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ORIGINAL ARTICLE

Case Control Study

miRNA-103: Molecular link between insulin resistance and nonalcoholic fatty liver disease

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Abstract

AIM: To investigate the associations between miRNA-103 (miR-103) and insulin resistance and nonalcoholic fatty liver disease (NAFLD).

METHODS: Serum samples were collected from 50 NAFLD patients who were overweight or obese (NAFLD group) and from 30 healthy subjects who served as controls (normal control group). Quantitative polymerase chain reaction was used to detect expression of miR-103. Fasting plasma glucose, fasting insulin, and triglyceride (TG) levels were measured. Homeostasis model assessment was used to evaluate basal insulin resistance (HOMA-IR). Patient height and weight were measured to calculate body mass index (BMI).

RESULTS: Compared with the normal control group, higher serum levels of miR-103 were expressed in the NAFLD group (8.18 ± 0.73 *vs* 4.23 ± 0.81, $P = 0.000$). When $P = 0.01$ (bilateral), miR-103 was positively correlated with HOMA-IR ($r = 0.881$), TG ($r = 0.774$) and BMI ($r = 0.878$), respectively. miR-103, TG and BMI were all independent factors for HOMA-IR ($\beta = 0.438/0.657/0.251$, $P = 0.000/0.007/0.001$). miR-103, TG, BMI and HOMA-IR were all risk factors for NAFLD (odds ratio = 2.411/16.196/1.574/19.11, $P = 0.009/0.022/0.01/0.014$).

CONCLUSION: miR-103 is involved in insulin resistance and NAFLD, and may be a molecular link between insulin resistance and NAFLD and a therapeutic target for these disorders.

Key words: miRNA-103; Insulin resistance; Nonalcoholic fatty liver disease

Core tip: Insulin resistance activates development of nonalcoholic fatty liver disease (NAFLD), however, the molecular mechanism is not fully understood. We determined fasting plasma glucose, fasting insulin, triglyceride (TG) and the levels of miRNA-103 (miR-103) in the serum of patients with NAFLD. We found that higher levels of miR-103 were expressed in the serum of patients with NAFLD. miR-103 was positively correlated with homeostasis model assessment and was used to evaluate basal insulin resistance (HOMA-IR),

TG and BMI, respectively. miR-103 was an independent factor for HOMA-IR and a risk factor for NAFLD. We conclude that miR-103 is involved in insulin resistance and NAFLD.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a clinical syndrome characterized by hepatic steatosis and fat deposition in hepatocytes in the absence of significant alcohol use. The incidence of NAFLD in the general Chinese population has almost doubled over the last 10-15 years and is highly prevalent in obese populations[1]. Obesity-associated insulin resistance is regarded as a factor that critically contributes to the development of NAFLD[2]. Insulin resistance resulting in a hyperinsulinemic state increases *de novo* lipogenesis, which further exacerbates hepatic lipid deposition and boosts the development of the disease.

miRNAs are endogenously expressed RNAs consisting of 20-24 nucleotides that affect the expression of hundreds of genes involved in numerous biological processes, including lipid metabolism, organ development, differentiation, brain morphogenesis, and apoptosis.

miRNAs are potent intracellular post-transcriptional regulators and are also selectively secreted into the circulation in a cell-specific fashion. miRNAs are now known to be stably expressed in serum[3], blood[4,5] and plasma[6]. Moreover, the unique expression patterns of these circulating miRNAs are related to specific human diseases[7]. miRNA-103 (miR-103) regulates insulin sensitivity and glucose homeostasis and is highly expressed in the liver of patients with NAFLD. Furthermore, there is a positive correlation between the patient's homeostatic model assessment (HOMA) index and miR-103 expression levels[8].

The clinical spectrum of NAFLD varies between steatosis with a benign clinical course and cirrhosis with serious complications, including hepatocellular carcinoma and liver failure, therefore, it is important to identify the molecular mechanisms and therapeutic targets of NAFLD. A "two-hit" mechanism has been proposed, however, the underlying molecular mechanism is not fully understood. In this study, we aimed to determine the levels of miR-103 expressed in the serum of patients with NAFLD to explore the associations between miR-103 and insulin resistance and NAFLD in order to identify new molecular therapeutic targets for these disorders.

