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Metabolic Circuit Involving Free Fatty Acids, microRNA 122, and Triglyceride Synthesis in Liver and Muscle Tissues

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BACKGROUND & AIMS: Effective treatments are needed for hepatic steatosis characterized by accumulation of triglycerides in hepatocytes, which leads to hepatocellular carcinoma. MicroRNA 122 (MIR122) is expressed only in the liver, where it regulates lipid metabolism. We investigated the mechanism by which free fatty acids (FFAs) regulate MIR122 expression and the effect of MIR122 on triglyceride synthesis. **METHODS:** We analyzed MIR122 promoter activity and validated its target mRNAs by transfection of Luciferase reporter plasmids into Huh7, BNL-1ME, and HEK293 cultured cell lines. We measured levels of microRNAs and mRNAs by quantitative real-time PCR analysis of RNA extracted from plasma, liver, muscle, and adipose tissues of C57BL/6 mice given the FFA-inducer CL316243. MIR122 was inhibited using an inhibitor of MIR122. Metabolic profiles of mice were determined using metabolic chambers and by histologic analyses of liver tissues. We performed RNA sequence analyses to identify metabolic pathways involving MIR122. **RESULTS:** We validated human Agpat1 and Dgat1 mRNAs, involved in triglyceride synthesis, as targets of MIR122. FFAs increased MIR122 expression in livers of mice by activating the retinoic acid-related orphan receptor alpha, and induced secretion of MIR122 from liver to blood. Circulating MIR122 entered muscle and adipose tissues of mice, reducing mRNA levels of genes involved in triglyceride synthesis. Mice injected with an inhibitor of MIR122 and then given CL316243, accumulated triglycerides in liver and muscle tissues, and had reduced rates of β-oxidation. There was a positive correlation between level of FFAs and level of MIR122 in plasma samples from 6 healthy individuals, collected before and during fasting. **CONCLUSIONS:** In biochemical and histologic studies of plasma, liver, muscle, and adipose tissues from mice, we found that FFAs increase hepatic expression and secretion of MIR122, which regulates energy storage vs expenditure in liver and peripheral tissues. Strategies to reduce triglyceride levels, by increasing MIR122, might be developed for treatment of metabolic syndrome.

Keywords: Posttranscriptional regulation; Transcription Factor; NAFLD; NASH.

Hepatic steatosis, characterized by accumulation of triglycerides (TG) in hepatocytes, can progress to nonalcoholic steatohepatitis (NASH), which can then progress to cirrhosis, and finally to hepatocellular carcinoma (HCC).[1,2] Therefore, understanding the mechanism of hepatic steatosis is important for applying caloric restriction diet strategies as well as the development of new therapeutic drugs.

Micro RNA 122 (miR-122) is the most abundant liver-specific miRNA (250,000 copies/hepatocyte) that is evolutionarily conserved, accounting for approximately 70% of total adult liver microRNAs (miRNAs).[3] miR-122 plays a role in cholesterol and free fatty acid (FFA) metabolism,[4] HCC growth,[5] and hepatitis C virus replication.[6] miR-122 is secreted into the blood stream and changes in its blood level were suggested as a predictive marker for NASH and chemical-induced liver injury.[7–9] Secreted miR-122 was found by our group to act as a hormone, targeting erythropoietin (EPO) production in the kidney, and to be responsible for inflammation-induced anemia.[10]

A number of transcription factors regulate miR-122 expression, including hepatocyte nuclear factors HNF1α, HNF3β, HNF4α, and C/EBPα (CAAT/enhancer-binding protein),[11–13] PPARγ (peroxisome proliferator-activated receptor gamma) /RXRα (retinoid X receptor alpha) complex,[14] and by Rev-ErbAα, suggesting that miR-122 is a circadian metabolic regulator.[15]

miR-122 inhibition by antisense oligonucleotide reduced hepatic and plasma levels of cholesterol and TG.[16]

Abbreviations used in this paper: **antagomiR, an inhibitor of miR; β-HB, β-hydroxybutyrate; BODIPY, boron-dipyrromethene; CL, CL316243; FFA, free fatty acid; HCC, hepatocellular carcinoma; HFD, high-fat diet; HNF, hepatocyte nuclear factor; LA, lauric acid; miR-122, microRNA 122; miRNA, microRNA; NASH, nonalcoholic steatohepatitis; OA, oleic acid; PCR, polymerase chain reaction; RORα, retinoid-related orphan receptor-alpha; SREBP-1c, sterol regulatory element-binding transcription factor 1c; TG, triglycerides; UTR, untranslated region.**

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EDITOR'S NOTES

BACKGROUND AND CONTEXT

Fatty liver and NASH are becoming a major healthcare burden. The mechanisms regulating hepatic and peripheral lipid biosynthesis and lipid accumulation are largely unknown.

