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ORIGINAL ARTICLE

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Serum microRNA panels predict bariatric surgery outcomes

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Abstract

Objective: The weight losses after bariatric surgery are modulated by multiple factors in people with obesity. MicroRNAs (miRNAs) have been reported to show significant regulatory roles in adipose tissue. However, a serum miRNA signature to serve as a biomarker of sustained weight losses following bariatric surgery has not yet been established.

Methods: MiRNA microarray was used to identify differentially expressed miRNAs in the serum of patients with an effective response after bariatric surgery compared with those without. Excess weight loss > 55% at 6 months after surgery was defined as an effective response.

Results: Three miRNAs were shown to have a significantly differential expression between patients with or without an effective response following bariatric surgery. The miR-31-5p was downregulated, whereas miR-328-3p and miR-181a-5p were upregulated in the patients with effective responses compared with those without effective responses. Panels of the serum ratios of miR-328-3p/miR-31-5p or miR-181a-5p/miR-31-5p and individual BMI value exhibited good performance in preoperative prediction of treatment effectiveness. Bioinformatic analysis depicted that predicted targets of these miRNAs were involved in the regulation of the AMP-activated protein kinase signaling pathway.

Conclusions: A circulating miRNA signature with clinical variables (BMI) can be a clinical biomarker to predict effectiveness following bariatric surgery.

Jih-Kai Yeh and Chia-Chun Chen contributed equally to this work.

INTRODUCTION

The prevalence of overweight and obesity has progressively increased worldwide in the past decades (1). Obesity not only contributes to insulin resistance and metabolic syndrome but also increases risks of cardiovascular diseases such as coronary heart disease, heart failure, and atrial fibrillation (2). Bariatric surgery is established as an effective weight control intervention for people with severe obesity (3), and it also exerts long-term effects on glycemic control, diabetes remission, and the reduction of cardiovascular events (4,5). However, considerable varying outcomes in terms of weight reduction and metabolic effects exist among individuals receiving bariatric surgery (6). This variance cannot be explained simply by surgical procedures, behavior changes, or adherence to dietary and exercise advice. A diversity of corresponding pathophysiological disturbances among individuals with obesity are believed to contribute to heterogeneous responses to this treatment.

There is growing interest in microRNAs (miRNAs) as epigenetic regulators of obesity and metabolic disorder. MiRNAs are endogenous, noncoding RNAs 21 to 25 nucleotides in length, and they regulate gene expression at the posttranscriptional level (7,8). The miRNAs are associated with many bioactive processes relevant to obesity development, such as adipocyte differentiation, insulin resistance, glucose homeostasis, cholesterol biosynthesis, and neural regulation of appetite (9,10). MiRNAs not only act intracellularly, but they are also found in the circulatory system. Thus, their serum levels can be used as diagnostic or therapeutic biomarkers of systemic diseases. Several applications in clinical settings, such as diabetes mellitus, myocardial infarction, heart failure, acute kidney injury, and cancer treatment, have been proposed (11-15).

Circulating miRNA signatures in individuals with obesity versus healthy individuals (16,17), as well as changes following the surgical procedure, have been previously published (18-21). However, there is a large discrepancy among these findings, and they have yet to give rise to a practical application. Identification of whether individuals benefit from bariatric surgery is an important clinical need that is unmet. Consequently, we aimed to explore whether serum miRNA levels were associated with individual treatment response following bariatric surgery and to provide a practical biomarker for differentiating weight loss outcomes of bariatric surgery.

Study Importance

What is already known?

- ► MicroRNAs (miRNAs) have been reported to show significant regulatory roles in adipose tissue metabolism and body weight regulation.
- ► Several miRNAs have significant roles in bariatric surgery outcomes.

What does this study add?

- ► Using a calculated miRNA panel together with BMI, but not singularly miRNA, can predict effectiveness following bariatric surgery.

How might these results change the direction of research or the focus of clinical practice?

- ► Personalized patient selection for better outcomes of bariatric surgery may be achieved with miRNA panels.

METHODS

Patient enrollment: OCEAN registry

The Obesity and Clock for Elegant AgiNg (OCEAN) registry is a prospective cohort study (NCT02674230) commenced in 2011 and designed to recruited patients with BMI > 35 kg/m^2. After 6 months of diet, exercise, and pharmacologic treatment programs, patients were candidates for bariatric surgery if they failed to achieve optimal weight reduction. Laparoscopic gastric sleeve or bypass surgeries were used in this study. Patients who were on steroids or those who had uncontrolled psychiatric diseases were excluded. Patients who could not sign the consent form were also excluded. From 2011 to 2018, patients who met indications and accepted bariatric surgery were screened for participation in this study. Surgeries were performed by the bariatric surgical team using the same surgical techniques at the Chang Gung Memorial Hospital in Taoyuan City, Taiwan. Patients who enrolled in this study have given and signed informed consent. This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital.

Sample collection and clinical evaluation

Blood samples were obtained from patients who participated in this study 1 day prior to the surgery or on the day of the surgery, with 12 hours of fasting. Serum was prepared immediately after blood collection and stored at −80 °C before analysis. At 1 month, 6 months, 1 year, and 2 years following surgery, the body weight, waist circumference, hip circumference, percentage of body fat, and lean mass (bioelectrical impedance) of the patients were measured. Blood pressure, fasting blood sugar, hemoglobin A_{1c}, spot urine of microalbumin, and creatinine were measured. Effective surgical treatment response was defined by percentage of excess body weight loss (%EBW) after surgery, which is defined using the following formula: (preoperative body weight − postoperative body weight)/(preoperative body weight − ideal body weight) × 100. Nonresponse

was defined as %EBW < 55%, and effective response was defined as %EBW > 55%.

