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Hepatocyte miRNA-21-Deficiency Promotes Hepatic Lipid Accumulation but Ameliorates Alcohol-Induced Liver Injury by Targeting DUSP16

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Background: Accumulation of lipid droplets and inflammatory cell infiltration in the liver is the pathophysiological hallmark of alcoholic liver disease (ALD). The cell-type specific role of miR-21 in ALD remains unknown due to the lack of cell-type specific knockout mice. The current study aims at elucidating the pathophysiological role of miR-21 in ALD using our newly generated hepatocyte specific miR-21 knockout mice.

Methods: We generated a new mouse model for hepatocyte specific deletion of miR-21 (Hep-KO). To induce alcoholic steatosis, Hep-KO and their wild-type (WT) littermates were fed with isovolumetric/isocaloric control liquid diet or Lieber-DeCarli ethanol liquid diet at different concentrations (1% to 6%) for 4 weeks followed by a single binge. Markers of steatosis, liver injury and inflammation were assessed. Lipidomics and metabolomics were conducted. Other methods: liver histology, 3'UTR luciferase reporter, qPCR, Western blots, Oil O red staining.

Results: Liver miR-21 RNA levels were highly induced in WT mice fed the ethanol diet compared with mice fed the control diet. Moreover, miR-21 levels were markedly upregulated in human patients with alcoholic cirrhosis vs normal controls, demonstrating their roles in the pathogenesis of ALD. Hep-KO mice fed the ethanol diet had increased hepatic triglyceride (TG) but decreased plasma TG levels, which was associated with increased lipogenesis (FASN, SCD1 and SREBP protein expression) but decreased VLDL secretion (MTTP). Lipidomics analysis revealed increased TG but decreased

phosphatidyl cholines (PC) and phosphatidyl ethanolamines (PE) lipid species in Hep-KO vs WT mice. Interestingly, inflammation was attenuated in Hep-KO, as evident by the decreased serum AST and ALT levels, and reduced expression of key hepatic pro-inflammatory (Il-6, Ccr2, MIP1a, LyG6) genes. At the cellular level, MAPKs, AMPK and PPARs signaling pathways were assessed to determine the key regulatory role of miR-21 in steatosis and inflammation, which showed that the activation of P38 and JNK were suppressed in Hep-KO. Using TargetScan, microrna and miRanda, we predicted a conserved miR-21 seed match region in mouse and human DUSP16 3'UTRs; a gene known as a phosphatase of P38 and JNK. Transfection of miR-21 mimics reduced the DUSP16 protein in HepG2 and Hepa1 cells. In addition, the mouse DUSP16 3'UTR containing the miR-21 seed region was cloned into a luciferase reporter and miR-21 mimic inhibited DUSP16 3'UTR activity in a dose-dependent fashion, which was relieved when the miR-21 seed region was mutated. Furthermore, DUSP16 protein expression was down-regulated in patients with alcoholic liver cirrhosis, where miR21, P-P38 and P-JNK levels were elevated.

Conclusion: Hepatocyte miR-21-deficiency promotes alcoholic steatosis, but ameliorates liver injury and inflammation through the miR-21/DUSP16/P38 pathway. Our work provides novel insights into the understanding of the molecular mechanism of non-coding RNAs as key regulators in alcoholic liver disease.

All authors have nothing to disclose.