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Title of project: Defining the link between hepatic NAD^+ salvage capacity and liver fibrosis.

ABSTRACT

The over-arching goal of this project is to understand the link between NAD^+ salvage capacity and liver fibrosis to provide novel insights into treatments for hepatic fibrosis/NASH. Non-alcoholic fatty liver disease (NAFLD) is emerging as a global pandemic as the prevalence of obesity and type 2 diabetes increases worldwide. NAFLD can progress to non-alcoholic steatotic hepatitis (NASH) with associated fibrosis, and can further progress to cirrhosis and hepatocellular carcinoma. No treatment for NASH currently exists, so novel treatments are needed. Increasing NAD^+ levels is an effective treatment for reducing hepatic lipid accumulation in rodent models for obesity. In the liver, NAD^+ levels are largely maintained by the NAD^+ salvage enzyme nicotinamide phosphoribosyltransferase (NAMPT). Our preliminary data show that hepatocyte-specific *Nampt* knockout (HNKO) mice have alterations in hepatic cholesterol efflux and develop hepatic fibrosis with focal necrosis and portal inflammation when fed a low-fat diet (LFD).
We propose a clear connection between NAD^+ salvage capacity and hepatic fibrosis development and that the HNKO mice have the potential to comprise a novel model for liver fibrosis. To validate this, we will determine the mechanism by which impaired NAD^+ salvage leads to hepatic fibrosis following LFD feeding by utilizing primary hepatocytes and *in vivo* models. Furthermore, the damage progression in our model will be assessed during an extended LFD-feeding period to determine whether the fibrosis reverses, reaches a steady state, or aggravates to hepatic cirrhosis. Finally, pharmacological studies will be undertaken to examine if NAD^+ repletion can enhance the effect of conventional NASH drugs and determine the applicability of the HNKO mice as a fibrotic liver disease model in general.
Our findings will provide novel insights into the link between NAD^+ salvage capacity and liver fibrosis development, as well as unravel the potential of the HNKO mice as a unique model of acute liver injury and fibrosis, thus aiding in development of new therapeutic strategies for the treatment of hepatic fibrosis/NASH.

ABSTRAKT

Det overordnede mål for projektet er at skabe en bedre forståelse for sammenhængen mellem NAD^+ gendannelseskapacitet og leverfibrose, som potentielt vil føre til bedre behandling af leverfibrose/NASH. Nonalkoholisk fedtleversygdom (NAFLD) har vist sig som en spirende global pandemi, efterhånden som prævalensen af fedme og type 2 diabetes stiger på verdensplan. NAFLD kan videreudvikle sig til nonalkoholisk steatohepatitis (NASH) med tilhørende fibrose, som igen kan føre til udviklingen af levercirrose og hepatocellulært karcinom. På nuværende tidpunkt eksisterer der ikke nogle effektive behandlinger for NASH, og der er derfor behov for nye behandlingsmetoder. Forøgelse af NAD^+ niveauer er en effektiv metode til at reducere hepatisk fedtakkumulering i fedmemodeller i mus. I leveren bliver NAD^+ niveauerne hovedsageligt

vedligeholdt af gendannelsesenzymet nikotinamid fosforibosyltransferase (NAMPT). Vores foreløbige resultater viser, at hepatocyt-specifikke *Nampt* knockout (HNKO) mus har forandringer i den hepatiske kolesterol efflux og udvikler leverfibrose med fokal nekrose og portal inflammation, når de fodres med en lavfedt diæt (LFD).

Vi foreslår, at der er en klar sammenhæng mellem NAD^+ gendannelseskapaciteten og udviklingen af leverfibrose, og at HNKO musene potentielt udgør en ny leverfibrosemodel. For at validere dette vil vi først fastslå mekanismen, hvormed nedsat NAD^+ gendannelse fører til udvikling af leverfibrose under LFD-fodring, ved at benytte både primære hepatocyt- og *in vivo*-modeller. Derudover vil vi undersøge skadesudviklingen i vores model under en forlænget LFD-fodringsperiode, for at fastslå om fibrosen forsvinder, stabiliseres eller forværres til levercirrose. Til sidst vil der blive foretaget farmakologiske studier for at undersøge om forøgelse af NAD^+ mængden kan forstærke effekten af konventionelle NASH behandlinger, samt fastslå anvendeligheden af HNKO musene som en model for fibrotiske leversygdomme i al almindelighed.

Vores fund vil skabe ny indsigt i betydningen af NAD^+ gendannelseskapacitet for udviklingen af leverfibrose, samt klarlægge potentialet for HNKO musene som en ny unik model til at studere akut leverskade og -fibrose. Alt dette vil bidrage til udviklingen af nye behandlingsmetoder til at bekæmpe leverfibrose/NASH.