Selection protocols of miRNA candidates

Unbiased high-throughput screening analyses of serum miRNA profiles were performed and compared among six patients with the highest or the lowest numbers of %EBW. MiRNAs with a significantly differential expression between patients were selected. Combined with the three miRNAs associated with adipocyte differentiation in our previous work (22), a total of 37 miRNA candidates were selected in this study.

RNA extraction and quantitative polymerase chain reaction analysis of miRNA

Before RNA extraction, hemolysis was examined in serum samples by detecting the absorbance of hemoglobin at 414 nm using the NanoDrop 2000c UV–Vis spectrophotometer (Thermo Fisher Scientific) to avoid the interference of hemolysis on the miRNA abundances. In order to calibrate the intersample variances, 250-μL serum samples were mixed with 1,000 μL of QIAZol Lysis Reagent (QIAGEN) and a spiked-in control of 10^7 copies of *Caenorhabditis elegans* miR-39 (5′-UCACCGGGUGUAAAUCAGCUUG; Integrated DNA Technologies, Inc.). Total RNA was extracted with an miRNeasy Mini Kit (QIAGEN). In order to avoid the heparin-related interference, 0.5 U of heparinase I (MilliporeSigma) was added to the pre-reverse transcription (RT) mixture containing 5.4 μL of serum RNA, 40 U of RNaseOUT (Invitrogen), 1 × polymerase chain reaction (PCR) buffer (Roche Diagnostics), and 2.5mM $MgCl_2$, and the mixtures were incubated at 25 °C for 1 hour.

The multiplex quantitative RT-PCR of miRNAs was performed as described previously (23). The cycle threshold (Ct) value of each miRNA was normalized with that of the spiked-in cel-miR-39 by adjusting the chmiRNA Ct values with the cel-miR-39 ratio (a ratio calculated based on the differences of the cel-miR-39 Ct value in each sample to a cel-miR-39 median of total samples). In order to adjust for variations in the total RNA amounts of individual samples, the cel-miR-39-normalized Ct value was further normalized to each individual RNA concentration, which was determined using a NanoDrop 3300 Fluorospectrophotometer (Thermo Fisher Scientific) and a Quant-iT RiboGreen RNA Assay (Invitrogen). MiRNAs with normalized Ct values > 40 were defined as "undetectable." Relative miRNA expression levels were represented as 40-Ct.

Target gene prediction and pathway analysis

The predicted target genes of miRNAs were identified with the MiRDB and TargetScan 7.1 databases. Pathway analysis for these miRNAs was predicted by using Metacore 6.13 pathway map analysis and QIAGEN Ingenuity Pathway Analysis.

Statistics

Continuous variables were expressed as the mean (SD). A variable's normality was first determined. Normally distributed variables were calculated by the Student *t* test, and non-normally distributed variables were compared by the Mann–Whitney *U* test. The area under the receiver operating characteristic curve (AUC) was used to determine the discriminatory power, and Youden's index was used to assess the optimal cutoff value. Algorithms of two or three miRNA panels were built by logistic regression (details in *Results*).

Categorical variables were compared using the χ^2 test or Fisher exact test. Correlation coefficients were calculated using Spearman rank correlation analysis between groups. The receiver operating characteristic analysis was performed to determine the best cutoff value of miRNA level to differentiate patients with effective or noneffective responses. All reported *p* values were two-sided, and $p < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS Statistics (IBM Corp.) and Prism (GraphPad Software, Inc.).

RESULTS

Patient clinical characteristics

In the first phase of the study, 77 patients were enrolled and they completed uncomplicated bariatric procedures in January 2016 through December 2018, of which 51 patients had a noneffective response, and 26 patients had an effective response. In the second phase, 73 patients were enrolled, and 34 patients had a noneffective response following surgery in January 2019 through December 2020. A total of 150 patients were enrolled for analysis ($n = 65$, $n = 85$ for effective vs. noneffective, respectively). As shown in Table 1, the median age was 36 years (10 years; range: 21-63 years), 60% of patients were female, and mean BMI was 40.9 (SD 5.5). Other laboratory data, including serum creatinine, fasting glucose, glycohemoglobin, and lipid profiles, are listed in Table 1. The average %EBW in the effective group was 64.6% (11.6%) compared with 37.5% (11.0%) in the noneffective group. The body weight and BMI were lower in the effective group compared with the noneffective group (Table 2).

Profiles of circulating miRNAs in patients who underwent bariatric surgery

A selection algorithm for the clinically applicable miRNAs is depicted in Figure 1. Of 37 candidate miRNAs, 4 were excluded

TABLE 1 Demographic and clinical characteristics of all patients

	Obesity (n = 150)
Age (y)	36 ± 10 (range 21-63)
Gender (female)	90 (60%)
Weight (kg)	113.1 ± 20.8
Height (cm)	165.8 ± 9.6
BMI (kg/m^2)	40.9 ± 5.5
Hypertension	63 (42%)
Smoking	32 (21%)
BUN (mg/dL)	12.8 ± 3.5
Serum creatinine (mg/dL)	0.7 ± 0.2
Total cholesterol (mg/dL)	190 ± 32
HDL (mg/dL)	46 ± 12
LDL (mg/dL)	119 ± 47
Triglyceride (mg/dL)	150 ± 87
Sugar AC	104 ± 32
HbA_{1c}	6.3 ± 1.2
Uric acid (mg/dL)	6.5 ± 1.7
Hemoglobin (g/L)	14.1 ± 1.6

Note: Data given as mean ± SD or number (percentage) for discrete variables.