MATERIALS AND METHODS

Subjects

This study enrolled a cohort of 50 patients with NAFLD who were treated at the Department of Endocrinology of the Second Affiliated Hospital of Medical College of Qingdao University, China from November 2011 to April 2013. Thirty age-matched healthy subjects were selected as controls. Blood pressure and electrocardiogram findings were normal in all patients. Patients with NAFLD were newly diagnosed and had not received any treatment. The diagnosis of NAFLD was based on the presence of an ultrasonographic pattern consistent with "bright liver" (brightness and posterior attenuation) with stronger echoes in the hepatic parenchyma than in the renal parenchyma, vessel blurring, and narrowing of the lumen of the hepatic veins in the absence of findings suggestive of other chronic liver disease. All cases of fatty liver were in accordance with the Chinese diagnostic criteria for NAFLD (alcohol consumption < 40 g per week and without consideration of alteration in liver enzymes). Body mass index (BMI) was calculated based on the following formula: BMI = weight/height^2. The study was approved by the Ethics Committee of the Second Affiliated Hospital of Medical College of Qingdao University.

Measurements

Fasting plasma glucose (FPG), fasting insulin (Fins), triglyceride (TG), and miR-103 levels were measured. Blood samples were obtained after an 8-h fasting period. Fins was measured by radioimmunoassay (RuiQi Biotechnology Corporation, Shanghai, China) with a sensitivity of 2 mU/L (normal range 0.5-25 mU/L). The insulin resistance index [homeostasis model assessment-insulin resistance (HOMA-IR)] was calculated using the HOMA model: (fasting insulin × fasting glucose)/22.5[9]. The expression of miR-103 was detected using real-time PCR.

Microarray profiling of serum miRNA

Total RNA was extracted from normal controls and patients with NAFLD (Biological Technology Co. Ltd., Chengdu, China). miRNA microarray analyses were carried out by Biological Technology using an ABI3730 Sequencer (Applied Biosystems, United States).

Statistical analysis

All statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, United States). Normally distributed data were expressed as mean ± SD. The *t* test was used to compare groups in the study. Pearson correlation analysis and stepwise regression analysis were used for simple correlation and multivariate analysis, respectively. $P < 0.05$ was considered statistically significant.

Table 1 Clinical characteristics of the study subjects (mean ± SD)

	NAFLD group (n = 50)	Normal control group (n = 30)
Age (yr)	50 ± 6.70	50 ± 6.81
Gender (male/female)	28/22	16/14
BMI (kg/m^2)	28.70 ± 3.12[b]	22.80 ± 3.07
ln (HOMA-IR)	1.72 ± 0.35[b]	0.38 ± 0.31
Fins (IU/mL)	16.3 ± 3.06[b]	8.55 ± 3.21
TG (mmol/L)	2.67 ± 1.23[b]	1.58 ± 1.19
TC (mmol/L)	5.01 ± 0.89[a]	4.61 ± 0.97
FPG (mmol/L)	5.95 ± 0.93[b]	4.85 ± 0.87
ALT (U/L)	38.10 ± 19.80[b]	26.30 ± 20.14

[a]P < 0.05, [b]P < 0.01 *vs* the control group (unpaired Student t test and χ^2 test). NAFLD: Nonalcoholic fatty liver disease; BMI: Body mass index; Fins: Fasting insulin; ln (HOMA-IR): Homeostasis model assessment-insulin resistance was log-transformed before analysis as it was not normally distributed; FPG: Fasting plasma glucose; TC: Total cholesterol; TG: Triglycerides; ALT: Alanine transaminase.

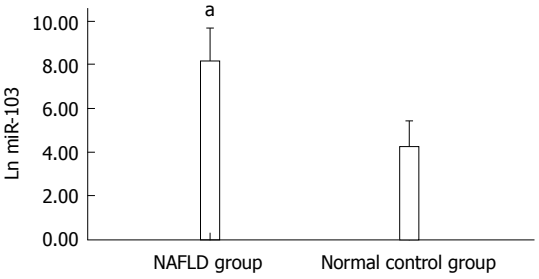

Figure 1 Differential expression of microRNA-103 levels in the serum of patients with nonalcoholic fatty liver disease. microRNA (miR)-103 was log-transformed before analysis because it was not normally distributed. [a]P < 0.05 *vs* normal control group (unpaired Student t test). NAFLD: Nonalcoholic fatty liver disease.

RESULTS

General characteristics

A total of 80 cases were included in this study. With the exception of age and gender (P > 0.05), BMI, HOMA-IR, Fins, TG, total cholesterol (TC), FPG, and alanine aminotransferase (ALT) in patients with NAFLD were higher than those in healthy controls (P < 0.05) (Table 1).

Differential expression of miR-103 levels in serum of patients with NAFLD

The levels of miR-103 expressed in the serum of NAFLD patients were higher than those in healthy controls (8.18 ± 0.73 *vs* 4.23 ± 0.81, P < 0.05) (Figure 1).

