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miR-122-regulated metabolic circuits:

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micro-management of lipid metabolism in the human liver

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25 **Abbreviations:** FFA: free fatty acids, HCC: hepatocellular carcinoma, HCV: hepatitis
26 C virus, HFD: high fat diet, LA: lauric acid; miRNA: microRNA, NAFLD: nonalcoholic
27 fatty liver disease, OA: oleic acid, PA: palmitic acid, PPAR: peroxisome proliferator-
28 activated receptor, ROR: retinoic-related orphan receptor; TG: triglycerides
29

30 The liver plays a key role in protein, carbohydrate and lipid metabolism. Alteration of
31 liver metabolic functions, e.g. by excessive alcohol intake, obesity, diabetes mellitus
32 or dyslipidemia can lead to accumulation of triglycerides (TG) in the liver, referred to
33 as fatty liver or hepatic steatosis. Hepatic steatosis can be accompanied by
34 inflammation, a condition called steatohepatitis that may progress to liver cirrhosis and
35 liver cancer (hepatocellular carcinoma (HCC)). Due to the increase of obesity,
36 nonalcoholic fatty liver disease (NAFLD) is one of the most important causes of liver
37 disease worldwide with a global prevalence estimated at 24% (1). With more than 100
38 million individuals affected in the US and Europe, it is expected that NAFLD-related
39 HCC will dramatically increase within the next decades (1, 2). However, to date no
40 effective pharmacological strategies are available to prevent progression of liver
41 cirrhosis and HCC development in the context of NAFLD.

42 Liver metabolic functions are tightly regulated by microRNA (miRNA) (reviewed
43 in (3)) and various forms of liver disease have been associated with altered intrahepatic
44 miRNA profiles. As the most abundant miRNA in the adult liver, miR-122 plays an
45 important role in the regulation of lipid metabolism and acts as a tumor suppressor in
46 the liver (reviewed in (4)). Moreover, since the hepatitis C virus (HCV) uses miR-122
47 to establish its infection (reviewed in (4)), miR-122 also represents an antiviral target
48 and anti-miR-122 strategies have been evaluated in clinical trials (5, 6). Of note, while
49 the administration of antisense miR-122 to chronic hepatitis C patients did not trigger
50 any dose-limiting adverse event in clinical trials, a sustained and reversible decrease
51 in serum cholesterol levels was observed in treated patients (5, 6) in line with the
52 important role of miR-122 in the regulation of hepatic cholesterol and fatty acid
53 metabolism (7-9). However, the molecular mechanism underlying the interplay
54 between miR-122 and lipid homeostasis has not yet been completely deciphered.

55 **Interplay between miR-122 and lipid metabolism in the human liver, white**
56 **adipose tissue and muscle**

57 While different studies have characterized the role of miR-122 in the mouse liver using
58 transgenic mice and anti-miR-122 strategies (8, 10, 11), the cellular pathways
59 regulated by miR-122 in the human liver are less well known. The study of tumoral and
60 nontumoral liver tissues from HCC patients revealed that liver miR-122 expression
61 inversely correlates with patient survival and metastasis (12). Indeed, miR-122
62 appears to be specifically repressed in poor prognosis HCC subsets exhibiting gene
63 expression profiles linked to the suppression of hepatic phenotype and the acquisition
64 of invasive properties (12). Most recently, it has been shown that miR-122 levels were
65 decreased in liver samples from patients with alcoholic liver disease (13) suggesting
66 that miR-122 networks may be deregulated in the course of different forms of liver
67 disease. In their recent study, Chai and colleagues have shed new light on the lipid
68 metabolic circuit involving miR-122 in the human liver (14) and have provided another
69 piece to the puzzle of the cellular pathways involving human miR-122. They showed
70 that some lipids can modulate miR-122 expression and secretion from liver-derived
71 cells, circulating miR-122 in turn being able to act remotely on non-liver derived tissues.

72 In contrast to other free fatty acids (FFA), lauric acid (LA), palmitic acid (PA) and
73 oleic acid (OA) enhanced the miR-122 promoter activity in human hepatoma Huh7
74 cells. Deciphering the underlying molecular mechanism using mutation of the predicted
75 ROR α binding site within the miR-122 promoter, a retinoic-related orphan receptor
76 alpha (ROR α) agonist and chromatin immunoprecipitation (ChIP) experiments, the
77 authors showed that binding of ROR α was involved in this process. These findings
78 were corroborated by mouse experiments where TG hydrolysis into FFA was induced
79 *in vivo* by the administration of the β 3-adrenergic receptor agonist CL316243. These

80 mice showed an increase in plasma FFA levels as well as hepatic TG, pre-miR-122
81 and miR-122 levels.