Abbreviations: AC, ante cibum (before meals); BUN, blood area nitrogen; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

TABLE 2 Demographic and clinical characteristics by effective and noneffective surgical response

	Noneffective (n = 87)	Effective (n = 63)	p value
Age (y)	36 ± 10 (22-63)	36 ± 9 (21-60)	0.99
Gender (female)	56 (64.4%)	34 (54%)	0.23
Weight (kg)	116.5 ± 21.4	108.4 ± 19.4	0.01
Height (cm)	164.7 ± 9.3	167.5 ± 10.1	0.13
BMI (kg/m^2)	42.7 ± 5.6	38.5 ± 4.5	<0.0001
Hypertension	40 (46%)	23 (36.5%)	0.22
Smoking	16 (18.4%)	16 (25.4%)	0.42
BUN (mg/dL)	12.9 ± 3.8	12.7 ± 3.1	0.91
Serum creatinine (mg/dL)	0.7 ± 0.2	0.7 ± 0.2	0.26
Total cholesterol (mg/dL)	186 ± 32	195 ± 32	0.05
HDL (mg/dL)	45 ± 12	47 ± 12	0.29
LDL (mg/dL)	115 ± 46	124 ± 49	0.14
Triglyceride (mg/dL)	141 ± 88	160 ± 87	0.11
Sugar AC	104 ± 30	104 ± 35	0.69
HbA_{1c}	6.3 ± 1.2	6.4 ± 1.4	0.99
Uric acid (mg/dL)	6.6 ± 1.5	6.5 ± 2.1	0.88
Hemoglobin (g/L)	14.1 ± 1.5	14.3 ± 1.8	0.78

Note: Data given as mean ± SD or number (percentage) for discrete variables.

Abbreviations: AC, ante cibum (before meals); BUN, blood urea nitrogen; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

for the nondetectable rate (Ct value > 40), and, in more than 50% of our samples, 15 were eliminated for their serum content altered by hemolytic samples. Among the 18 remaining candidate miRNAs, 6 had significant associations with bariatric surgery efficacy. Furthermore, we identified three (miR-328-3p, miR-181a-5p, and miR-31-5p) that had a significantly differential expression between patients in the effective and noneffective groups.

The distribution and differences of these three miRNAs in effective versus noneffective groups are plotted in Figure 2. The expression levels of miR-328-3p (p = 0.016) and miR-181a-5p (p = 0.036) were significantly upregulated in the effective group compared with the noneffective group. In contrast, the serum miR-31 level (p = 0.01) was downregulated in the effective group compared with the noneffective group.

Diagnostic accuracy of circulating miRNAs in predicting effectiveness after bariatric surgery

The receiver operating characteristic curve analysis was used for evaluating the discriminating performance of individual circulating miRNA values (Table 3). Considering that some miRNAs may work their biologic effects in a synergistic or competitive inhibition model, we used simple mathematic equations to integrate individual miRNA values and clinical factors into an miRNA panel with better diagnostic performance. At first, we tried to combine effects of these miRNAs and use serum miR-328-3p/miR31-5p (Ratio 1) and miR-181a-5p/miR-31-5p (Ratio 2), respectively. The AUC of miR-328-3p/miR31-5p (Ratio 1) was 0.680, with a sensitivity of 71.4% and specificity of 59.8% at the cutoff value 5.194. The AUC of miR-181a-5p/miR-31-5p (Ratio 2) was 0.670, with a sensitivity of 57.1% and specificity of 78.2% at the cutoff value 5.314. In order to improve the performance of the diagnostic tool for clinical application, we added baseline BMI to Ratio 1 or Ratio 2 as new predictive panels. The diagnostic performance of Ratio 1 + BMI or Ratio 2 + BMI improved AUC to 0.77 and 0.76, respectively (Figure 3).

Target gene prediction and pathway analysis

Next, we searched and analyzed the target genes of three miRNAs (miR-328-3p, miR-181a-5p, and miR31-5p) that were highly associated with weight reduction outcomes after bariatric surgery. Target gene prediction by MiRDB and TargetScan 7.2 databases identified