Pearson correlation analysis of associations between miR-103 and HOMA-IR, BMI and TG

Positive correlations were observed between miR-103 and HOMA-IR (r = 0.881), BMI (r = 0.878) and TG (r = 0.774) (Figure 2).

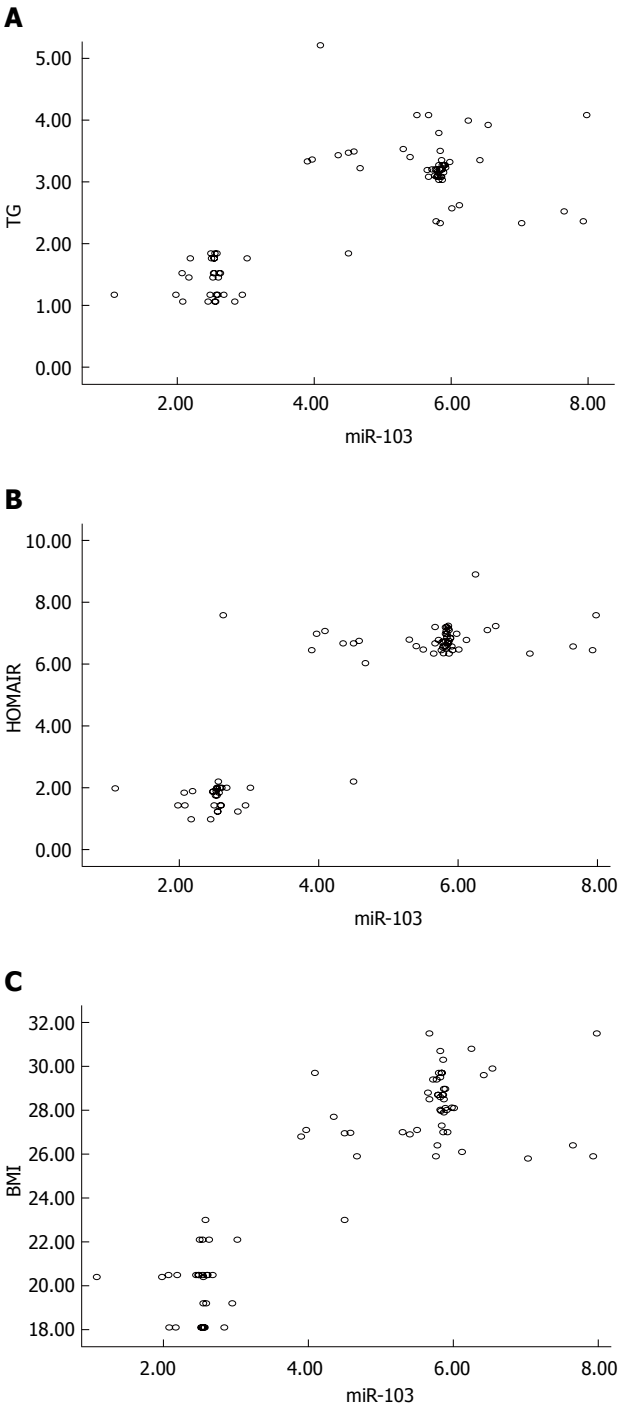

Figure 2 Correlation between microRNA-103 and Triglycerides (A), Homeostasis model assessment-insulin resistance (B), and body mass index (C). A: microRNA (miR)-103 was positively correlated with Triglycerides (TG) (r = 0.774, P = 0.01, bilateral); B: miR-103 was positively correlated with Homeostasis model assessment-insulin resistance (HOMA-IR) (r = 0.881, P = 0.01, bilateral); C: miR-103 was positively correlated with body mass index (BMI) (r = 0.878, P = 0.01, bilateral).

Multivariate analysis of miR-103, BMI, TG and HOMA-IR

HOMA-IR was a dependent variable and miR-103, TG and BMI were independent variables. Multivariate

Table 2 Linear regression analysis of factors affecting homeostasis model assessment-insulin resistance

	Independent variables	Independent variables	SE standard regression	*t* value	*P* value
BMI	0.251	0.072	0.431	3.510	0.001
miR-103	0.438	0.113	0.302	3.890	0.000
TG	0.657	0.236	0.258	2.779	0.007

BMI: Body mass index; miR-103: MicroRNA-103; TG: Triglyceride; HOMA-IR: Homeostasis model assessment-insulin resistance.