NEW FINDINGS

miR-122 is regulated by the transcription factor RORα. Free fatty acids (FFA) activate RORα to increase the expression and secretion of miR-122. miR122 targets triglyceride biosynthesis, both in the liver as well as in adipose and muscle tissues. Upon fasting, FFA increases miR-122 expression reducing TG biosynthesis and increases α-oxidation, shifting lipid accumulation to an energy source.

LIMITATIONS

This investigation was performed in animal models and needs further proof in humans to underline its clinical significance.

IMPACT

This investigation shows that microRNAs behave as hormones and are expressed, secreted and function in a circuitry homeostatic manner.

Similar effects were observed in African green monkeys treated with an inhibitor of miR-122 (antagomiR-122).[17] However, the underlying mechanism of this effect is unknown.

Mice with a germline or a conditional deletion of miR-122 in the liver are viable and fertile. However, with age, they develop steatohepatitis, liver fibrosis, and HCC.[18] The serum lipid profiles of both liver-specific knockouts and germline knockouts of miR-122, showed reduction in total cholesterol, low-density lipoprotein, high-density lipoprotein, and serum TG. However, the livers of both had progressive steatohepatitis, a feature not seen in the previous antisense oligonucleotide studies. The authors noted up-regulation of genes known to be involved in hepatic lipid synthesis, including *Agpat1* (1-acyl-sn-glycerol-3-phosphate acyltransferase-beta-1), which is part of the TG synthesis pathway, and led to TG accumulation in their liver. The authors confirmed that mouse *Agpat1* is a direct miR-122 target gene. Notably, none of the human genes involved in lipid metabolism are proven miR-122 targets, and the mechanism by which miR-122 regulates lipid metabolism remains undetermined.

It is not yet known whether miR-122 expression is affected by lipid metabolism. It was shown that the FFA palmitic acid induces the expression and activity of miRNAs, such as miR-29a and miR-195, in myocytes and hepatocytes.[19,20]

In this study, we determined that FFAs enhance miR-122 promoter activity and its secretion to the blood. We show that this effect is retinoid-related orphan receptor alpha (RORα)-dependent. In addition, we identified novel miR-122 target genes, which are involved in the TG synthesis pathway. Using RNA-seq pathway analysis together with molecular, histological, and biochemical approaches, we established the significant effect of FFA-induced miR-122 on lipid metabolism in the liver and also in peripheral tissues. We provide evidence for a metabolic circuit comprising FFAs, miR-122, and TG. Taken together, our results expand our knowledge regarding the regulation of miR-122, describe the systemic "hormonal" effect of this miRNA, and emphasize the pivotal role of miR-122 in energy and lipid metabolism. These understandings could pave the way for the development of novel therapeutic approaches for liver steatosis.

Materials and Methods

Mice

Male C57BL/6 mice, 7 to 8 weeks old, were purchased from Harlan Laboratories (Jerusalem, Israel). Mice were kept in a pathogen-free facility, under a 12-hour light/dark cycle. Research on mice was approved by the Hebrew University Institutional Animal Care and Ethics Committee.

Cell Culture

HCC-derived human and mouse cell lines: Huh7, BNL-1ME, and human nonhepatic cell lines HEK293-embryonic kidney cells, C2C12-mouse myoblast cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin. Primary hepatocytes isolated from livers of C57BL/6 mice were cultured in Dulbecco's modified Eagle's medium/F12 supplemented with 5% fetal calf serum, 1% penicillin/streptomycin, 1% nonessential amino acids, and 1% insulin-selenium-transferrin.

CL316243 and AntagomiR Injections to Mice

C57BL/6 mice, 7 to 8 weeks old, were injected subcutaneously with 1 mg/kg CL316243 (CL) (R&D Systems-Tocris, Minneapolis, MN) dissolved in saline. Saline was injected as control. Mice were killed between 1 and 24 hours and the livers, white adipose, and skeletal muscle tissues were frozen in liquid nitrogen or in optimum cutting temperature embedded frozen blocks, for further RNA and histologic analysis. In experiments of miR-122 repression by antagomiR, mice were hydrodynamic tail vain injected with antagomiR-122 or antagomiR-control (5 μg/mouse in 1.5 mL saline) 48 hours before CL injection. In the experiment with antagomiR treatment without CL, antagomiR-18 was used as control. AntagomiRs were obtained from Sigma-Aldrich (St Louis, MO); see Supplementary Table 3.

Study Subjects and Ethics Statement

For the measurement of miR-122 and FFA analysis in blood samples collected from patients undergoing plasmapheresis or from healthy volunteers after overnight fasting, we received institutional review board approvals from the Hadassah Hebrew University Hospital. Informed consent and permission to use biological materials for research were obtained from all subjects. The RNA samples of patients with NASH were obtained from the "Liver.net" biobank, established by the Collaborative Research Centre 841 at Hamburg University Medical Center.