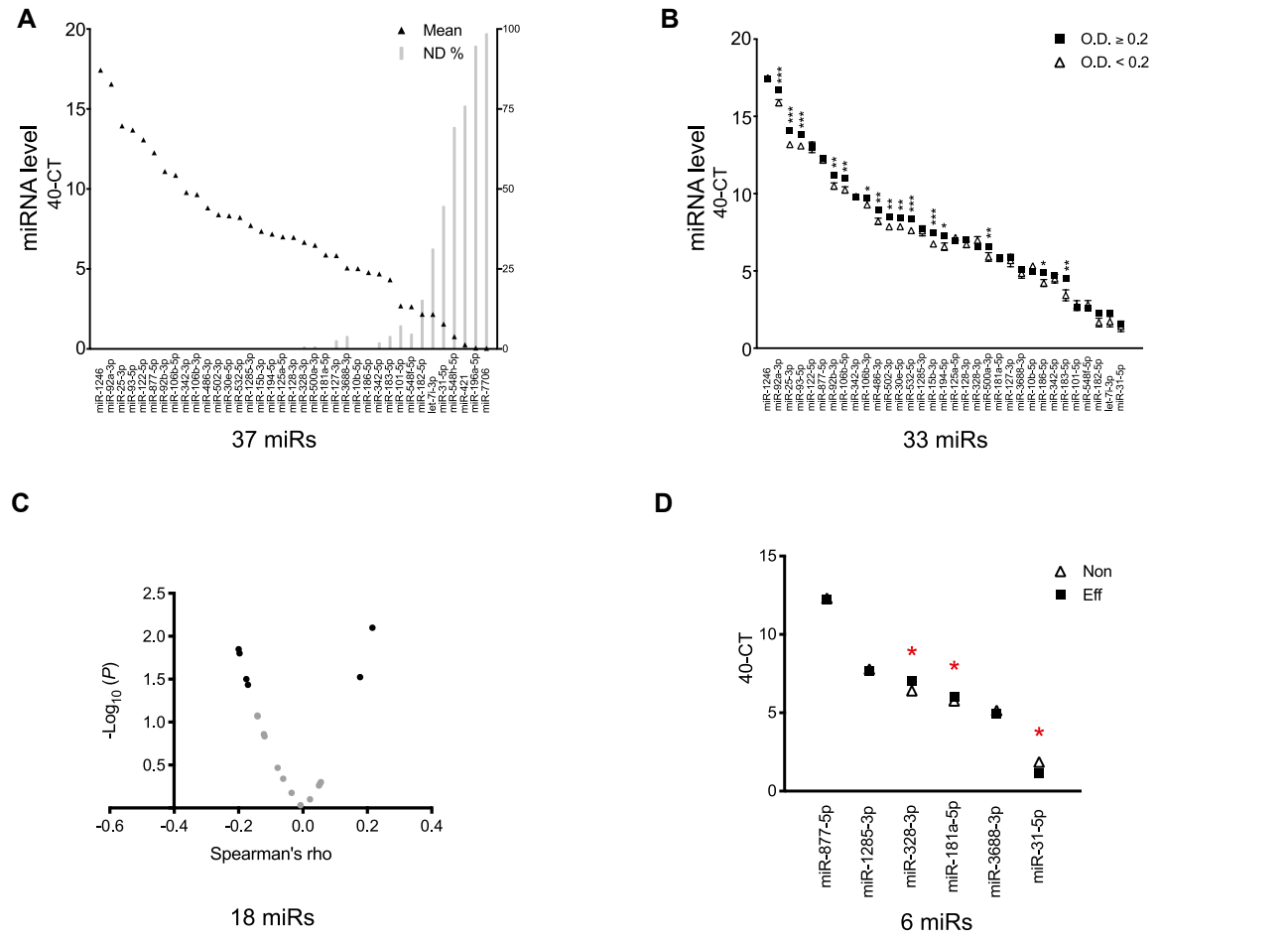

FIGURE 1 Algorithm to identify circulating miRNAs with the best performance in discriminating the effective vs. noneffective groups. (A) Quantification of 37 miRNAs by quantitative RT-PCR in 150 serum samples. The means of individual miRNA expressions are presented as 40-Ct and sorted in a descending order. The gray bars indicate the nondetected rate (percentage) of each miRNA, which was defined as the percentage of samples with Ct > 40. (B) Sample hemolysis was determined by the absorbance of hemoglobin at 414 nm greater than 0.2. The means and standard errors for the expression levels of 33 miRNAs in the hemolytic and the nonhemolytic groups were calculated. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. (C) A total of 18 miRNAs with reasonable abundances and their correlations with effectiveness of bariatric surgery calculated by Spearman rank correlation. Volcano plot shows the log *p* value vs. the Spearman correlation. (D) The data distribution of six correlated miRNAs between patients with or without an effective response after bariatric surgery. The miRNA expressions are presented as 40-Ct. $^{*}p < 0.05$. (E) Flowchart displays the stepwise algorithm for miRNA selection. Ct, cycle threshold; miRNAs, microRNAs [Color figure can be viewed at wileyonlinelibrary.com]

69, 827, and 220 common targets of miR-328-3p, miR-181a-5p, and miR31-5p, respectively (Figure 4A). The pathway analysis revealed the top 10 enriched pathways for each miRNA (Figure 4B). Notably, several predicted target genes were involved in the AMP-activated protein kinase (AMPK) signaling pathway, which is known as a metabolic coordinator and which is activated for adaptative changes in growth and metabolic control in tissues of liver, muscle, and fat under low-energy conditions (24). The schematic overview of the targets of miR-328-3p, miR-181a-5p, and miR31-5p in the regulatory mechanism of the AMPK signaling pathway is shown in Figure 5.

