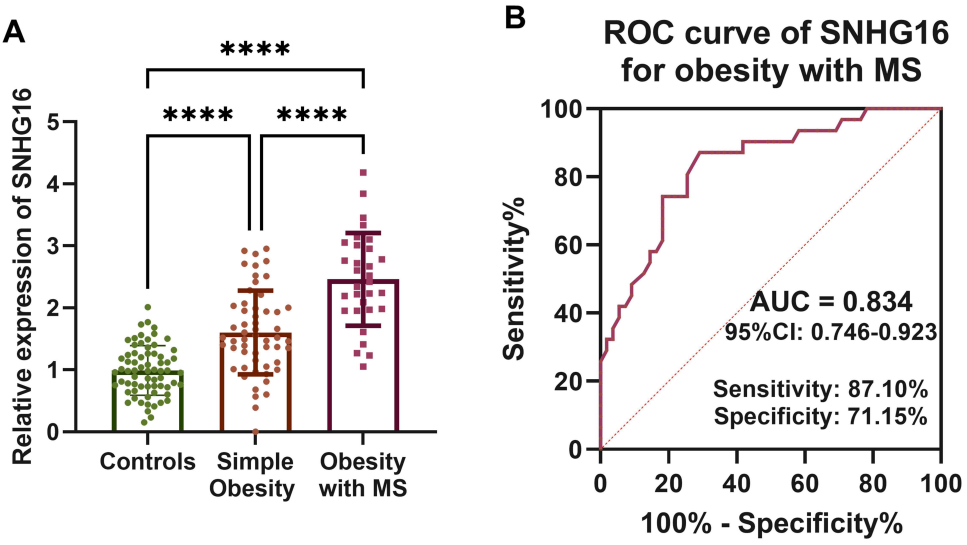

Figure 2 SNHG16 levels predict metabolic syndrome in obese children. (**A**) The expression of SNHG16 in children with simple obesity and those with obesity-MS. (**B**) Predictive significance of SNHG16 in obese children with MS using ROC curves. **** $P < 0.0001$ vs simple obesity group.

Table 4 Potential Risk Factors for the Development of Metabolic Syndrome in Obese Children

Indicators	OR	95% CI	P value
Age (y)	3.604	0.576–22.559	0.171
Gender (M/F)	1.534	0.264–8.924	0.634
BMI (kg/m^2)	6.390	1.118–36.507	**0.037**
WC (cm)	4.617	0.756–28.215	0.098
FBG (mmol/L)	3.508	0.779–15.789	0.102
HOMA-IR	7.514	1.242–45.479	**0.028**
TG (mmol/L)	6.043	1.241–29.416	**0.026**
TC (mmol/L)	2.686	0.380–18.975	0.322
HDL-C (mmol/L)	2.197	0.468–10.309	0.318
LDL-C (mmol/L)	5.110	0.927–28.178	0.061
SBP (mmHg)	3.118	0.450–21.617	0.250
DBP (mmHg)	1.948	0.427–8.882	0.389
SNHG16	8.519	1.432–49.690	**0.019**

Note: Values in bold suggest a statistically significant difference where $P < = 0.05$.

Abbreviations: BMI, body mass index; FBG, fasting blood glucose; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment-insulin resistance index. OR, odds ratio; 95% CI, confidence interval.

Functional and Signaling Pathway Analysis of miR-27a-3p Affecting Children with Simple Obesity and Obesity with MS

In an attempt to explore the potential mechanism of action of the SNHG16/miR-27a-3p axis affecting children's obesity and obesity-MS, their downstream targets were predicted. As depicted in Figure 4A, Venn identified 65 overlapping targets from five different databases. Then, the PPI network was screened for these overlapping targets, resulting in 65 nodes (genes) and 27 PPI edges (PPI relationships), with a significant enrichment P-value of 0.0073 (Figure 4B). From the PPI network, the top 10 hub genes with the highest degree were identified using the Degree algorithm (Figure 4C). GO enrichment analysis of overlapping targets identified enriched BP, MF, and CC terms, primarily focused on "response to peptide hormone", "cellular response to insulin stimulus", and "insulin response", as shown in Figure 4D. Subsequently, the KEGG pathway enrichment analysis of overlapping targets indicated significant enrichment within the "MAPK signaling pathway", "Ras signaling pathway", "mTOR signaling pathway", and "ErbB signaling pathway" (Figure 4E).

