

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

Mirjam B. Zeisel<sup>1,2</sup>, Sébastien Pfeffer<sup>2,3</sup> and Thomas F. Baumert<sup>1,2,4</sup>

<sup>1</sup> Inserm, U748, Strasbourg, France; <sup>2</sup> Université de Strasbourg, Strasbourg, France; <sup>3</sup> Architecture et Réactivité de l'ARN, Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France; <sup>4</sup> Pôle Hépato-digestif, Nouvel Hôpital Civil, Strasbourg, France

**Word count:** 800

**References:** 10

**Table:** 1

**Corresponding authors:** Mirjam B. Zeisel, PhD, PharmD, Inserm U748, Université de Strasbourg, 3 Rue Koeberlé, F-67000 Strasbourg, France; Phone: (++33) 3 68 85 37 03, Fax: (++33) 3 68 85 37 24, e-mail: [Mirjam.Zeisel@unistra.fr](mailto:Mirjam.Zeisel@unistra.fr) and Thomas F. Baumert, M. D., Inserm U748, Université de Strasbourg, 3 Rue Koeberlé and Pôle Hépato-digestif, Nouvel Hôpital Civil, F-67000 Strasbourg, France; Phone: (++33) 3 68 85 37 03, Fax: (++33) 3 68 85 37 24, e-mail: [Thomas.Baumert@unistra.fr](mailto:Thomas.Baumert@unistra.fr)

**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; MTTP: microsomal triglyceride transfer protein; TG: triglyceride; TNF: tumor necrosis factor; VLDL: very-low density lipoprotein; WT: wild-type

Keywords: hepatocellular carcinoma, miR122, tumor suppressor

**Comment on** 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. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. *J Clin Invest.* 2012 Aug 1;122(8):2871-83. doi: 10.1172/JCI63539. Epub 2012 Jul 23.

and 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. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. *J Clin Invest.* 2012 Aug 1;122(8):2884-97. doi: 10.1172/JCI63455. Epub 2012 Jul 23.

### **Abstracts**

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.

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 (MTTP) contributed to steatosis, which was reversed by in vivo restoration of Mttp 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<sup>-/-</sup> 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<sup>-/-</sup> 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.

## Comment

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 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 down-regulated in HCC [4] and hepatocyte-specific *Dicer1* knock-out (KO) mice spontaneously develop HCC [5]. However, its exact role in hepatocarcinogenesis and the underlying molecular mechanisms had not yet been investigated.

Two independent laboratories now reported arresting conclusions regarding the metabolic, anti-inflammatory and anti-tumorigenic roles of miR-122 by generating germline KO, either called *MiR122*-KO [6] or *MiR122a*<sup>-/-</sup> [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*<sup>-/-</sup> along with reduced TG levels [7]. In contrast to antisense miR-122-treated mice, these miR-122 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*<sup>-/-</sup> 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*<sup>-/-</sup> mice allowed to restore MTTP levels, to increase serum lipids and to reduce 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*<sup>-/-</sup> mice than in females [7], in line with observed HCC incidences 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 $\alpha$  [6, 7]. In order to assess whether the anti-inflammatory function of miR-122 may be dissociated from its tumor 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 for the understanding of HCC-development. These data clearly confirm an important role for 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 a 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 miR122 acts as a tumor suppressor in hepatocytes will have to be taken into account for the treatment of chronic HCV infection.

## **Acknowledgements**

Work in T.F.B.'s laboratory is supported by the European Union (ERC-2008-AdG-233130-HEPCENT, INTERREG-IV-Rhin Supérieur-FEDER-Hepato-Regio-Net 2009), Laboratoire d'Excellence HEPSYS (Investissement d'Avenir; ANR-10-LAB-28), ANR-05-CEXC-008, ANRS (2008/354, 2009/183, 2011/132), Région Alsace, Inserm, and University of Strasbourg. Work in S.P.'s laboratory is supported by the European Research Council (ERC-2010-StG-260767-ncRNAVIR), Agence Nationale de la Recherche (ANR-08-MIEN-005 and ANR-10-LABX-36-netRNA), the Institut National du Cancer (INCa-PLBIO-2009-173) and CNRS.

## **Conflict of interest**

The authors declare no conflict of interest.

## References

- [1] El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012;142:1264-1273 e1261.
- [2] Wang XW, Heegaard NH, Orum H. MicroRNAs in liver disease. *Gastroenterology* 2012;142:1431-1443.
- [3] Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, et al. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. *Cell Metab* 2006;3:87-98.
- [4] Bai S, Nasser MW, Wang B, Hsu SH, Datta J, Kutay H, et al. MicroRNA-122 inhibits tumorigenic properties of hepatocellular carcinoma cells and sensitizes these cells to sorafenib. *J Biol Chem* 2009;284:32015-32027.
- [5] Sekine S, Ogawa R, Ito R, Hiraoka N, McManus MT, Kanai Y, et al. Disruption of Dicer1 induces dysregulated fetal gene expression and promotes hepatocarcinogenesis. *Gastroenterology* 2009;136:2304-2315 e2301-2304.
- [6] Hsu SH, Wang B, Kota J, Yu J, Costinean S, Kutay H, et al. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. *J. Clin. Invest.* 2012;122:2871-2883.
- [7] Tsai WC, Hsu SD, Hsu CS, Lai TC, Chen SJ, Shen R, et al. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. *J. Clin. Invest.* 2012;122:2884-2897.
- [8] Beer S, Zetterberg A, Ihrie RA, McTaggart RA, Yang Q, Bradon N, et al. Developmental context determines latency of MYC-induced tumorigenesis. *PLoS Biol* 2004;2:e332.
- [9] Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science (New York, NY)* 2005;309:1577-1581.
- [10] Janssen HL, Reesink HW, Zeuzem S, Lawitz E, Rodriguez-Torres M, Chen A, et al. A randomized, double-blind, placebo (plb) controlled safety and anti-viral proof-of-concept

study of miravirsen (mir), an oligonucleotide targeting miR122, in treatment naive patients with genotype 1 (gt1) chronic HCV infection. Hepatology 2011;54:101A (abstract LB106).