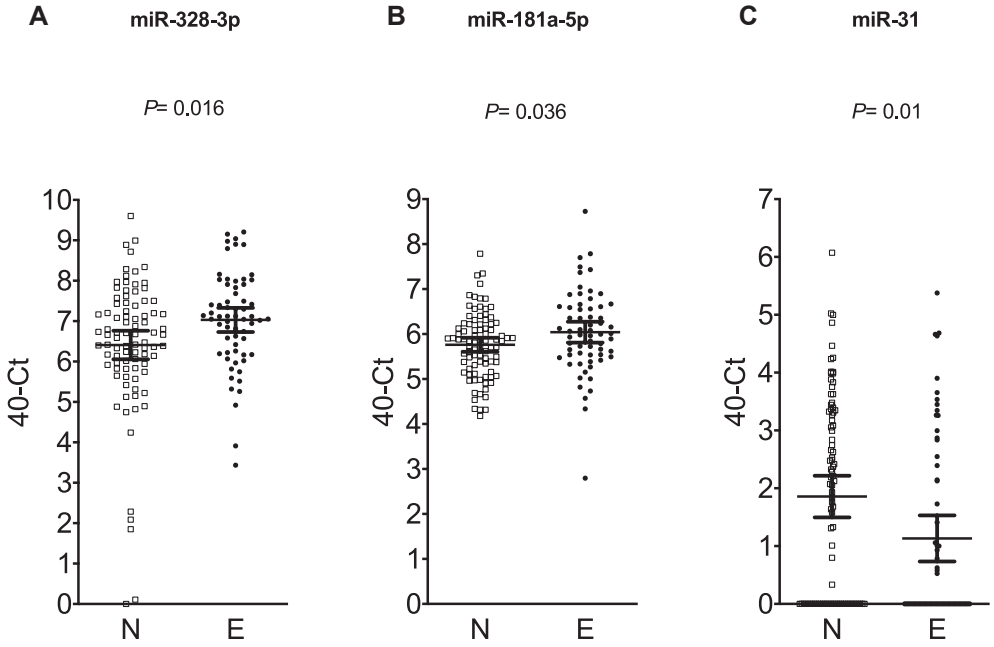

FIGURE 2 Scatterplots representing the distributions for the expression levels of miR-328-3p, miR-181a-5p, and miR-31 in the noneffective vs. effective groups. *p* value was calculated by Mann–Whitney *U* test. Ct, cycle threshold

TABLE 3 Selected miRNAs and miRNA ratios with significantly differential expression between the noneffective and effective groups

	N		E					
miRNA	Mean	SD	Mean	SD	Fold (E/N)	*p* value (MWU)	AUC	95% CI
miR-328-3p	6.41	1.66	7.03	1.18	1.54	0.016	0.615	0.524-0.706
miR-181a-5p	5.76	0.73	6.04	0.92	1.21	0.036	0.600	0.508-0.693
miR-31-5p	1.86	1.69	1.13	1.59	0.61	0.014	0.617	0.526-0.708
miR-328-3p/miR-31-5p	4.55	2.30	5.90	1.75	2.54	0.000	0.680	0.593-0.766
miR-181a-5p/miR-31-5p	3.90	1.68	4.91	1.69	2.01	0.000	0.669	0.580-0.758

Note: Ratio 1 represented as the ratio of miR-328-3p/miR31-5p levels; Ratio 2 represented as the ratio of miR-181a-5p/miR-31-5p levels. Algorithms of two miRNA panels were built by logistic regression; Algorithm 1: Logit(P[Y = 1]) = Ln([P(Y=1)]/[P(Y = 0]) = 4.579 + (0.3574) × (miR-328-3p/miR-31-5p) + (−0.1684)*BMI; Algorithm 2: Logit(P[Y = 1]) = Ln([P(Y = 1)]/[P(Y = 0)]) = 4.735 + (0.3635) × (miR-181a-5p/miR-31-5p) + (−0.1652) × *BMI.

Abbreviations: AUC, area under the ROC curve; E, effective group; MWU, Mann-Whitney *U* test; N, noneffective group; ROC, receiver operating characteristic.

DISCUSSION

In post-bariatric surgery patients, the disparity of outcomes among individuals remains an area of uncertainty in clinical practice. Genetic or epigenetic differences are believed to determine obesity and the response to obesity treatment. In this study, we assessed the feasibility of circulating miRNA panels to recognize possible nonresponders in a clinical setting of obesity treatment. We demonstrated that two miRNA panels (BMI + ratio of miR-328-3p/miR31-5p; BMI + ratio of miR-181a-5p/miR-31-5p) are indicative of an effective or noneffective response in terms of weight reduction at 6 months after bariatric surgery, with reasonable accuracy. These miRNA panels facilitate informed decision-making of severe obesity treatment in clinical practice. For patients with a genetic profile consistent with poor outcomes after bariatric surgery, they may be more appropriately treated with other modalities or informed about risks of weight regain and the need for further revisional interventions. Moreover, these three miRNAs are implicated in the regulation of the AMPK signaling pathway from database analyses, which is relevant to obesity and metabolic disorders. AMPK, a critical regulator of cellular metabolism, is activated in response to many types of physiologic stresses under lower intracellular ATP levels to promote catabolic and inhibit anabolic

