

miR-122 acts as a tumor suppressor in hepatocarcinogenesis *in vivo*

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COMMENTARY ON:

Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. Hsu SH, Wang B, Kota J, Yu J, Costinean S, Kutay H, Yu L, Bai S, La Perle K, Chivukula RR, Mao H, Wei M, Clark KR, Mendell JR, Caligiuri MA, Jacob ST, Mendell JT, Ghoshal K. *J Clin Invest.* 2012 Aug 1;122(8):2871–83. Copyright (2012). Reprinted with permission of American Society for Clinical Investigation.

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Abstract: miR-122, an abundant liver-specific microRNA (miRNA), regulates cholesterol metabolism and promotes hepatitis C virus (HCV) replication. Reduced miR-122 expression in hepatocellular carcinoma (HCC) correlates with metastasis and poor prognosis. Nevertheless, the consequences of sustained loss of function of miR-122 *in vivo* have not been determined. Here, we demonstrate that deletion of mouse *Mir122* resulted in hepatosteatosis, hepatitis, and the development of tumors resembling HCC. These pathologic manifestations were associated with hyperactivity of oncogenic pathways and hepatic infiltration of inflammatory cells that produce pro-tumorigenic cytokines, including IL-6 and TNF. Moreover, delivery of miR-122 to a MYC-driven mouse model of HCC strongly inhibited tumorigenesis, further supporting the tumor suppressor activity of this miRNA. These findings reveal critical functions for miR-122 in the maintenance of liver homeostasis and have important therapeutic implications, including the potential utility of miR-122 delivery for selected patients with HCC and the need for careful monitoring of patients receiving miR-122 inhibition therapy for HCV.

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Abbreviations: ALP, alkaline phosphatase; ALT, alanine transferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDL, high density lipoprotein; IL, interleukin; KO, knock-out; LDL, low density lipoprotein; LKO, liver-specific knock-out; mRNA, messenger RNA; microRNA, miRNA; MTP, microsomal triglyceride transfer protein; TG, triglyceride; TNF, tumor necrosis factor; VLDL, very-low density lipoprotein; WT, wild type.

MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. Tsai WC, Hsu SD, Hsu CS, Lai TC, Chen SJ, Shen R, Huang Y, Chen HC, Lee CH, Tsai TF, Hsu MT, Wu JC, Huang HD, Shiao MS, Hsiao M, Tsou AP. *J Clin Invest.* 2012 Aug 1;122(8):2884–97. Copyright 2012. Reprinted with permission of AMERICAN SOCIETY FOR CLINICAL INVESTIGATION.

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Abstract: MicroRNA-122 (miR-122), which accounts for 70% of the liver's total miRNAs, plays a pivotal role in the liver. However, its intrinsic physiological roles remain largely undetermined. We demonstrated that mice lacking the gene encoding miR-122a (*Mir122a*) are viable but develop temporally controlled steatohepatitis, fibrosis, and hepatocellular carcinoma (HCC). These mice exhibited a striking disparity in HCC incidence based on sex, with a male-to-female ratio of 3.9:1, which recapitulates the disease incidence in humans. Impaired expression of microsomal triglyceride transfer protein (MTP) contributed to steatosis, which was reversed by *in vivo* restoration of *Mtpp* expression. We found that hepatic fibrosis onset can be partially attributed to the action of a miR-122a target, the *Klf6* transcript. In addition, *Mir122a*^{-/-} livers exhibited disruptions in a range of pathways, many of which closely resemble the disruptions found in human HCC. Importantly, the reexpression of miR-122a reduced disease manifestation and tumor incidence in *Mir122a*^{-/-} mice. This study demonstrates that mice with a targeted deletion of the *Mir122a* gene possess several key phenotypes of human liver diseases, which provides a rationale for the development of a unique therapy for the treatment of chronic liver disease and HCC.

