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Original Article

Expression of microRNAs differed in the omental adipose tissue of obese rats

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Abstract: Objective: To establish obese rat models by high-fat diet, screen microRNAs by microarray in the omental adipose tissue, and find out differential expression of microRNAs in obese rats, for further understanding the role of microRNAs as regulating molecules in obesity-induced lipid metabolism disorders. Methods: 40 male SD rats were randomly divided into normal diet group and high-fat diet group, respectively. After fed for 8 weeks, rats were weighted, measured length and other characteristics were observed. Eye blood was taken to test blood glucose level, blood lipids level, insulin level and other indicators. The omental adipose tissue was measured by electronic analytical scales and saved at -80°C liquid nitrogen. Fat cells were stained by oil red to observe their morphology under microscopy. The expression of microRNAs was screened by microarray, and verified by Real-Time PCR. Results: After high-fat diet for 4 and 8 weeks, some fatty indicators changed, including increased body weight, omental fat weight, triglycerides, total cholesterol, low-density lipoprotein, blood glucose level and insulin level, and decreased high-density lipoprotein, and differential phenotype of fat cells. Besides, by microarray techniques and Real-Time PCR, 13 differential expression microRNAs were identified, including 7 up-regulated microRNAs (microRNA30a, microRNA7e, microRNA30c, microRNA335, microRNA103, microRNA107, microRNA139-5p), and 6 down-regulated microRNAs (microRNA494, microRNA140, microRNA342-5p, microRNA382, microRNA17-1-3p, microRNA92a). Conclusion: Changes in the expression of microRNAs contribute to the pathogenesis of many diseases, including obesity disorders. These alterations can be due to various mechanisms, such as cell proliferation, apoptosis, migration, and differentiation, providing new therapies for diseases.

Keywords: microRNA, microarray, obese rats

Introduction

The prevalence of overweight and obese individuals is obviously increasing in developing countries [1]. In obese individuals, adipose tissue releases increased amounts of non-esterified fatty acids, glycerol, hormones, pro-inflammatory cytokines and other factors that are involved in the development of insulin resistance. When insulin resistance is accompanied by dysfunction of pancreatic islet cells that release insulin-failure to control blood glucose levels results [2]. microRNAs are small non coding RNAs frequently regulated dysfunction in human malignancies [3]. Hundreds of microRNAs are encoded in animal genomes, where they provide important regulatory functions in development, apoptosis, life span, and metabolism [4, 5]. Their expression is specific to cells and tissues and is temporally regulated. MicroRNAs are known to be involved in developmental and physiological processes, and their dysregulation leads to the development of diseases [6]. Evidence from in vitro studies, and, more recently, from human studies has identified specific microRNAs that are associated with obesity [7]. However, currently, there were few reports using the chip to screen differential microRNAs in the adipose tissue of obese rats. In our paper, to study the expression of microRNAs in the omental adipose tissue, a high-fat diet induced obesity rat model was established to screen microRNAs through microarray techniques, which would help to understand the regulation of fat cell growth mechanism. Furthermore, it would have a great economic and social value to determine novel therapeutic targets for the treatment of obesity, insulin resistance associated with type 2 diabetes mellitus, and coronary heart disease.

Table 1. Nutrient and Calorie ratios of the normal diet and high-fat diet

Diet		Protein (%)	Fat (%)	Carbohydrate (%)
Normal diet	Nutrient ratios	30	5.7	64.3
	Calorie ratios	28	12	60
High-fat diet	Nutrient ratios	30	40	30
	Calorie ratios	20	60	20

Table 2. Primer sequences and reaction conditions

Gene	Primer	Length (bp)	Tm (°C)
microRNAs-17	5-ACAGCAGAGAAGGCACAAGAGG-3	22	60.09
microRNAs-342	5-AGGGGAGCAAACAGAGAAAGAG-3	22	59.97
microRNAs-92a	5-AAAAGCACAAGACCCGGCCAG-3	21	59.97
microRNAs-382	5-GAAGAAGAACGAGGAGGAAACG-3	22	59.97
microRNAs-30c	5-AGAAAACAACCAACACACACAGC-3	23	59.97
microRNAs-30a	5-CAAACAGACGGAAGAAAGCAGC-3	22	60.04
microRNAs-139	5-ACAACAGAGCACGAGACACCAG-3	22	60.04
microRNAs-335	5-ACAAGAGCAAAAACGAAAAAAGA-3	23	60.04
GAPDH	5-CTGGGCTACACTGAGCACC-3	101	62.00
	5-AAGTGGTCGTTGAGGGCAATG-3		62.90