82 Interestingly, the effect on miR-122 secretion from Huh7 cells appeared to be
83 independent on the effect of FFA on miR-122 expression since FFA that demonstrated
84 no effect on the miR-122 promoter (i.e. decanoic and myristic acids) were able to
85 increase the secretion of miR-122 from these cells. Like miR-122 expression, miR-122
86 secretion is under the control of ROR α since a ROR α agonist and antagonist
87 respectively increased and decreased LA-induced miR-122 secretion from Huh7 cells.
88 This process appears to also take place *in vivo* since increased serum miR-122 levels
89 were observed prior to increased miR-122 expression in CL316243-treated mice as
90 well as in mice following a 24h-starvation period. To further investigate the clinical
91 relevance of these findings, the authors assessed serum miR-122 levels in individuals
92 subjected to plasmapheresis, a procedure that has been reported to increase plasma
93 FFA levels (15), as well as following fasting. Plasmapheresis indeed increased serum
94 FFA and miR-122 levels and a positive correlation was observed between FFA and
95 miR-122 levels in healthy individuals following fasting. Noteworthy, secreted miR-122
96 was able to be transferred to miR-122-negative cells and to repress miR-122-target
97 genes in these cells as shown by experiments using supernatants from LA-treated
98 Huh7 cells and non-liver-derived recipient cells that do not express miR-122. The
99 underlying molecular mechanism was not studied but miR-122 may be included in
100 exosomes or be associated with lipoproteins (16). Furthermore, the addition of miR-
101 122 mimics to miR-122-negative non-liver-derived cells triggered a decrease of LA-
102 induced lipid droplet formation underscoring that exogenous miR-122 can modulate
103 lipid pathways in non-liver-derived cells.

104 To corroborate their findings *in vivo*, Chai and colleagues treated mice with
105 antagomiR-122 and/or the β 3-adrenergic receptor agonist CL316243 and monitored
106 TG synthesis pathway gene expression as well as TG and miR-122 levels in the liver,
107 white adipose tissues and skeletal muscle. CL316243 increased miR-122 levels in the
108 serum, liver, white adipose tissue and skeletal muscle while the concomitant
109 administration of antagomiR-122 reduced miR-122 levels in those compartments and
110 lead to an increase of TG levels in the liver and skeletal muscle in line with an increase
111 in the expression of miR-122-target genes involved in TG synthesis. Next-generation
112 RNA-sequencing and pathway analysis revealed that miR-122 is likely involved in
113 several metabolic pathways that regulate TG levels, including circadian rhythm
114 pathways and adipogenesis. Furthermore, antagomiR-122-treated mice exhibited
115 increased food uptake and reduced voluntary activity, behaviors that favor TG
116 accumulation and steatosis. It has to be pointed out that in contrast to the study by
117 Chai and colleagues, a previous study reported a reduction of steatosis following short-
118 term administration of antagomiR-122 to mice fed with high-fat diet (HFD) (8). These
119 different observations may be due to different experimental conditions and analysis
120 timelines since different strains of miR-122 knock-out mice were shown to exhibit
121 extensive lipid accumulation in the liver (10, 11).

122 Chai and colleagues uncovered novel direct targets of miR-122 in humans,
123 namely *Agpat1* and *Dgat1* that play a major role in TG synthesis. This is in line with
124 previously published data demonstrating that mouse *Agpat1* is a miR-122 target (10,
125 11). They also validated *Dgat1* as miR-122 target in mice. Based on Ingenuity pathway
126 analysis (IPA) and GeneAnalytics analysis of their elegant *in vivo* gain-of-function and
127 loss-of-function experiments, the authors hypothesized that miR-122 decreases *Foxo1*
128 and *PPAR γ* , transcription factors that have been previously linked to hepatic steatosis