Table 3 Binary logistic regression analysis of factors affecting nonalcoholic fatty liver disease

Variables	*P* value	OR	95%CI
miR-103	0.009	2.411	1.25-4.652
TG	0.022	16.196	1.507-174.013
BMI	0.010	1.574	1.117-20.219
HOMA-IR	0.014	19.11	1.808-202.001

BMI: Body mass index; miR-103: MicroRNA-103; TG: Triglyceride; HOMA-IR: Homeostasis model assessment-insulin resistance.

linear regression analyses showed that miR-103, TG and BMI were all independent factors for HOMA-IR (β = 0.438/0.657/0.251, P = 0.000/0.007/0.001) (Table 2).

Binary logistic regression analysis of miR-103, BMI, TG, HOMA-IR and NAFLD

NAFLD was a dependent variable and miR-103, TG, BMI and HOMA-IR were independent variables. Binary logistic regression analysis showed that miR-103, TG, BMI and HOMA-IR were all risk factors for NAFLD (OR = 2.411/16.196/1.574/19.11, P = 0.009/0.022/0.01/0.014) (Table 3).

DISCUSSION

In this study, we found that HOMA-IR, Fins, TG, and FPG levels in patients with NAFLD were higher than those in healthy controls. These results suggest that these higher levels exist in patients with insulin resistance, and lipid and glucose abnormalities. To date, despite significant efforts, the accurate pathogenesis of NAFLD is not fully understood.

NAFLD, the prevalence of which is increasing in obesity, is one of the most frequent causes of chronic liver diseases and is characterized by the accumulation of lipids in hepatic cells. It is closely associated with hypertriglyceridemia, insulin resistance and intestinal microbiota changes[10,11]. More specifically, the input of lipid exceeds the output of lipid from the liver, which induces storage of lipid in the liver contributing to the development of hepatic steatosis. According to the two-hit hypothesis, insulin resistance results in increased intrahepatic triglyceride accumulation and this is the first hit, followed by the second step. The latter likely involves cytochrome P450 activation, oxidative stress, increased inflammatory cytokine production, lipid peroxidation, activation of hepatic stellate cells, and apoptosis. França *et al*[12] found that hypertriglyceridemia and liver steatosis were associated with increased microsomal triglyceride transfer protein expression. Another study reported that phospholipid ω-3 polyunsaturated fatty acids may play an important role in the development of NAFLD[13]. Therefore, many of the mechanisms underlying this association are still unclear.

Insulin resistance, described as the inability of insulin to stimulate glucose uptake, is a risk factor for the development of NAFLD[14]. Insulin resistance results in a reduction in lipolysis inhibition by insulin, which leads to fatty acid accumulation contributing to altered mitochondrial function, increased lipid intermediates and hepatic steatosis[15]. Insulin activates sterol regulatory element-binding protein (SREBP)1c, a master regulatory transcription factor in lipid synthesis, through stimulation of the mammalian target of rapamycin complex 1, which leads to increased lipogenesis[16]. Therefore, insulin resistance characterized by a hyperinsulinemic state as observed in patients with NAFLD increases *de novo* lipogenesis, which further exacerbates hepatic lipid deposition and accelerates development of the disease.

Recent studies have indicated that miRNAs are involved in the development of NAFLD, and serum levels of miRNAs are correlated with the severity of liver steatosis[17,18] and may represent novel, noninvasive biomarkers of diagnosis and histological disease severity in patients with NAFLD[19,20]. Hoekstra *et al*[21] reported that fatty liver development in low-density lipoprotein receptor knockout mice was associated with a significant change in the hepatocyte miRNA profile, a fivefold decrease in miR-302a expression was reported, which predisposed the liver to insulin resistance.

miR-103 results in insulin resistance. Trajkovski *et al*[8] reported that silencing of miR-103 led to improved insulin resistance. In contrast, gain of miR-103 function in either liver or fat was sufficient to induce insulin resistance. Further studies confirmed that high expression of miR-103 led to insulin resistance by downregulating caveolin-1, which is the direct target gene of miR-103 and a critical regulator of the insulin receptor. In addition, silencing of miR-103 decreased total fat by reducing adipocyte size. Furthermore, adiponectin levels were increased in anti-miR-103-injected *ob/ob* mice. Smaller adipocytes were associated with increased insulin sensitivity in human and rodent models[22], and adiponectin levels were positively correlated with insulin sensitivity[23]. In our study, we also found that miR-103 was positively correlated with HOMA-IR and was an independent

factor in overweight or obese patients with NAFLD. These data indicate that miR-103 is indirectly involved in the development of NAFLD due to insulin resistance.