Table 3. Comparison for body weight and length of rats

	0 week	4 weeks	8 weeks
Body weight			
Normal diet	244.55 ± 8.73	320.22 ± 5.05	385.92 ± 26.52
High fat diet	246.56 ± 6.02	351.72 ± 5.99	473.25 ± 48.5*
Body length			
Normal diet	18.94 ± 1.73	23.17 ± 1.38	25.679 ± 1.31
High fat diet	18.72 ± 1.45	25.06 ± 1.08	28.89 ± 1.90

*P < 0.01 vs. Normal diet group at 8 weeks.

Table 4. Ometal fat weight changed in high-fat diet group after fed for 8 weeks

	Normal diet group	High-fat diet group
Omental fat weight (g)	13.61 ± 1.20	30.11 ± 1.60*

*P < 0.01 vs Normal diet group.

Materials and methods

Animals

40 healthy male SD rats (Slack companies, license number: SCXK (Shanghai) 2012-0002) were weaned (6-7 weeks old), weighed (220 g ± 25 g); and randomly divided into control group (normal diet) and experimental group (high-fat diet). Those rats were raised in SPF-classified cages, 3-8 rats per cage, and their food, water, litter and cages were strictly sterilized by a standard protocol reviewed and approved by the Animal Committee. The light was given in a cycle of 12 hours bright and 12 hours night, and those rats were free to both food and water.

Diets

The normal diet used in this experiment was synthetic diet, which contained enough nutrient and energy to meet requirements for rats' growth and development. Additionally, a high-fat diet was performed on the base of the normal diet by adjusting the proportion of fat and starch. The normal diet was provided by Slack Company with a ratio including 28% protein, 12% fat, and 60% carbohydrate. And the high-fat diet was also offered by Slack Company with a ratio including 20% protein, 60% fat, and 20% carbohydrate (**Table 1**).

Establishment of obese rat models

40 male SD rats were divided into normal diet group and the high-fat diet group, fed by normal diet and high-fat diet for 8 weeks, respectively. Every week, those rats were weighted, measured length and observed characteristics of mental states, water intake, urine volume, appetite, feces, hair and fur brightness and eyes. Modeling evaluation criteria: rats weighing 20% more than the standard weight were considered to match the obesity standard. After 4 weeks, tail vein blood was taken to test blood lipids, blood glucose and other indicators. After 8 weeks, the rats were sacrificed to collect orbital blood, testing serum lipids and blood glucose levels. The weight of rats was measured by animal electronic weighting scales, and, the omental adipose tissue was kept and measure by electronic analytical scales and saved at -80°C liquid nitrogen.

Table 5. Lipid levels including triglycerides etc. compared in both groups

Lipid	0 week		4 weeks		8 weeks	
	Normal diet	High fat diet	Normal diet	High fat diet	Normal diet	High fat diet
Triglycerides	0.94 ± 0.12	0.99 ± 0.09	1.68 ± 0.16	2.05 ± 0.11	2.00 ± 0.14	3.17 ± 0.19*
Total cholesterol	2.38 ± 0.1	2.42 ± 0.11	2.88 ± 0.08	3.46 ± 0.14	3.75 ± 0.12	4.20 ± 0.15*
High-density Lipoprotein	1.11 ± 0.13	1.17 ± 0.26	1.02 ± 0.15	0.94 ± 0.14	0.83 ± 0.18	0.54 ± 0.19*
Low-density lipoprotein	1.07 ± 0.10	1.05 ± 0.22	1.52 ± 0.11	1.82 ± 0.11	2.72 ± 0.10	3.02 ± 0.07*

*P < 0.01 vs Normal diet group at 8 weeks.

