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MicroRNA-192* impairs adipocyte triglyceride storage

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ABSTRACT

We investigated the expression of miR-192* (miR-192-3p) in the visceral adipose tissue (VAT) of obese subjects and its function in cultured human adipocytes. This miRNA is a 3′ arm derived from the same pre-miRNA as miR-192 (miR-192-5p) implicated in type 2 diabetes, liver disease and cancers, and is predicted to target key genes in lipid metabolism. In morbidly obese subjects undergoing bariatric surgery preceded by a very low calorie diet, miR-192* in VAT correlated negatively ($r = -0.387$; $p = 0.046$) with serum triglyceride (TG) and positively with high-density lipoprotein (HDL) concentration ($r = 0.396$; $p = 0.041$). In a less obese patient cohort, the miRNA correlated negatively with the body mass index ($r = -0.537$; $p = 0.026$). To characterize the function of miR-192*, we overexpressed it in cultured adipocytes and analyzed the expression of adipogenic differentiation markers as well as cellular TG content. Reduced TG and expression of the adipocyte marker proteins aP2 (adipocyte protein 2) and perilipin 1 were observed. The function of miR-192* was further investigated by transcriptomic profiling of adipocytes expressing this miRNA, revealing impacts on key lipogenic genes. A number of the mRNA alterations were validated by qPCR. Western analysis confirmed a marked reduction of the lipogenic enzyme SCD (stearoyl coenzyme A desaturase-1), the fatty aldehyde dehydrogenase ALDH3A2 (aldehyde dehydrogenase 3 family member A2) and the high-density lipoprotein receptor SCARB1 (scavenger receptor B, type I). SCD and ALDH3A2 were demonstrated to be direct targets of miR-192*. To conclude, the present data identify miR-192* as a novel controller of adipocyte differentiation and lipid homeostasis.

1. Introduction

MicroRNAs (miRNAs) are short (19–23 nt) non-coding single-stranded RNA molecules that contribute to regulation of gene expression via mRNA deadenylation or destabilization and translational repression or activation [1]. Bioinformatic analyses have suggested that more than 50% of all genes are subject to regulation by miRNAs [2]. Pri-miRNA transcripts typically contain several miRNA precursors [3]. During the process of maturation, generally one arm of each processed double-stranded miRNA precursor becomes functional. The other, 'passenger' strand denoted with an asterisk (*) is normally degraded. However, in some cases both strands of the duplex become functional miRNAs that target different mRNA populations [3].

Visceral or abdominal adipose tissue represents a fat depot located within the abdominal cavity and accumulates in subjects with abdominal-type obesity. Visceral adipocytes have a higher rate of lipolysis, secrete pro-inflammatory cytokines, and are more insulin-resistant than subcutaneous adipocytes [4]. VAT drains directly through the portal circulation to the liver [5], and it has been hypothesized that non-esterified fatty acids as well as adipocytokines released by VAT may contribute to hepatic triglyceride storage, insulin resistance and elevated serum triglyceride (TG) concentration [6].

Human VAT miRNAs have been investigated in several studies, focusing on miRNA expression patterns in type 2 diabetes [7], obese

Abbreviations: ALDH3A2, aldehyde dehydrogenase 3 family member A2; aP2, adipocyte protein 2; BMI, body mass index; GLUT4, glucose transporter type 4; miRNA, microRNA; NT, non-targeting; PLIN, perilipin 1; qPCR, quantitative reverse transcriptase PCR; SCARB1, scavenger receptor B, type I; SCD, stearoyl coenzyme A desaturase-1; SGBS, Simpson–Golabi–Behmel syndrome; TG, triglyceride; VAT, visceral adipose tissue.

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subjects [8–10], or subjects with non-alcoholic fatty liver disease/steatohepatitis [11,12]. In several cases, there is a negative correlation between the expression of miRNAs and their predicted protein targets in the VAT of obese subjects, consistent with physiologically relevant gene regulation by the miRNAs [15]. Yu et al. [13] demonstrated that peroxisome proliferator activated receptor-γ (PPAR-γ) agonist stimulation has in the subcutaneous and visceral fat depots distinct but overlapping effects on the expression of miRNAs that target the transforming growth factor-β (TGF-β), insulin and Wnt/β-catenin signaling pathways with key roles in the control of adipogenesis. Moreover, in vitro experiments with preadipocyte/adipocyte cultures have provided evidence for functional roles of a number of miRNAs in adipogenesis: miR-103, miR-320, and miR-1908 enhance adipogenesis while miR-146 and miR-194 decrease adipocyte differentiation [9,14–18]. Of note, correlation was shown between dysregulation of miRNAs targeting white adipose tissue adipocytokine expression and non-alcoholic steatohepatitis [11], consistent with the hypothesis that adipocytokines regulated by miRNAs and secreted by adipose tissue impact the pathogenesis of non-alcoholic fatty liver disease.