129 and adipogenesis in mice (17-19). Furthermore, they also provided novel evidence for
130 the regulation of miR-122 expression in human liver-derived cells. In line with its liver-
131 enriched expression profile, miR-122 expression is known to be driven by liver-
132 enriched transcription factors. The miR-122 promoter can be stimulated by hepatocyte
133 nuclear factor (HNF) 1 α , 4 α and 6 as well as FoxA2 and CCAAT/enhancer binding
134 protein α (20, 21). Chai and colleagues now provided evidence that ROR α plays a role
135 in FFA-mediated miR-122 expression. Interestingly, ROR α knock-out mice have been
136 reported to exhibit a dysregulation of PPAR γ signaling and increased hepatic glucose
137 and lipid metabolism rendering them more susceptible to the development of hepatic
138 steatosis, obesity and insulin-resistance upon feeding with HFD than control mice (19,
139 22). These data indicate the existence of a complex network involving FFA, miR-122
140 and several transcription factors including ROR α and PPAR γ that regulates lipid
141 metabolism in the liver with remote effects on TG levels in peripheral tissues.

142 Taken together, this study increases our understanding about the role of miR-
143 122 in hepatic lipid metabolism and highlights its role as a secreted signaling molecule
144 to regulate lipid storage in adipose tissue and skeletal muscle.

145

146 **Perspectives of increasing hepatic miR-122 levels for prevention and treatment** 147 **of liver disease**

148 Several therapeutic strategies targeting miRNAs are in development (for review see
149 (23)) including for metabolic diseases. The safety, tolerability and pharmacokinetics of
150 anti-miR-103/107 will be evaluated in diabetic patients with NAFLD (ClinicalTrials.gov
151 Identifiers: NCT02826525). Due to the lack of strategies to slow down/reverse
152 progression of liver disease and to prevent HCC in cirrhotic patients in the context of
153 NAFLD as well as the key role of miR-122 as a tumor suppressor and a regulator of

154 lipid metabolism in the liver, novel preventive and therapeutic strategies aiming to
155 increase miR-122 levels may provide perspectives for patients with advanced liver
156 disease. In line with this, a recent study showed that restoration of miR-122 levels
157 reduced ethanol-induced liver injury in mice (13).

158 Therapeutic approaches using antisense miR-122 strategies were shown to be
159 well tolerated in chronic hepatitis C patients (5, 6) but so far miR-122 mimics have not
160 been used in the clinic. Several miRNA mimics (miR-16, miR-29 and miR-34) have
161 reached phase I clinical trials (ClinicalTrials.gov Identifiers: NCT02369198,
162 NCT02603224 and NCT01829971) and miR-34 mimics (MRX34) were evaluated in
163 cancer patients including patients with liver cancer. However, this study was
164 terminated due to immune related serious adverse events as announced by Mirna
165 Therapeutics on September 20, 2016 and indicated on ClinicalTrials.gov. It will thus
166 be very important to carefully evaluate the potential risks associated with the
167 modulation of miR-122 levels in patients with liver disease.

168 Another approach to increase miR-122 levels in metabolic disorders without
169 using miRNA mimics or viral vector gene therapy could be through activation of ROR α
170 as suggested by Chai and colleagues (14). This appears to be an interesting strategy
171 since small molecule drugs could be used (24). However, it has to be kept in mind that
172 ROR α does not only regulate glucose and lipid metabolism but also T helper 17 (Th17)
173 cell development that plays an essential role in many autoimmune disorders;
174 moreover, ROR α is involved in the stabilization of p53 /apoptosis and has been
175 suggested as an anti-cancer target (reviewed in (24)). Further preclinical and clinical
176 studies are needed to define and carefully characterize novel therapeutic targets in
177 order to improve the management of patients with liver disease.

178

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184

185 **Conflict of interest**

186 The authors declare no conflict of interest.

187

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- 248
- 249

250 **Figure legend**

251 **Figure 1. Impact of miR-122 on the triglyceride biosynthesis pathway.** An increase
252 of free fatty acids (FFA) in the bloodstream due to triglyceride (TG) hydrolysis, e.g.
253 following starvation or treatment with a β 3-adrenergic receptor agonist, enhances the
254 promoter activity of miR-122 through retinoic-related orphan receptor alpha (ROR α) in
255 hepatocytes. In turn, miR-122 regulates the TG biosynthesis in the liver by inhibiting
256 *Agpat1* and *Dgat1*, two genes involved in the production of TG. FFA also increase the
257 secretion of miR-122 from hepatocytes into the bloodstream. Secreted miR-122
258 remotely decreases the TG levels both in adipose tissues and muscle cells as well as
259 the formation of lipid droplets in muscle cells (14). (Images adapted from SMART
260 (Servier Medical Art))

β 3-adrenergic receptor agonist CL316243
starvation