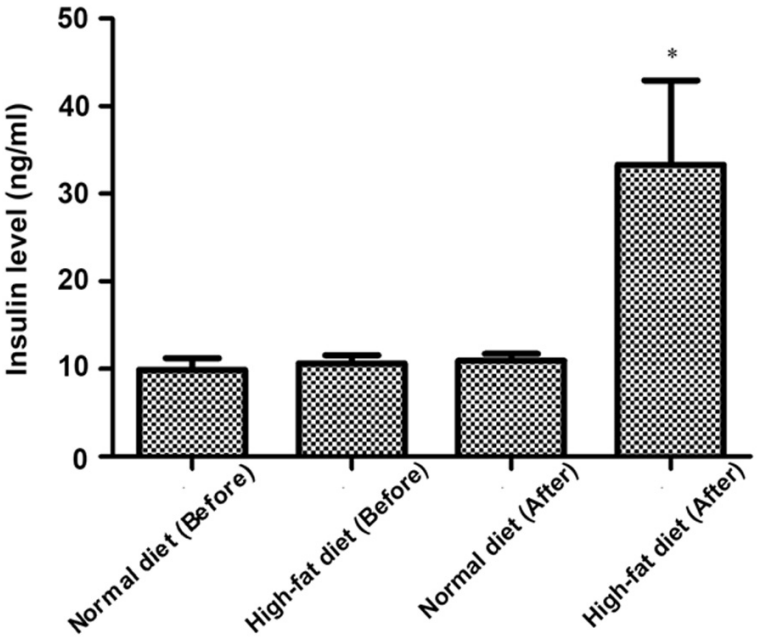

Figure 1. Insulin levels increased before and 8 weeks after normal or high-fat diet. *P < 0.01 vs. normal-fat diet group.

MicroRNA microarray

The microRNA chip was provided by Shanghai Kangcheng Bioengineering Company. Total RNA in the omental adipose tissue was removed by Trizol reagent (Invitrogen Corporation) according to their instructions, and tested by ultraviolet absorption spectrometry and deformation agarose gel electrophoresis. Furthermore, microRNAs were labeled by miRCURYTM Array Labeling Kit according to their instructions, concentrated by RNeasyMini Kit according to their instructions, and hybridized by miRCYRYTM Array Microarray Kit and Hybridization Chamber IIKit according to their instructions. Finally, images were scanned by Gene-pix 4 000 B, exciting with 635 nm sight light wavelength, saved as r11F files and analyzed by Genepix Pro 6.0. It was considered as a significant upward or downward trend when the standard value of high-fat diet group was higher than 2 times or less than 0.5 times compared with normal diet group.

Real-Time PCR verification

Total RNA was extracted according to instructions: the sequences and reaction conditions of PCR primers for cDNA synthesis were outlined in **Table 2**.

Protein tested by Western-Blot

Protein of rats was extracted, concentrated and tested by methods including: SDS-PAGE electrophoresis; protein transferred; protein imaging; immunoassay; and chemiluminescence detection.

Data analysis

Data were analyzed by SPSS 20, described as means ± standard deviation for measurement, and compared by ANOVA analysis between groups. When P < 0.05, the difference was statistically significant.

Results

Body weight and length

Before the experiment, there was no significant difference in weight or length of rats between normal diet group and high-fat diet group (P > 0.05, n = 18). After fed for 8 weeks, compared with normal diet group (385.92 ± 26.52 g), the weight of rats in high-fat diet group (473.25 ± 48.5 g) were significantly increased more than 22.91% (P < 0.01, n = 18), in **Table 3**.