Dysregulation of miR-192 has been associated with a number of pathologic conditions including type 2 diabetes, liver diseases, and cancers [19–21]. Our preliminary analyses indicated that both miR-192 and its 'passenger' arm miR-192* are expressed at substantial and highly similar levels in the VAT of obese human subjects. However, the function of miR-192* has not been investigated, although target predictions suggest that this species regulates the expression of a number of key genes in lipid metabolism. We therefore surmised that miR-192* could serve as a regulator of adipocyte differentiation and/or lipid metabolism, and set out to investigate its function in a cultured human adipocyte model. The study identifies miR-192* as a novel controller of adipogenesis and adipocyte lipid homeostasis.

2. Materials and methods

2.1. Study subjects

The morbidly obese subjects (BMI 46.8 ± 6.3) were recruited amongst patients undergoing laparoscopic bariatric surgery at the Peijas Hospital of the Hospital District of Helsinki and Uusimaa. The following inclusion criteria were applied: (i) age 18–65 years; (ii) no known acute or chronic disease except for obesity or obesity related diseases such as type 2 diabetes, non-alcoholic fatty liver disease, cardiovascular diseases and/or hyperlipidemia; based on history, physical examination, standard laboratory tests (complete blood count, liver functions tests, creatinine, TSH, electrolyte and lipid concentrations as well as glucose, C-peptide, and insulin) and electrocardiogram. Exclusion criteria were: (i) excessive use of alcohol (over 20 g/day), (ii) use of hepatotoxic medications or herbal products, and (iii) pregnancy or lactation. The subjects were studied in the morning after an overnight (10–12 h) fast one to two weeks prior to surgery. Weight, height, waist and hip circumferences were recorded as previously described [22]. Blood samples were taken for measurement of concentrations of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglyceride, glucose, C-peptide, and insulin, as well as of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) activities as described [22]. Before the operation, the patients adhered to a 6–12 week very low calory diet (<800 kcal/day).

In the less obese (BMI 31.2 ± 2.5) female cohort, 17 women undergoing elective gynecological surgery for non-malignant reasons in the department of Obstetrics and Gynecology, Helsinki University Hospital, were recruited. Patients were scheduled for abdominal or laparoscopic hysterectomy, salpingo-oophorectomy, diagnostic laparoscopy, enucleation of uterine myoma, enucleation of ovarian cyst, or laparoscopic colpo-sacro-rectopexy. Reasons for surgery included uterine myomas accompanied with menorrhagia and/or pain, benign ovarian cysts, infertility and dysmenorrhea, or rectocele. Blood samples were drawn before the operation. Serum was isolated by centrifugation and stored at −20 °C until the analyses (see above). The characteristics of the subjects are shown in Table 1.

2.2. VAT biopsies

At the time of surgery, biopsies of omental VAT were taken and snap-frozen in liquid N_2. The adipose tissue samples were stored at −80 °C until further processing.

2.3. Ethics

The nature and potential risks of the study were explained to all subjects prior to obtaining their written informed consent. The protocol was approved by the Medicinal Ethics Committee of the Helsinki and Uusimaa Hospital District. The Ethics committee operates according to the principles of good clinical research practice (ICH-GCP-E6) and in accordance with the international obligations concerning the status of research subjects and the rules and guidelines that govern research [Medical Research Act 488/1999, chapter 2a (23.4.2004/295), section 5 and 10a].

2.4. Isolation of total RNA from VAT

Frozen visceral fat (approximately 200 mg) was homogenized and total RNA was isolated and purified by using the Lipid Tissue miRNeasy Mini® Kit (Qiagen, Valencia, CA). The quality of the RNA was verified by Agilent 2100 Bioanalyzer® (Agilent, Santa Clara, CA). The isolated RNA was stored at −80 °C.

Table 1
Characteristics of the study subjects.

Subjects undergoing bariatric surgery.	
Gender (females/males)	18/9
Age (yrs)	48.0 ± 9.7
Body composition	
Body weight (kg)	133.5 ± 25.9
Body mass index (kg/m^2)	46.8 ± 6.3
Biochemical parameters	
fP-glucose (mmol/L)	5.8 ± 0.9
fS-insulin (mU/L)	11.9 (7.3–20.0)
fS-C-peptide (nmol/L)	1.1 ± 0.40
fS-LDL cholesterol (mmol/L)	2.8 ± 0.9
fS-HDL cholesterol (mmol/L)	1.2 ± 0.3
fS-TG (mmol/L)	1.3 ± 0.5
fS-ALT (U/L)	34 (22.0–51.5)
fS-AST (U/L)	30 (24.0–39.5)
fS-ALP (U/L)	69 (56.0–82.5)
Subjects undergoing gynecologic surgery	
Gender (females/males)	17/0
Age (yrs)	46.0 ± 9.5
Body composition	
Body weight (kg)	85.0 ± 9.0
Body mass index (kg/m^2)	31.2 ± 2.5
Biochemical parameters	
fS-LDL cholesterol (mmol/L)	3.4 ± 0.8
fS-HDL cholesterol (mmol/L)	1.4 ± 0.4
fS-TG (mmol/L)	1.6 ± 2.0
fS-AST (U/L)	33 (21–145)

Abbreviations: f, fasting; P, plasma; S, serum; LDL low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; ALT alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase. Data are shown as mean ± SD or median (25%–75% percentile), as appropriate.