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Deletion of microRNA-34a alleviates endothelial dysfunction and inflammatory response during experimental cholestasis

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Background: Sustained cholestasis results in injury to the biliary epithelium with subsequent ductular reaction, angiogenesis, pericholangitis, and fibrogenesis. *The* early event in chronic *cholestatic liver disease,* portal hypertension, is induced by an increased intrahepatic resistance, a major consequence of liver cirrhosis. Endothelial dysfunction in liver sinusoidal endothelial cells (LSECs) decreases the production of vasodilators, such as nitric oxide (NO) and favors vasoconstriction. We aimed to characterize microRNA regulated sinusoidal endothelial dysfunction (SED) in the mouse model of cholestatic liver injury. **Methods:** Bile duct ligation (BDL) and MDR2 knockout mice ($MDR2^{-/-}$) were used as animal models of cholestatic liver injury (CLI). The upstream modulators and downstream mediators of sinusoidal injury were defined in TLR-4, constitutive and liver specific miR-34a knockout mice *in vivo* and cultured human hepatic sinusoidal endothelial cells with miR-34a modifications *in vitro* by real-time PCR array/assay, immunohistochemistry and Western blot analysis. **Results:** Using BDL and $MDR2^{-/-}$ mouse model of cholestatic liver injury, results showed the significant increase in serum ALT and hepatic miR-34a, severe inflammation, pericellular fibrosis, and intensified nitrosative stress induced by a 24- or 31-fold induction of nitric oxide synthase (NOS2), respectively. The upregulation of miR-34a in human sinusoidal endothelial cells led to a time-dependent repression of its target protein Sirt1 levels as shown by western blot analyses, and a significant increase of the expression of NOS2. SED markers ICAM-1, VEGFR-2, and E-selectin were significantly up-regulated in the progressive phases of CLI. Lack of miR-34a in vivo, reversed the serum ALT level, and restored the levels of Sirt1 coupled with decreased NOS2 mRNA expression as well as SED dysfunction markers. Depletion of miR-34a in vivo also decreased overall vessel formation and neutrophil infiltration. This was significant in portal areas of CLI mice and was associated with a significant down-regulation of profibrogenic genes and matrix metalloproteinases. Interestingly, in mice lacking TLR-4 with CLI, significantly reduced miR-34a levels, increased Sirt1 repression and decreased NOS2 mRNA expression were observed. In isolated sinusoidal endothelial cells by laser capture microdissection (LCM) from CLI mice, enhanced expression of miR-34a was observed and SED was discovered. Finally liver specific knockout of miR-34a in CLI mice also showed reduced NOS2 mRNA level and reversed SED. **Conclusion:** The discovery that the activation of miR-34a plays a significant role in the process of cholestatic liver injury through sinusoidal endothelial dysfunction for an exciting field in which the epigenomic microRNAs of hepatic endothelial cells may be manipulated with potential therapeutic benefits to liver inflammation and fibrosis.

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