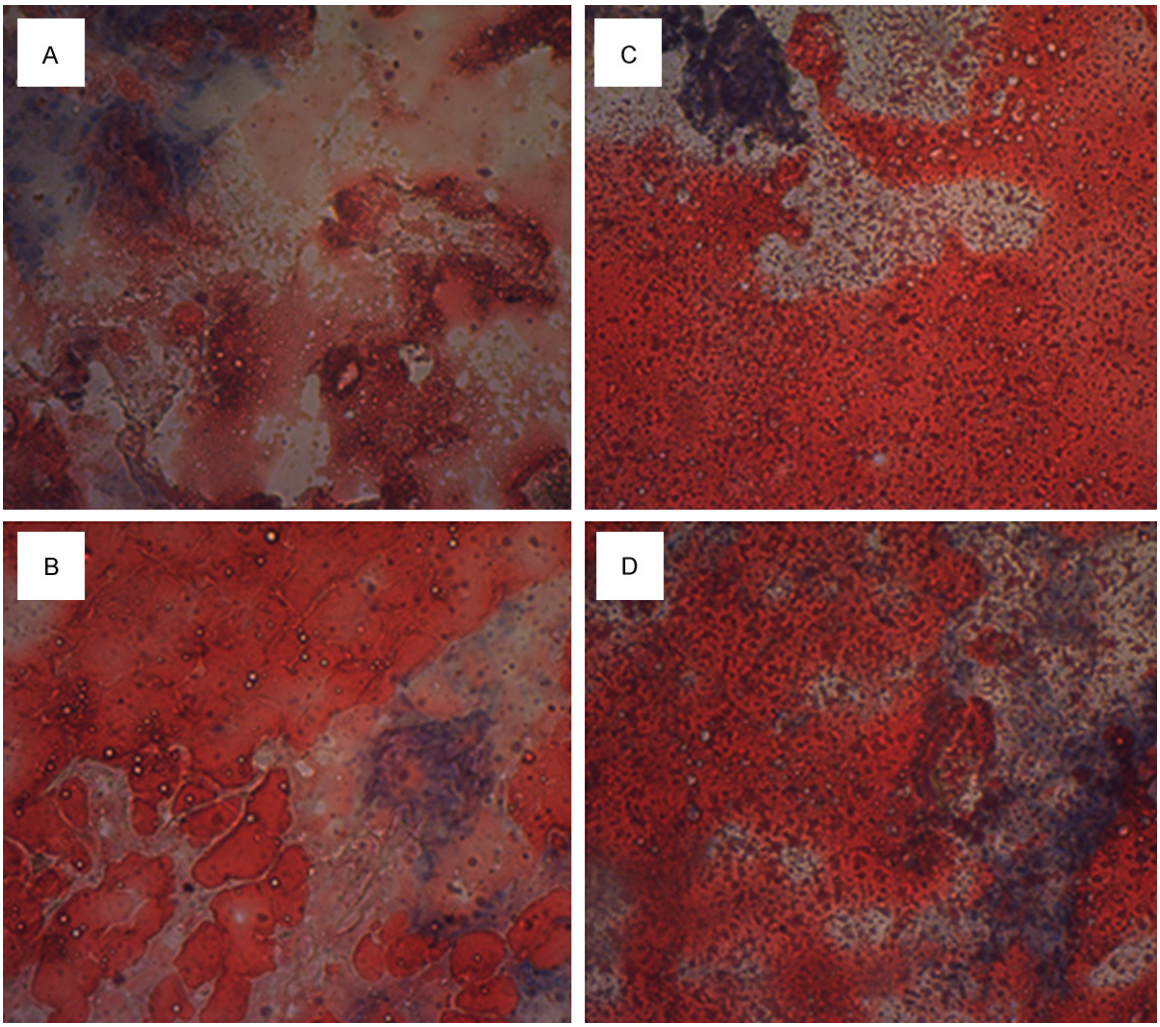

Figure 2. Fat cells with oil red staining in normal diet group and high-fat diet group. Compared with normal diet group (A), fat cells of rats in high-fat diet group (B-D) were more fully, rounded, and dense.

Omental fat weight

After fed for 8 weeks, the omental fat weight of rats in high-fat diet group (30.11 ± 1.60 g) was significantly increased more than 3 times($P < 0.01$, $n = 18$) when compared with normal diet group (13.61 ± 1.20 g), in **Table 4**.

Lipid levels

After fed for 8 weeks, compared with normal diet group, the lipid levels of rats in high-fat diet group changed ($P < 0.01$, $n = 18$), including increased triglycerides, total cholesterol, and low-density lipoprotein and decreased high-density lipoprotein (**Table 5**).

Blood glucose levels

Before the experiment, there was no difference in blood glucose levels of rats between normal diet group (46.39 ± 3.21 mmol/L) and high-fat diet group (44.4 ± 3.00 mmol/L). After fed for 8 weeks, compared with normal diet group (43.38 ± 2.56 mmol/L), the blood glucose level of rats in high-fat diet group (97.44 ± 2.12 mmol/L) was significantly increased ($P < 0.05$, $n = 18$).

Insulin levels

Before the experiment, there was no significant difference in insulin levels of rats between normal diet group (9.88 ± 1.34 ng/ml) and high-fat diet group (10.63 ± 0.92 ng/ml). After fed for 8 weeks, compared with normal diet group (10.95 ± 0.77 ng/ml), the insulin level of rats in high-fat diet group (33.33 ± 9.59 ng/ml) was significantly increased ($P < 0.01$, $n = 18$, **Figure 1**). The changed data described above, one of the main indicators for insulin resistance, indicated

Table 6. Alteration of microRNA expression in obese rats

Expression	microRNAs	Value change	P
Up-regulated microRNAs	microRNA30a	3.17 ± 0.38	0.039
	microRNA7e	2.68 ± 0.24	0.038
	microRNA30c	1.72 ± 0.12	0.038
	microRNA335	2.23 ± 0.15	0.037
	microRNA139-5p	4.54 ± 0.67	0.009
Down-regulated microRNAs	microRNA494	0.76 ± 0.11	0.044
	microRNA140	0.67 ± 0.06	0.044
	microRNA342-5p	0.42 ± 0.08	0.027
	microRNA382	0.31 ± 0.07	0.024
	microRNA17-1-3p	0.58 ± 0.04	0.023
	microRNA92a	0.38 ± 0.13	0.018

that obese rat models were successfully established.