Discussion

Childhood obesity usually develops from an early age, predicts negative health outcomes in adulthood, and is strongly associated with obesity-MS. Therefore, effective diagnosis and intervention in this period can prevent MS progression. This preliminary research novelly identified a significant elevation of SNHG16 in obese children, particularly those with MS. Furthermore, elevated SNHG16 correlated with obesity clinical indicators and effectively differentiates healthy from obese children. Crucially, SNHG16 levels emerged as a potential risk factor for MS in obese children and significantly predicted its development.

LncRNAs, a diverse class of regulatory RNAs, control gene expression via various mechanisms and are involved in biological processes, such as glycolipid metabolism. For example, LncRNA HEM2ATM is highly expressed in adipose tissue M2 macrophages and regulates obesity-related inflammation and insulin resistance.[1] LncRNA LINK-A promotes obesity progression by remodeling the adipose tissue inflammatory microenvironment.[9] Diabetes is a chronic disease associated with obesity. Inhibition of SNHG16 can suppress pathological angiogenesis in high-glucose environments.[19] The findings manifested that SNHG16 is highly expressed in diabetic foot patients,[20] and serves as a potential diagnostic biomarker for diabetic retinopathy.[1] High-fat diet (HFD)-induced obesity, primarily due to adipose tissue dysfunction, is

