ABSTRACT

Charles University in Prague, Faculty of Pharmacy in Hradec Králové, Department of Biological and Medical Sciences
Candidate: Mgr. Ester Dohnalkova
Consultant: prof. PharmDr. Petr Nachtigal, Ph.D.
Title of Thesis: Hepatocyte ROCK1 kinase activity incites liver inflammation in murine non-alcoholic fatty liver disease

Background: Rho-associated protein kinase 1 (ROCK1) activity has been previously implicated in lipotoxicity-induced extracellular vesicle (EV) release from hepatocytes. In turn, lipotoxic hepatocyte-derived EVs incite inflammatory response in monocytes and macrophages. In addition, we previously found that activated ROCK1 is a distinct histologic feature of inflammatory stage of murine and human nonalcoholic fatty liver disease (NAFLD). However, it is currently unknown whether activated ROCK1 may directly contribute to NAFLD pathogenesis. Therefore, we aimed to examine the effect of hepatocyte ROCK1 kinase activity on the development of inflammation in a model of murine NAFLD.

Methods: To assess the role of ROCK1 in NAFLD, we have adopted an *in vivo* gain-of-function approach. C57BL/6J mice were injected with either: **a)** vehicle; **b)** adeno-associated virus, serotype 8 (AAV8)-packaged, TBG-driven constitutively active ROCK1 (CA-ROCK1) construct; or **c)** AAV8-packaged, TBG-driven kinase-dead ROCK1 (KD-ROCK1) as a negative control. Two weeks later, mice were placed on either chow or diet high in fat, fructose and cholesterol (FFC) for 8 weeks; this short-term feeding represents model of isolated steatosis with minimal tissue inflammation. At the end of the study, livers, adipose tissue and blood were harvested for downstream analyses and mass cytometry by time-of-flight (CyTOF).

Results: Chow-fed mice expressing ROCK1 mutants showed normal liver histology and function. All three groups of FFC-fed mice developed similar body weight, liver weight, and epididymal white adipose tissue weight. Metabolic phenotype (fasting glucose, fasting insulin) of all mice was assessed and there were no statistically significant differences between KD-ROCK1 and CA-ROCK1 in FFC-fed groups. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated and FFC-fed mice expressing CA-ROCK1 showed increased HOMA-IR compare to KD-ROCK1 mice. Liver injury, as assessed by serum alanine aminotransferase, did not differ between the three FFC groups. In contrast, FFC-fed CA-ROCK1 mice displayed a significant increase in markers of macrophage activation (CCL3, CCL4, CCL5, CXCL10), macrophage surface markers (CD68, F4/80), and macrophage abundance (Mac-2 by immunohistochemistry) in the liver. These macrophage-associated inflammation markers were significantly lower in FFC-fed KD-ROCK1 mice and FFC-fed controls. CyTOF analysis was performed on isolated intrahepatic leucocytes and demonstrated significant alterations in immune cell subpopulations caused by both the FFC feeding as well as CA-ROCK1 expression. Finally, hallmarks of liver fibrogenesis (αSMA, collagen 1a1, fibronectin) were increased in FFC-fed CA-ROCK1 mice compared to FFC-fed KD-ROCK1 mice.

Conclusion: Induction of ROCK1 kinase activity on the background of isolated steatosis incites steatohepatitis characterized by macrophage-associated inflammation. In addition, the expression of CA-ROCK1 in hepatocytes also promoted liver fibrogenesis. Given the evidence that ROCK1 promotes disease progression, we speculate that inhibition of ROCK1 kinase activity maybe salutary in human NAFLD.