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Hepatocellular carcinoma (HCC) is the fifth most prevalent and the third leading cause of cancer death worldwide. Although early-stage tumors can be curated using surgical approaches, treatment options for advanced HCC are limited. Virus- and alcohol-induced cirrhosis represent major causes of HCC, but the molecular mechanisms underlying HCC are still only incompletely understood [1]. Several microRNAs (miRNAs) have been implicated in hepatocarcinogenesis and may represent promising new anti-cancer targets (reviewed in [2]). miRNAs are small non-coding regulatory RNAs resulting from Dicer-mediated processing of miRNA precursors. They act by modulating target messenger RNA translation and/or stabilization and are involved in a wide variety of biological processes. miR-122, a



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miRNA predominantly expressed in hepatocytes, accounts for approximately 70% of liver-expressed miRNAs. Evidence from sequestering miR-122 *in vivo* suggests that this miRNA is involved in cholesterol biosynthesis and liver function [3]. It may also play a role in hepatocarcinogenesis as its expression is downregulated in HCC [4] and hepatocyte-specific *Dicer1* knockout (KO) mice spontaneously develop HCC [5]. However, its exact role in hepatocarcinogenesis and the underlying molecular mechanisms had not been investigated yet.

Two independent laboratories now report arresting conclusions regarding the metabolic, anti-inflammatory, and anti-tumorigenic roles of miR-122, by generating germline KO, called either *Mir122* KO [6] or *Mir122a*^{-/-} [7], and liver-specific KO (*Mir122* LKO) mice [6]. These mice developed normally and were characterized by reduced serum cholesterol levels in line with previous studies reporting antisense miR-122 sequestration *in vivo* [3]. In *Mir122* KO and *Mir122* LKO mice, total serum cholesterol was reduced by 30% with concomitant decrease in LDL and HDL while TG levels remained unchanged [6]. Reduced serum cholesterol (total cholesterol, VLDL and HDL) was also observed in *Mir122a*^{-/-} along with reduced TG levels [7]. In contrast to antisense miR-122-treated mice, these *Mir122* KO mice developed steatosis and hepatitis that progressed to fibrosis and HCC and thereby displayed a phenotype resembling liver-specific *Dicer1* KO mice [5] (Table 1). Hepatic gene expression analysis indicated that numerous genes involved in lipid metabolism were differentially regulated in LKO mice [6]. *Mir122a*^{-/-} mice also demonstrated impaired expression of several proteins

involved in lipid metabolism, among which microsomal triglyceride transfer protein (MTTP). Although miR-122 does not directly target MTTP messenger RNA, prolonged injection of MTTP- or miR-122-expression constructs in *Mir122a*^{-/-} mice allowed restoration of MTTP levels, increase of serum lipids and reduction of hepatic steatosis, inflammation and fibrosis [7]. These functional experiments indicate that lack of MTTP and/or miR-122 expression leads to liver disease. In addition, both groups validated several novel direct targets of miR-122, among which *Klf6* or *Agpat1*, that could also account for the observed phenotype [6,7].

Moreover, in both models, prolonged lack of miR-122 in mouse hepatocytes associates with HCC [6,7]. While spontaneous tumors were 3.9-fold higher in male *Mir122a*^{-/-} mice than in females [7], in line with observed HCC incidence in humans, Hsu *et al.* observed equal penetration in *Mir122* KO males and females although males displayed higher tumor weight and grades [6]. To ascertain that lack of miR-122 indeed accounts for HCC development, the authors elegantly demonstrated through *in vivo* rescue experiments that tumor development may be prevented by restoration of hepatic miR-122 expression [6,7]. These experiments provide definite conclusions regarding the tumor suppressor function of miR-122 in the liver.

Noteworthy, HCC-resembling tumors were associated with hepatic infiltration of inflammatory cells and increased levels of pro-inflammatory/pro-tumorigenic cytokines such as IL-6 and TNF α [6,7]. In order to assess whether the anti-inflammatory function of miR-122 may be dissociated from its tumor

Table 1. Phenotype of *Mir122* knockout mice and effect of *Mir122* restoration. This table summarizes the characteristics of *Mir122a*^{-/-} as well as *Mir122* KO and *Mir122* LKO mice [6,7]. n.d., not determined; mo, months; wk, week.