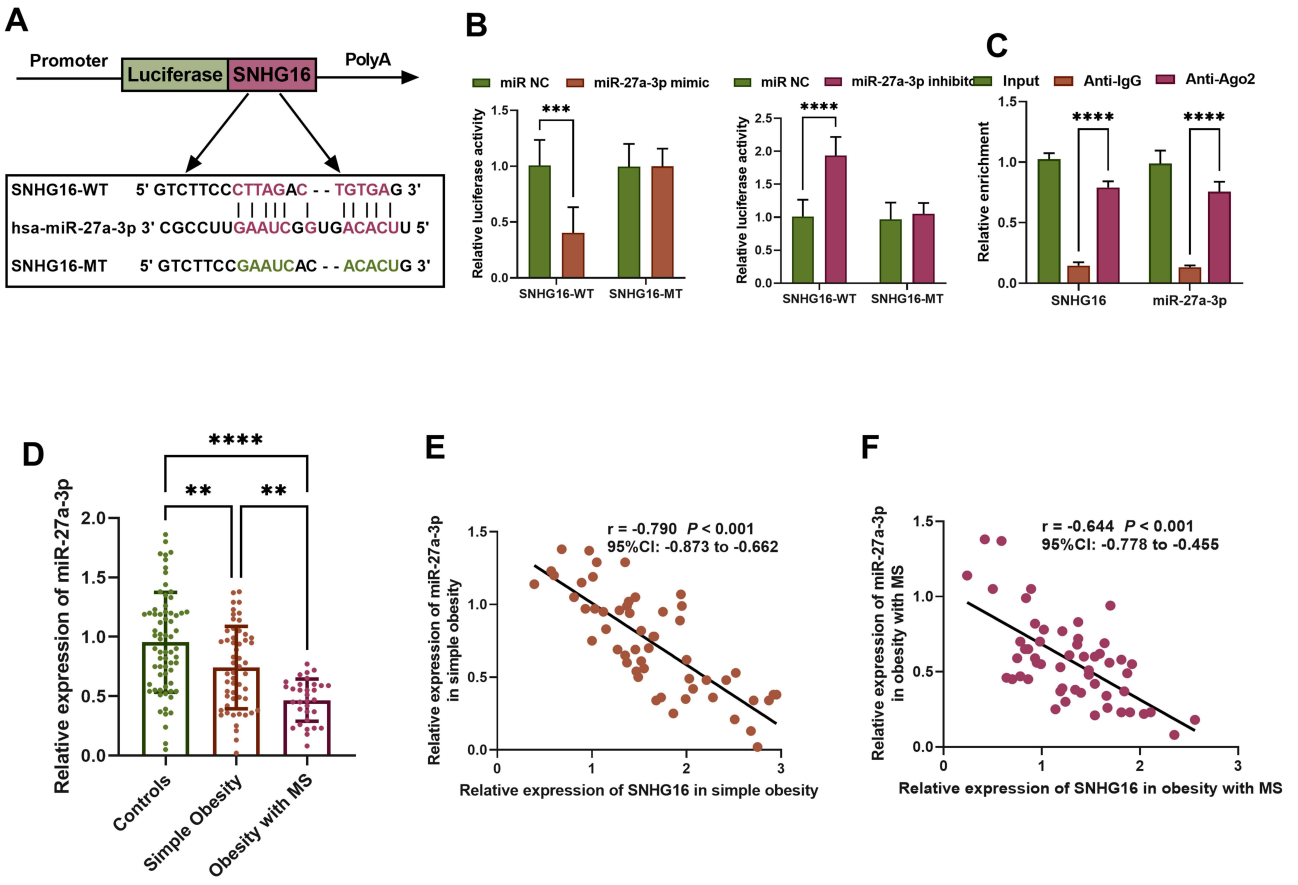

Figure 3 miR-27a-3p was the direct target miRNA of SNHG16. (**A**) The putative binding sequences are presented. DLR assay (**B**) and RIP assay (**C**) were conducted to examine the target relationship between SNHG16 and miR-27a-3p. (**D**) The expression of serum miR-27a-3p in the subjects. Pearson coefficient was conducted to examine the correlation between miR-27a-3p and SNHG16 in children with simple obesity (**E**) and obesity with MS (**F**). ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ vs miR NC or controls.

a major risk factor for MS. SNHG16 levels are significantly elevated in HFD-fed mice and oxLDL-treated vascular smooth muscle cells.[15] Transcriptome analysis of human visceral adipocytes identified SNHG16 as a differentially expressed gene in obese individuals.[16] Our study revealed for the first time that serum SNHG16 levels are significantly elevated in obese children. A meta-analysis showed that high BMI children are 5 times more likely to become obese adults than normal-weight children.[21] Our study found a significant positive correlation between SNHG16 and BMI in obese children. Obese children exhibit hyperglycemia, hyperinsulinemia, and impaired glucose tolerance, and our study found a significant association between SNHG16 and blood glucose, lipids, and HOMR-IR. Previous studies show obesity causes physiological changes due to excess body fat accumulation.[22] Adipocytes secrete cytokines and hormones affect lipid metabolism. In obese children, insulin resistance increases liver fatty acid synthesis and decreases oxidation, raising blood TG levels. Elevated TGs disrupt LDL-C production and metabolism via cholesterol transporter proteins, increasing LDL-C. Obesity-related metabolic disorders accelerated LDL-C catabolism, lowering HDL-C. Consistent with prior research, we found significantly higher TC, TG, and LDL-C, and lower HDL-C in obese children. Also, high SNHG16 expression was associated with higher TG and LDL-C. Research findings indicate that SNHG16 may play a role in obesity-induced lipid metabolism dysfunction.

In our research, serum SNHG16 typically distinguishes obese children from healthy controls and may be a risk factor for metabolic syndrome. Emerging evidence has shown that SNHG16 affects lipid metabolism in colorectal cancer.[23] Blocking SNHG16 can increase drug sensitivity in gastric cancer by affecting glucose metabolism.[24] SNHG16 also protects against vascular endothelial damage in cardiovascular disease by affecting glutamine metabolism.[25] Despite the scarcity of reports related to SNHG16 in MS, we demonstrated for the first time that elevated SNHG16 is a potential risk factor and predictive marker for childhood obesity-MS. Age- and gender-stratified analysis revealed that SNHG16