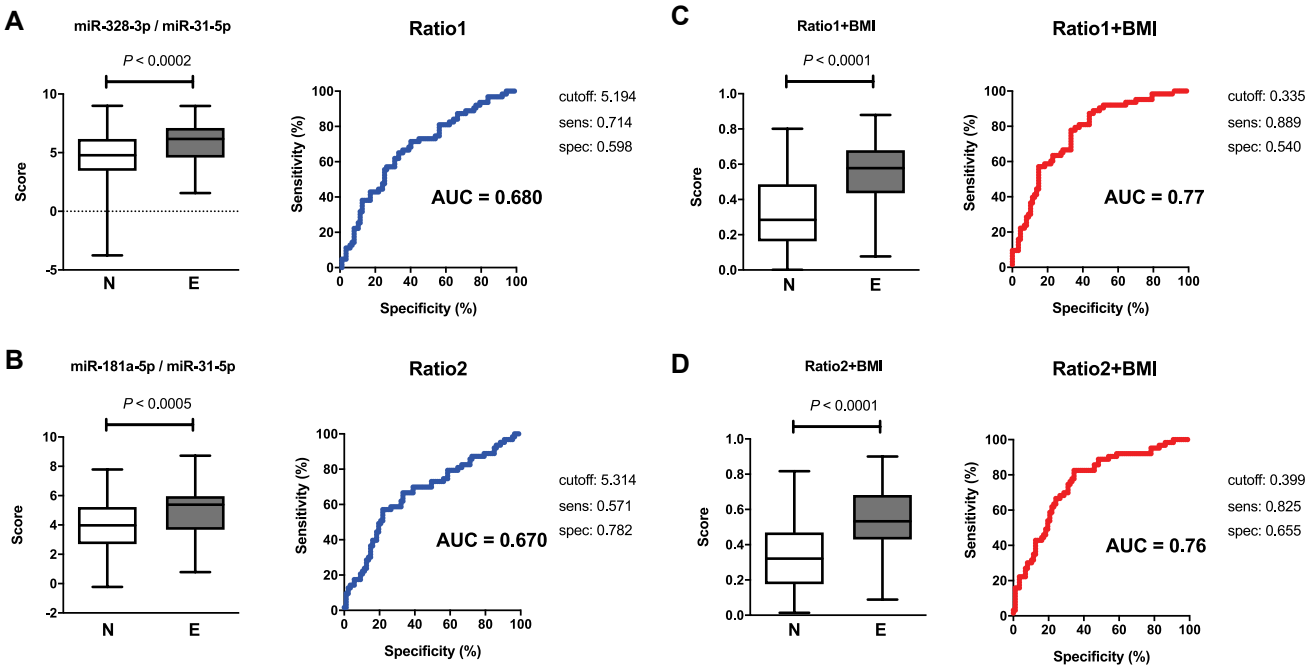

FIGURE 3 The ROC analysis and the AUC values were performed to discriminate between the effective vs. noneffective groups. (A) Ratio 1 (miR-328-3p/miR31-5p); (B) Ratio 2 (miR-181a-5p/miR-31-5p); (C) BMI + Ratio 1; and (D) BMI + Ratio 2. AUC, area under receiver operating characteristic curve, ROC, receiver operating characteristic [Color figure can be viewed at wileyonlinelibrary.com]

processes. In insulin-responsive muscle and fat tissue, AMPK upregulated solute carrier family 2 (facilitated glucose transporter), member 4 (GLUT4) trafficking to meet energy challenges via the intermediates of Rab GTPase activating proteins TBC1 domain family members 1 and 4 (25). In addition, AMPK is the upstream kinase of many critical metabolic enzymes such as acetyl-CoA carboxylase, 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA) reductase, and adipocyte triglyceride lipase (26,27). Therefore, we speculate that individuals with severe obesity who benefit from bariatric surgery may have an imbalanced AMPK signal pathway to modulate metabolic status and maintain energy balance, which is manifested by specific miRNAs involved in the regulatory mechanism. Nevertheless, there is limited knowledge about the relevant targeted genes and regulatory pathways, which are directly affected by miRNAs identified in the study, linking to the response to specific treatment modality. Therefore, further research is needed to identify their connections.

Functional implication of these miRNAs in prior studies supports their relevant roles in disease mechanisms. MiR-31 has been reported to be significantly upregulated in plasma of children with obesity and it directly targets CCAAT/enhancer binding protein, alpha, which, concurrently with retinoic acid receptor gamma, is involved in insulin sensitivity, lipogenesis, and adipocyte differentiation and maturation (28-30). In concordance with our finding, it was reported that miR-328 activated adipogenesis in brown adipocytes that are metabolically active and produce heat to maintain body temperature and energy balance (31). Moreover, miR-328-3p mediated the beneficial effects of dietary methionine restriction via alleviating oxidative stress and improving the efficiency of protein metabolism in a high-fat diet-induced obesity mouse model (32). Regarding miR-181a-5p, gut microbiota-derived metabolites controlled the expression of the miR-181 family in white adipocytes in mice to regulate energy expenditure. However, dysbiotic gut microbiota promoted adipocyte inflammation, insulin resistance, and the development of obesity (33).

Previous studies have reported that obesity-associated miRNA profiles are altered following bariatric surgery and correlated with clinical metabolic improvement. For example, Ortega et al. observed marked changes in the circulating miRNA profile, including a decrease in miR-16-1, miR-122, miR-140-5p, and miR-193a-5p and an increase in miR-221 and miR-199a-3p before and after a gastric bypass procedure (10). Nunez-Lopez et al. identified miR-151-5p (increased), miR-122-5p (decreased), and miR-34-5p (decreased) in response to Roux-en-Y gastric bypass surgery-induced weight loss, which correlated with clinical indices of insulin sensitivity, β-cell function, and glucose metabolism in people with severe obesity (34). Changes in miRNA expression have also been observed in the adipose tissue, with different patterns between visceral and subcutaneous sites. Our group reported miRNA expression patterns in subcutaneous and visceral adipose tissue in patients undergoing bariatric surgery. One of the most significant signatures, miR-122, was upregulated in visceral adipose tissue and affected peroxisome proliferator-activated receptor gamma (PPAR-γ) signaling and