	<i>Mir122</i> KO and <i>Mir122</i> LKO mice [6]	<i>Mir122a</i> ^{-/-} mice [7]
Development and fertility	Normal	Normal
Serum lipids	Reduced total cholesterol, LDL, HDL (VLDL n.d.) Normal TG	Reduced total cholesterol, HDL, VLDL (LDL n.d.) Reduced TG
ALP and ALT	Elevated ALP (ALT n.d.)	Elevated ALP and ALT
Serum inflammation markers	Elevated IL-6, TNF α	Elevated IL-6
Liver histology	Extensive lipid accumulation Reduced glycogen storage Inflammation (infiltrating inflammatory cells expressing <i>Ccr2</i> and producing IL-6 and TNF α , increase of <i>Ccl2</i> expression in hepatocytes) Fibrosis Proliferation of bile duct Spontaneous tumors (higher in LKO males in 12-17 mo; equal penetration in KO males and females after 11-15 mo but males display higher tumor weight and grades)	Extensive lipid accumulation Reduced glycogen storage Inflammation Fibrosis Portal fibrosis Spontaneous tumors (3.9-fold higher in males)
Hepatic lipid metabolism	Elevated TG Upregulated expression of TG biosynthesis (<i>Agpat1</i> , <i>Mogat1</i> , <i>Agpat3</i> , <i>Agpat9</i> , <i>Ppap2a</i> , <i>Ppap2c</i> and <i>Dgat1</i>) Abnormal expression of genes involved in development, cellular proliferation and death, and cancer (<i>Epcam</i> , <i>c-Myc</i> , <i>Mapre1</i> and <i>Rhoa</i>)	Elevated cholesterol and TG Reduced expression of genes involved in lipid metabolism (<i>Acaca</i> , <i>Acy1</i> , <i>Fasn</i> , <i>Scd1</i> , <i>Cpt1a</i> , <i>Cpt2</i> , <i>Gsta2</i> , <i>Pklr</i> , <i>Slco1a</i> , <i>Slco1a4</i> , <i>Mttp</i> , <i>Slc27a5</i> , <i>Foxa1</i> , <i>Foxa2</i> , <i>Mxipl</i> and <i>Nr1h2</i>) Increased expression of genes involved in immune response, fibrogenesis (<i>Klf6</i>), epithelial-mesenchymal transition, signal transduction, cell survival, cell death, and cancer
Rescue of liver pathology and serum lipids	n.d.	Restoration of MTTP or miR-122 expression by hydrodynamic injection
Prevention of HCC	Restoration of liver miR-122 expression by adenoviral delivery in 11-wk-old mice	Restoration of miR-122 expression in 3-mo-old mice by hydrodynamic injection

suppressor function, Hsu *et al.* used a previously described non-inflammatory HCC mouse model (tet-o-MYC; LAP-tTA mice) [8]. Interestingly, miR-122 levels were markedly reduced in tumors from these mice and adenoviral-mediated delivery of miR-122 strongly reduced tumor burden in treated mice [6]. Thus, these experiments clearly demonstrate that miR-122 possesses an anti-tumorigenic function that is independent of its role in preventing liver disease and inflammation.

In conclusion, these new mouse models provide an important step forward in the understanding of HCC development. These data clearly confirm an important role of miR-122 in hepatocarcinogenesis. Further studies are needed to explore the tumor suppressor function of miR-122. These results have also profound implications regarding the therapeutic potential of miR-122. While they indicate that complementing the low expression of miR-122 could be a valid strategy against HCC, it is important to note that miR-122 is also an essential host factor for hepatitis C virus (HCV) replication [9]. In contrast to the physiological role of miR-122 in negatively regulating expression of proteins involved in lipid metabolism, miR-122 positively regulates HCV translation and replication. Antiviral strategies targeting miR-122 are therefore in clinical development [10]. Given that chronic HCV infection is a major cause of HCC, the fact that miR-122 acts as a tumor suppressor in hepatocytes will have to be taken into account for the treatment of chronic HCV infection.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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