Fat cells morphology

Fat cells were observed under microscope by oil red staining (**Figure 2**). Compared with normal diet group, fat cells of rats in high-fat diet group were more fully, rounded, and dense.

MicroRNA expression

The microRNAs in the omental adipose tissue of rats were identified and verified by methods of microRNA microarray and Real-Time PCR. In obese rats, among total 468 kinds of microRNAs, 13 expressed differentially, including 7 up-regulated microRNAs (microRNA30a, microRNA7e, microRNA30c, microRNA335, microRNA103, microRNA107, microRNA139-5p), and 6 down-regulated microRNAs (microRNA494, microRNA140, microRNA342-5p, microRNA-382, microRNA17-1-3p, microRNA92a) (**Table 6**).

Discussion

Obesity has emerged as a global health problem with more than 1.1 billion adults to be classified as overweight or obese, and is associated with type 2 diabetes, cardiovascular disease, and several cancers. Since obesity is marked by an increased size and/or number of adiposity, elucidating the molecular events governing adipogenesis is of utmost importance. Recent findings indicate that microRNAs-small non-protein-coding RNAs that function as post-transcriptional gene regulators-are involved in the regulatory network of adipogenesis [8]. Insulin signal transduction defects are the most common defects causing type 2 diabetes in the early development stage for individuals, and microRNAs have been identified as new regulating molecules affecting many biological functions including metabolic processes [9-11]. However, the direct regulation of insulin sensitivity by microRNAs has not been demonstrated in vivo yet [12]. Markus Stoffel and his colleagues, physiologists from Swiss Federal Institute of Technology, found that microRNA 103 and 107 were both up-regulated in mice and humans suffering from fatty liver disease [13, 14]. It was then determined that insulin sensitivity was reduced by microRNAs, closing a protein called Catholic -1, which could stabilize insulin receptors on the membrane of fat cells [14, 15]. Kajimoto et al. tested the microRNA expression map in 3T3-LI preadipocytes differentiation, and they found that 21 microRNAs were up-regulated or down-regulated during the process [16].

To study human obesity, many animal models are established including high-fat, high-energy food induced obese rat model, which is consistent with the pathogenesis of human obesity [17]. Currently, there are no reports to screen microRNAs by micro array chip methods, in order to identify the differential expression of microRNAs in adipose tissue in obese rats. Recently, many researches focus on the function of microRNA in many diseases, which becomes a hot point for investigating pathogenesis. However, present studies are only the tip of the iceberg, so there are still many unresolved problems. MicroRNA is generally recognized as a new regulatory molecule affecting a variety of biological functions, but there is no enough evidence to prove it has a direct role in regulation in obesity and is in association with in insulin sensitivity or type 2 diabetes, and coronary heart diseases [18-20].

The aim of our investigation was to determine whether the expression map of microRNAs differed in the presence or absence of obesity. By feeding high-fat diet, obese rat models were established, compared with normal diet group, fatty indicators changed in high-fat diet group, including increased body weight, omental fat

weight, triglycerides, total cholesterol, low-density lipoprotein, blood glucose level and insulin level, and decreased high-density lipoprotein, and differential phenotype of fat cells. Furthermore, by microarray techniques and Real-Time PCR, 13 differential expression microRNAs were identified, including 7 up-regulated microRNAs (microRNA30a, microRNA7e, microRNA30c, microRNA335, microRNA103, microRNA107, microRNA139-5p), and 6 down-regulated microRNAs (microRNA494, microRNA140, microRNA342-5p, microRNA382, microRNA17-1-3p, microRNA92a). Finally, all results proved in our study will help to better understand the mechanism of regulating fat cells growth, to know the role of microRNA in obese rats, and to lay foundations for further studies on mciroRNA-induced lipid metabolism disorders.

Disclosure of conflict of interest

None.

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