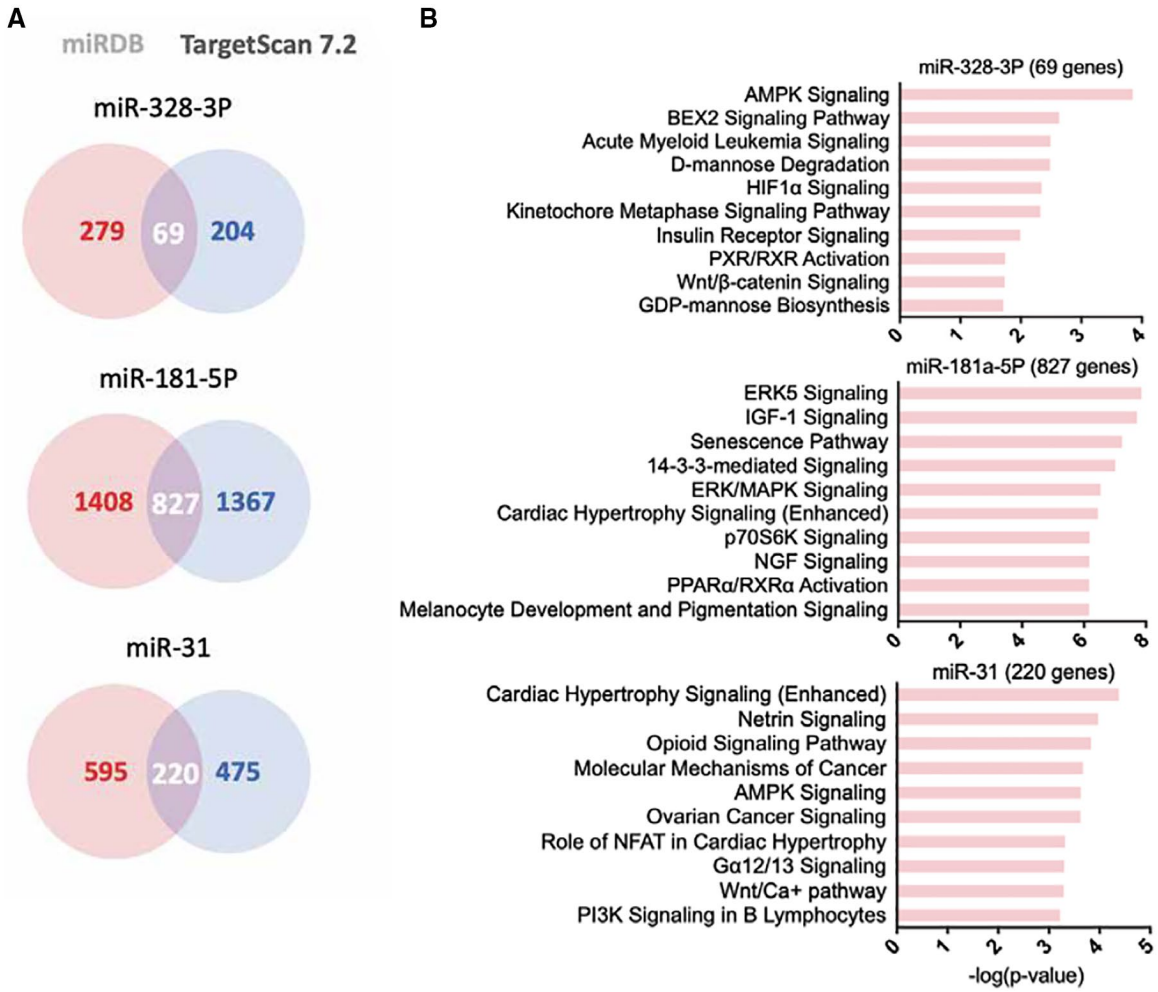

FIGURE 4 Target gene prediction and pathway analysis of miR-328-3p, miR-181a-5p, and miR-31-5p. (A) Venn diagrams showed the potential miRNA targets predicted from databases of TargetScan 7.1 and MiRDB; (B) the intersecting genes predicted from both databases were further examined by Metcore pathway map analysis. The top 10 significantly enriched pathways are listed with their −log *p* values [Color figure can be viewed at wileyonlinelibrary.com]

adipocyte differentiation *in vitro* (22). On the other hand, different components of miRNA profile changes (down-expressed miR-519d, miR-299-5p, miR-212, and miR-671-3p; up-expressed miR-370 and miR-487a) in subcutaneous adipose tissue after laparoscopic adjustable gastric banding also were reported. The level of PPAR-α protein, which is the target gene of miR-519d, increased after surgery (35).

However, marked discrepancies between these studies have been noticed, which have been attributed to differences in sample size, study population, type of intervention, miRNA profiling methods, follow-up duration, and collected samples (36). Most previous studies had small sample sizes (less than 30 participants). Our results represented a larger cohort of 150 Asian patients who underwent consistent weight loss reduction surgeries and 6 months of a postoperative maintenance program by a single specified medical team. For miRNA isolation, profiling, and normalization methods, we used an miRNeasy isolation kit to produce a higher RNA quantity in serum samples to avoid failure of detecting low-abundance miRNAs. In addition, profiling by multiplex quantitative PCR and normalization with a synthetic miRNA as a spike-in control between each miRNA and each individual sample make our results reproducible and reliable.