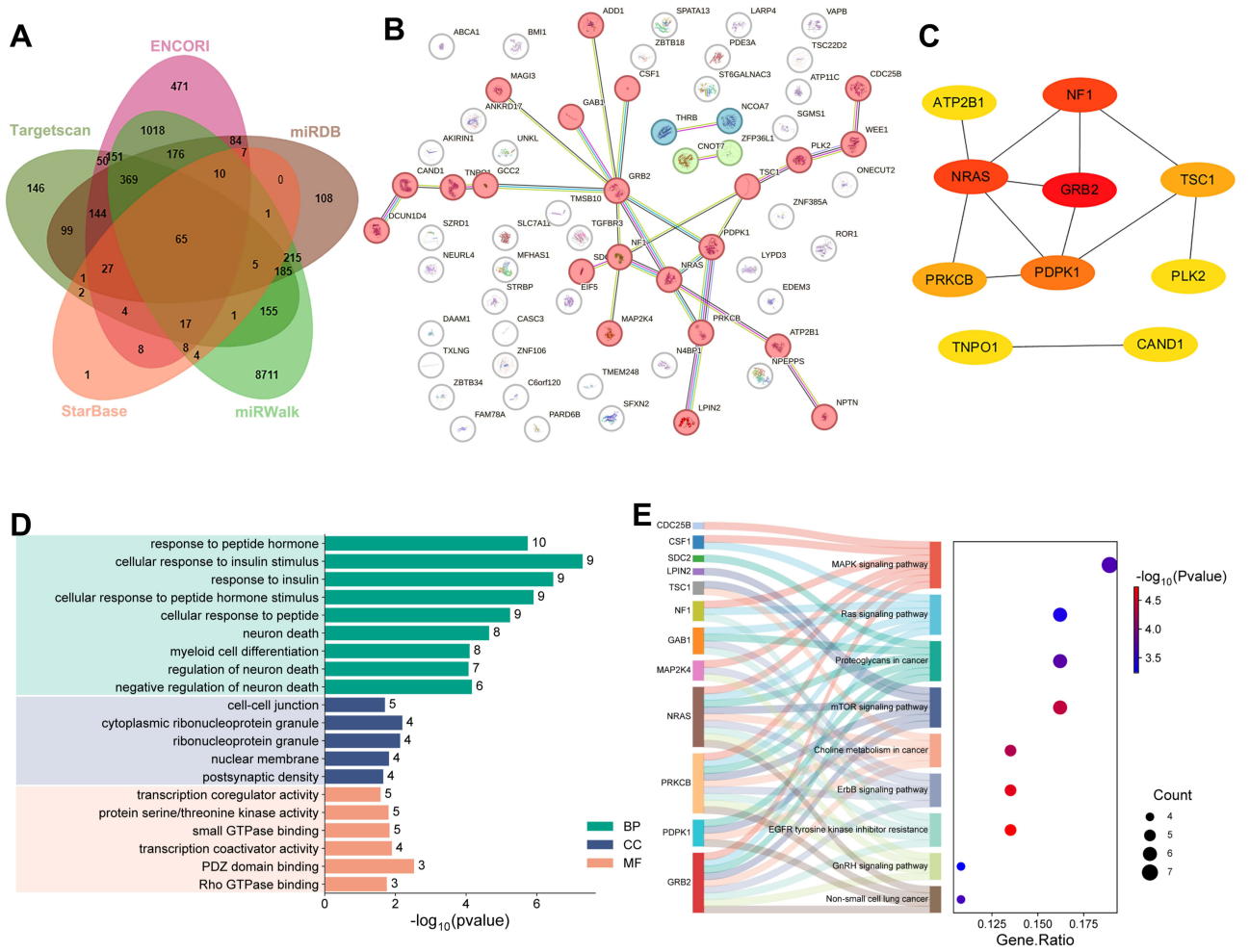

Figure 4 GO enrichment and KEGG enrichment of overlapping target of miR-27a-3p. (**A**) Venn diagram presents target mRNAs from five databases predicting miR-27a-3p. (**B**) The PPI network was established by the overlapping targets. (**C**) Top 10 hub genes with higher degrees screened from a PPI network. (**D**) The top 20 enriched BP, MF, and CC terms for the overlapping targets were analyzed by the GO enrichment. (**E**) Sankey dot pathway enrichment of the top 10 KEGG pathway enrichment for the targets.

expression was elevated in obese children compared to controls, across all gender and age subgroups. Notably, a statistically significant difference in SNHG16 levels was observed between children with obesity-associated MS and those with simple obesity in several subgroups. This indicates that SNHG16 levels are not influenced by sex or age and represent a stable biological marker of obesity and obesity-MS. This stability underscores the potential of SNHG16 as a biomarker for early screening or assessment of MS in obese pediatric patients.

The ceRNA hypothesis unveils a novel RNA interaction mechanism, where lncRNAs function as miRNA sponges, inhibiting their expression to regulate diverse biological processes. For example, miR-221-3p can be used as a biomarker of response to weight loss interventions in girls with abdominal obesity.[21] miR-34a and miR-93 are potent biomarkers for identifying adverse phenotypes of childhood obesity and are strongly associated with MS.[6] MiR-27a-3p has been reported as an anti-obesity gene in previous studies. For example, miR-27a was significantly reduced in the serum and adipose tissue of obese human patients and in the adipose tissue of mice on an HFD.[26] Adipose-derived exosomes in obese individuals significantly reduced miR-27a-3p expression and correlated with immunotherapy outcomes in lung adenocarcinoma patients.[27] A research found that miR-27a-3p was significantly reduced in differentially expressed miRNAs in plasma exosomes of obese mice induced by an HFD.[28] Our findings align with previous studies, showing significant downregulation of serum miR-27a-3p in obese children. Notably, we identified miR-27a-3p as a novel target of SNHG16, implicated in obesity. Additionally, serum miRNA sequencing showed miR-27a-3p downregulation with a whole-grain diet, suggesting it is a potential biomarker for lipid metabolism.[29] Quercetin and EGCG exhibit