Increased hepatic expression of miR-103 is directly involved in the development of NAFLD. NAFLD is highly associated with obesity and insulin resistance and is accompanied by hypertriglyceridemia, histologically characterized by hepatic TG accumulation of > 5%, resulting in steatosis. Xie *et al*[24] reported that miR-103 accelerates adipogenesis when expressed ectopically. miR-103 was increased in the liver of patients with NAFLD[8]. Our results showed that NAFLD patients had higher levels of miR-103 expression in serum compared with normal controls, and miR-103 was positively correlated with TG. Taken together, these results indicate that high expression of miR-103 may be directly involved in the development of NAFLD by increasing adipogenesis in hepatocytes leading to ectopic lipid deposition, thus contributing to hepatic steatosis.

miR-103 links insulin resistance and NAFLD. It has been reported that BMI and TG are the main factors related to the severity of NAFLD[25] and TG is regarded as an independent parameter associated with NAFLD[26]. Our results showed that miR-103 was positively correlated with HOMA-IR, TG and BMI, respectively, and miR-103, TG and BMI were all independent factors associated with HOMA-IR. MiR-103, TG, BMI and HOMA-IR were all risk factors for NAFLD. Therefore, miR-103 may be a potential molecular link between insulin resistance and NAFLD.

In conclusion, our results indicated that high expression of miR-103 directly increases adipogenesis in hepa-tocytes and indirectly results in hypertriglyceridemia due to insulin resistance, thus contributing to the development of NAFLD. Therefore, miR-103 is involved in insulin resistance and NAFLD, and may be regarded as a potential molecular link between them and a therapeutic target of these disorders. However, miR-103 as the bridge between insulin resistance and NAFLD requires further evidence. As our study included a small sample size and no liver biopsies were obtained, the above results require to be confirmed in a larger study which includes liver biopsies.

COMMENTS

Background

The pathogenesis of nonalcoholic fatty liver disease (NAFLD) remains obscure. Insulin resistance activates the development of NAFLD, however, the molecular mechanisms involved are not fully understood. miRNAs are involved in the development of NAFLD, and are known to be stably expressed in serum, blood and plasma. Moreover, the unique expression patterns of these circulating miRNAs are correlated with specific human diseases. miRNA-103 (miR-103) regulates insulin and glucose homeostasis and is highly expressed in the liver of patients with NAFLD.

Research frontiers

Insulin resistance activates the development of NAFLD, and miR-103 regulates insulin sensitivity. miRNAs are involved in the development of NAFLD, and serum levels of miRNAs are correlated with the severity of liver steatosis and may represent novel, noninvasive biomarkers of diagnosis and histological disease severity in patients with NAFLD.

Innovations and breakthroughs

Previous studies have shown that miR-103 regulates insulin sensitivity in obese mice and is increased in the liver of patients with NAFLD. In this study, the authors determined fasting plasma glucose, fasting insulin, triglyceride (TG) and expressed miR-103 levels in the serum of patients with NAFLD. The levels of miR-103 expressed in serum were higher in patients with NAFLD than in controls. miR-103 was positively correlated with homeostasis model assessment-insulin resistance (HOMA-IR), TG and body mass index (BMI), respectively. miR-103 was an independent factor of HOMA-IR and a risk factor for NAFLD. Therefore, miR-103 is involved in insulin resistance and NAFLD and may be regarded as the link between them and a therapeutic target in both disorders.

Applications

The study results suggest that high expression of miR-103 directly increases adipogenesis in hepatocytes and indirectly results in hypertriglyceridemia due to insulin resistance, thus contributing to the development of NAFLD. miR-103 is involved in insulin resistance and NAFLD, and may be regarded as a potential molecular link between them and a therapeutic target in both disorders.

Terminology

Insulin resistance is a physiological condition in which cells fail to respond to the normal actions of the hormone insulin. The body produces insulin, but the cells in the body become resistant to insulin and are unable to use it effectively, leading to hyperglycemia. Pancreatic β cells subsequently increase their production of insulin, further contributing to hyperinsulinemia, which is involved in the development of type 2 diabetes, hypertension, NAFLD and cancer. miR-103 belongs to the family of miRNAs, which are noncoding, highly conserved regulatory RNAs, which help to regulate gene expression at the post-transcription level. miR-103 is involved in the development of insulin resistance, cancer, and other diseases.

Peer review

This was an interesting study showing the relationship between miR-103, insulin resistance and NAFLD and an interesting paper reporting novel findings regarding the role of miR-103 in the pathogenesis of NAFLD, but miR-103 as the bridge of insulin resistance and NAFLD, may need more evidence.

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