Metabolic reprogramming induced by pravastatin prevents polycystic liver disease progression improving mitochondrial bioenergetics in cystic cholangiocytes

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Background and Aims: Polycystic liver diseases (PLDs) are hereditary genetic disorders marked by progressive development of intrahepatic fluid-filled biliary cysts, representing a primary source of morbidity. The current pharmacological treatment for advanced disease involves the chronic administration of somatostatin analogs, aimed at reducing the elevated intracellular cAMP levels in cystic cholangiocytes. Nevertheless, this approach demonstrates limited effectiveness. Cystic cholangiocytes exhibit abnormal proteostasis and endoplasmic reticulum (ER) stress, promoting cystogenesis. Considering the interplay between ER and mitochondria, we investigated mitochondrial dynamics, bioenergetics, and metabolism within cystic cholangiocytes, further exploring their potential therapeutic regulatory value in vitro and in vivo.

Method: Human cystic cholangiocytes (ADPKD¹⁶GANAB⁻/⁻ or ADPLD¹⁶PRKCSH⁻/⁻) and normal human cholangiocytes (NHC) as controls were studied. Mitochondrial ultrastructure and its interaction with the ER were analyzed by transmission electron microscopy. Mitochondrial mass, dynamics, and functionality were evaluated using flow cytometry and qPCR. Mitochondrial bioenergetic activity was assessed by Seahorse Analyzer, and protein levels of electron transport chain (ETC) complexes through immunoblotting. Metabolic fluxes including de novo synthesis and oxidation of palmitate, glucose, and glutamine were analyzed in radioassays. The effect of pravastatin was investigated in male PCK rats (Pkhd1⁻/⁻).

Results: Cystic cholangiocytes exhibited higher mitochondria-ER distance and increased mitochondrial mass. The expression of mitochondrial dynamics (i.e., fusion, fission, and mitophagy) and biogenesis-related genes was found upregulated in cystic tissue and cells from PLD patients, compared to gallbladder tissue and NHCs, respectively. Cystic cells displayed higher mitochondrial membrane potential and abnormal bioenergetic capacities compared to NHCs. Consequently, ATP and mitochondrial reactive oxygen species levels were elevated in cystic cells, correlating with upregulation of ETC complexes. Both ADPKD and ADPLD cholangiocytes exhibited enhanced rates of glutamine
and palmitate oxidation compared to NHCs, respectively, along with an increased capacity for the *de novo* synthesis of cholesterol. The chronic administration of pravastatin reduced liver volume and weight in PCK rats compared to controls. *In vitro*, pravastatin mitigated mitochondrial metabolic hyperactivity and decreased the proliferation of cystic cholangiocytes.

**Conclusion:** Cystic cholangiocytes exhibit altered mitochondrial dynamics, bioenergetics, and metabolism, with lipids playing a crucial role as energy substrate. Rewiring lipid metabolism with pravastatin halts hepatic cystogenesis, arising as a novel therapeutic opportunity.