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antidiabetic and anti-obesity effects, alleviating insulin resistance by regulating miR-27a-3p.[30] 1,25-dihydroxyvitamin D3 may inhibit lipogenesis by upregulating miR-27a-3p.[31] Our study revealed novel downregulation of miR-27a-3p in obese children with MS, inversely correlated with SNHG16 levels. In summary, we found for the first time that SNHG16 could regulate the development of childhood obesity-MS by targeting miR-27a-3p.

Growth factor receptor-bound protein 2 (GRb2), also known as ASH, NCKAP2, MST084, is located on human chromosome 17q25.1 and functions as a component of the integrin adhesion complex, linking activated RTK to Ras/MAPK signaling.[32] GRb2 also shows therapeutic potential in diabetes, obesity, and cardiovascular disease.[33] Additionally, mutations in the GRb2-related binding protein 2 gene are linked to drinking behavior and obesity.[34] Furthermore, suppression of GRb2 expression was associated with improved hepatic steatosis.[35] In our study, among the Top 10 hub genes enriched in overlapping target genes of miR-27a-3p were GRB2, in addition to these, overlapping target genes were mainly enriched in "response to peptide hormone", "response to insulin", "cellular response to insulin stimulus". Furthermore, neurofibromin 1 (NF1) is one of the overlapping targets, also known as WSS, NFNS, and VRNF, located on human chromosome 17q11.2, and is often reported as a tumor suppressor gene.[36] NF1 heterozygous mice avoid diet-induced obesity and hyperglycemia and have enhanced glucose clearance.[37] Another study found that mice with NF1 haploinsufficiency exhibited metabolic alterations, including decreased adipose energy, increased glucose clearance, and insulin sensitivity, and decreased susceptibility to diet-induced obesity and hyperglycemia.[38] In turn, studies of model organisms have demonstrated that loss of NF1 also prevents obesity, increases metabolic rate reduces visceral and subcutaneous fat mass in Drosophila, and acts as an anti-obesity and hyperglycemia-specific disease in mice.[39] In our study, we found that the top 10 Hub genes in the SNHG16/miR-27a-3p axis overlapping gene PPI network included FN1. Dietary polyphenols prevent obesity by activating the mTOR signaling pathway,[40] and mTOR activity is associated with obesity-related insulin resistance.[41] In addition, NF1 targets the mTOR pathway through regulation and has been associated with obesity and type 2 diabetes.[39] Our study found that miR-27a-3p's target genes are mainly enriched in signaling pathways like mTOR and MAPK. However, further investigation is needed to determine if the SNHG16/miR-27a-3p axis plays a role in obesity-related metabolic syndrome by targeting FN1 to regulate the mTOR pathway. In addition, this study has certain limitations. Even though T1DM is the most preventive form of diabetes in children, we did not focus on the correlation between T1DM and serum SNHG16. This decision was made due to our focus on the relationship between SNHG16, childhood obesity, and obesity-related MS. Moreover, within the spectrum of diabetes, MS is mainly associated with T2DM. It should be noted that current research reports also lack data on the correlation between T1DM and SNHG16. Therefore, we plan to focus on this aspect in future studies. Furthermore, a limitation of this study is the omission of the duration of obesity in children. This indicator was not incorporated into the experimental design at the initial stages of this preliminary study, despite being a potentially crucial factor in understanding the progression of obesity-MS and their association with SNHG16 levels. Future research will aim to include this variable to gain a comprehensive understanding of the underlying mechanisms.

Conclusion

All in all, this study revealed that serum SNHG16 levels were markedly increased in children with obesity, particularly those with concurrent MS, and positively correlated with BMI. SNGH16 demonstrated diagnostic potential for obesity and emerged as a potential risk factor for obesity-MS. Mechanistically, SNHG16 targets miR-27a-3p, modulating its downstream pathways and suggesting its promise as a novel diagnostic biomarker and therapeutic target.

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Disclosure

The authors report no conflicts of interest in this work.

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