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## Role of methylation-controlled J-protein, endogenous repressor of the mitochondrial respiratory chain, in cholestatic liver disease

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**Background and Aims:** Mitochondrial dysfunction contributes to CLD pathogenesis, in fact bile acids are cytotoxic, capable of emulsifying lipid membranes such as mitochondrial, causing ROS overproduction and hepatocyte death. MCJ is a mitochondrial protein that represses the function of the mitochondrial respiratory chain (MRC), so that its deficiency could mitigate the oxidative stress caused by cholestasis. Our aim is to study the effect of MRC inhibition by MCJ on bile acids induced liver toxicity.

**Method:** Bile duct ligation (BDL) in WT and MCJ-KO mice was used as an animal model of cholestasis. Liver injury was assessed by histology. The protein and gene expressions were measured by Western blot and qPCR respectively. The MCJ expression in WT mice was transiently knockdown injecting MCJ siRNA (siMCJ). *In vitro* studies, primary hepatocytes were treated with deoxycholic acid (DCA), MCJ was silenced using a ShRNA for MCJ (shMCJ), assessing the apoptosis by caspase3 activity. Total ROS and superoxide production, and active mitochondria were measured by CellROX<sup>®</sup>, MitoSOX<sup>®</sup> and MitoTracker<sup>®</sup> stain respectively. Mitochondrial membrane potential was analysed by flow cytometry, and ATP levels were measured by luminescence. MCJ levels were analysed by immunohistochemistry in patient liver biopsies with primary biliary cholangitis (PBC).

**Results:** Hepatic MCJ expression are significantly induced in PBC patients and WT mice after BDL. *In vivo*, we saw greater survival of MCJ-KO mice after BDL compared with WT, and lower inflammatory hepatic infiltrate at 48 hours after BDL. The liver injury was evaluated 7 days after BDL showed minimal levels of necrotic areas and inflammation in mice treated with siMCJ relative to those that did not receive siMCJ. Both *in vivo* and *in vitro*, MCJ-KO have lower JNK activation. In fact, we found less apoptosis after adding DCA in hepatocytes MCJ-KO and ShMCJ. This lower apoptosis is due to lower depolarization of mitochondrial membrane, lower ROS production and higher ATP production that we saw in hepatocytes MCJ-KO after DCA treatment. In addition, MCJ-KO hepatocytes showed higher expression of genes related to bile acids transport (MDR2, FXR), and lower expression of inflammatory genes (IL1b, TNF).

**Conclusion:** Loss of MCJ protects hepatocytes against JNK activation, ROS production, mitochondrial membrane depolarization, and ATP depletion as a result of bile acid toxicity. Our results identify MCJ as a potential therapeutic target to mitigate liver injury in CLD.