There are several limitations to this study. First, the assessment of effective or noneffective response was determined only by the facts of excess weight loss at 6 months, not correlated results with a common cardiometabolic function index such as insulin sensitivity, peak oxygen consumption, or body composition. Second, only 37 candidate miRNAs, which had a differential expression in six individuals at the extreme end of %EBW, were selected for circulating miRNA profile analyses in the whole study cohort. Our goal of this study was not to provide a comprehensive genetic analysis but rather to demonstrate the feasibility of using simple serum profiles to provide additional information of responsiveness to the specific treatment.

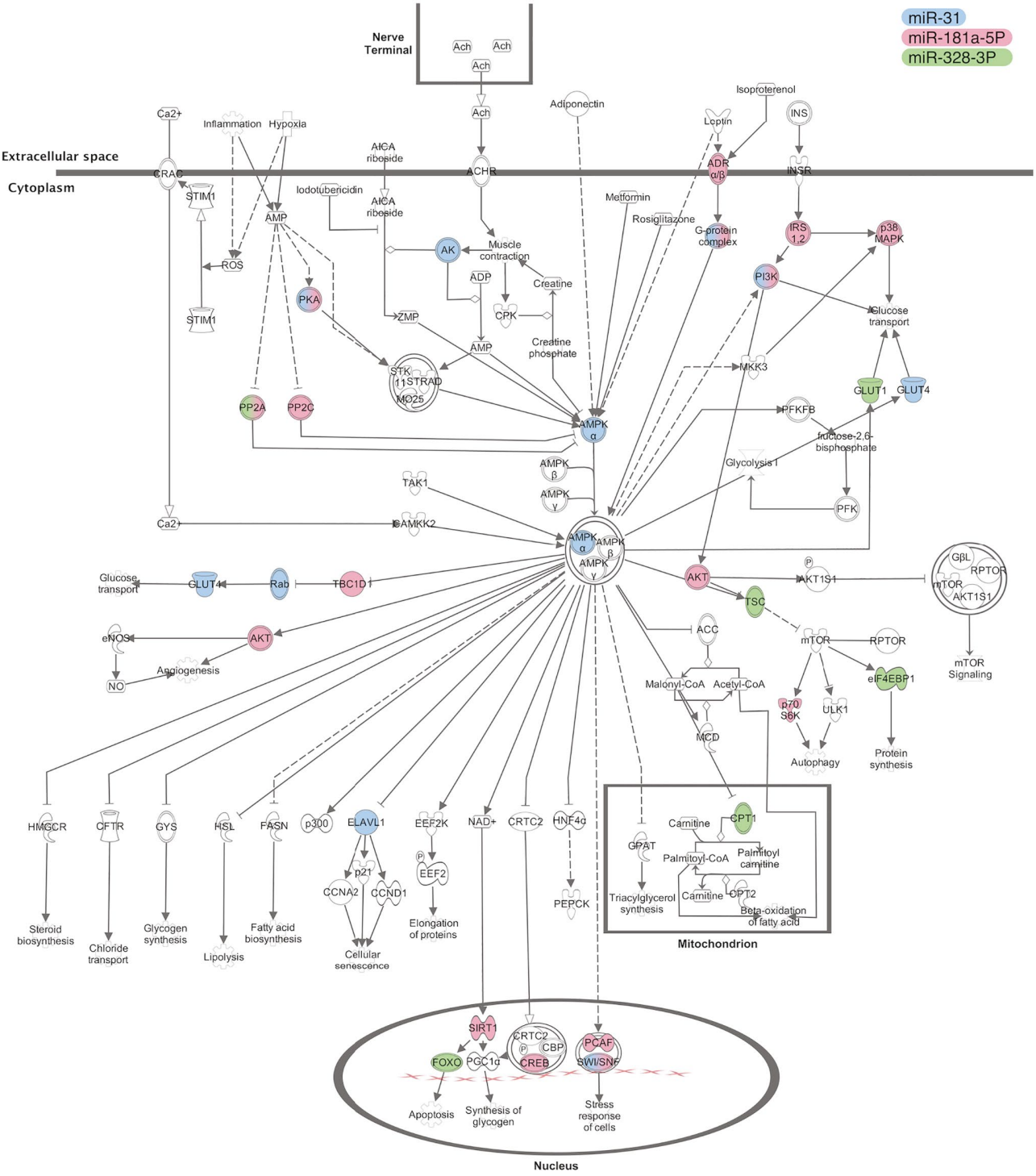

FIGURE 5 Schematic overview for the targets of miR-328-3p, miR-181a-5p, and miR-31-5p in the AMPK signaling pathway. AMPK, AMP-activated protein kinase

CONCLUSION

Obesity and obesity-related morbidities have become major public health concerns over the decades. Understanding the molecular mechanisms of metabolic disorders is essential to develop effective therapeutic treatments. Although bariatric surgery has made remarkable progress in obesity treatment, there is considerable heterogeneity of response among individuals. Several miRNAs as epigenetic modulators have been found to be differentially expressed in individuals with obesity and are modified by weight reduction management. Using the fact that miRNA expression in circulation differs substantially between responders and nonresponders to bariatric surgery, we demonstrated that serum miRNA panels could distinguish patients with effective or noneffective response in terms

of sustained weight loss at 6 months. In clinical practice, we look forward to the predictive panels that will help patients and physicians in the decision-making of obesity treatments.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHOR CONTRIBUTIONS

CYW and TSY were involved in all aspects of the project. CCC, YSC, KHL, CCP, and TAL researched data. JKY, MSW, TSY, and CYW researched data, contributed to the discussion, and reviewed/edited the manuscript. JKY and CYW contributed to the discussion and reviewed/edited the manuscript.

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