Lack of FGF21 promotes hepatic steatosis and insulin resistance (IR) leading to de novo lipogenesis

Steven C. Nguyen-Ho[1], Xiaoju Shi[1], Yujia Chen[1], Youxi Yu[1], Suping Li[1], Robert C. Martin[1,2], Yan Li[1,2,*]

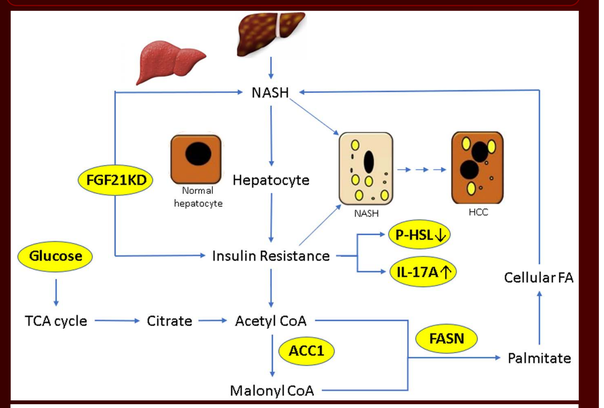
[1]Department of Surgery, School of Medicine, University of Louisville, Louisville, KY 40202, USA

[2]Department of Pharmacology & Toxicology, School of Medicine, University of Louisville, Louisville, KY 40202, USA

Introduction

- Non-alcoholic steatohepatitis (NASH) is the most severe form of non-alcoholic fatty liver disease (NAFLD) and is a risk factor for hepatocellular carcinoma (HCC).
- Accumulating evidence indicates that insulin resistance (IR) is associated with NASH and HCC carcinogenesis.
- Hepatic lipid accumulation results from a combination of enhanced lipid delivery and uptake, increased de novo lipogenesis (DNL), and changes in fatty acid oxidation (FAO).
- DNL is a risk factor linking NASH to HCC.
- Our previous study demonstrates that fibroblast grow factor 21 (FGF21) inhibits the hepatocyte-Toll Like Receptor 4 (TLR4)-Interleukin 17A(IL-17A) signaling against NASH-HCC transition, but the effects of FGF21 on IR and DNL are unknown.
- This study aims to investigate the role of FGF21 on hepatocyte IR and DNL.

Working Hypothesis



Lack of FGF21 could promote hepatic steatosis and insulin resistance (IR) leading to de novo lipogenesis via upregulation of fatty acid synthase (FASN) and acetyl CoA carboxylase (ACC1), which might place an important role contributing to NASH-HCC transition.

Methods

- Using the fourth-generation lentivirus packing system (Lenti-X, Takara-Clontech), FGF21 gene was knocked down (FGF21KD) in a mouse hepatocyte line, FL83B (ATCC CRL-2390), and a mouse hepatoma cell line, Hepa1-6 (ATCC CRL-1830).
- Hepa1-6 and Hepa1-6 FGF21KD cells were grown in DMEM medium, while FL83B and FL83B-FGF21KD cells were grown in F12K medium. Cells were treated with 50mM glucose and 25, 50, and100μM of sodium palmitate (FFA) as well as lipopolysaccharides (LPS), and insulin.
- Oil Red O staining was performed to determine the lipid accumulation in the cells with treatments of FFA and IL-17A.
- Western blot analysis was performed to detect protein expressions of phosphorylated hormone sensitive lipase (p-HSL) and IL-17A.
- Total RNA was extracted from the cells and qPCR was performed to determine mRNA expression for fatty acid synthase (FASN) and acetyl CoA carboxylase (ACC1).

Results

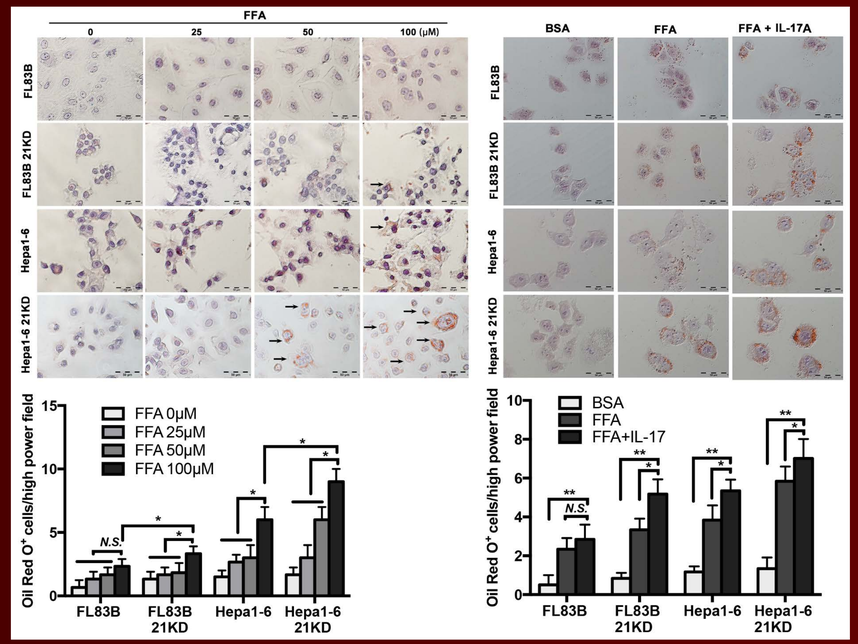


Figure 1 & 2: Positive Oil Red O staining cell was counted in high power field (40X), and the indexes of Red O staining positive cells were calculated. BSA: bovine serum albumin; *N.S.*: no statistical significance; *, P<0.05; **, P<0.01.

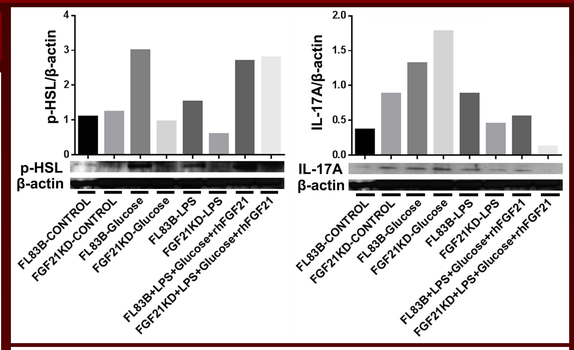


Figure 3: Protein levels of p-HSL and IL-17A in FL83B cells and FL83B FGF21KD cells treated with insulin, glucose, LPS, and recombinant human (rh) FGF21.

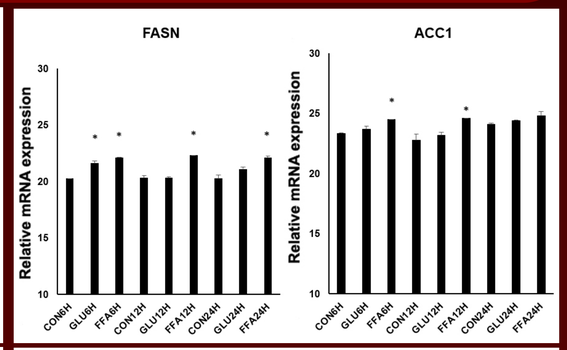


Figure 4: qPCR to detect mRNA levels of FASN and ACC1 in Hepa1-6 cells treated with FFA for 6hours, 12hours, and 24hours. H: hour. *, p<0.05 vs control.

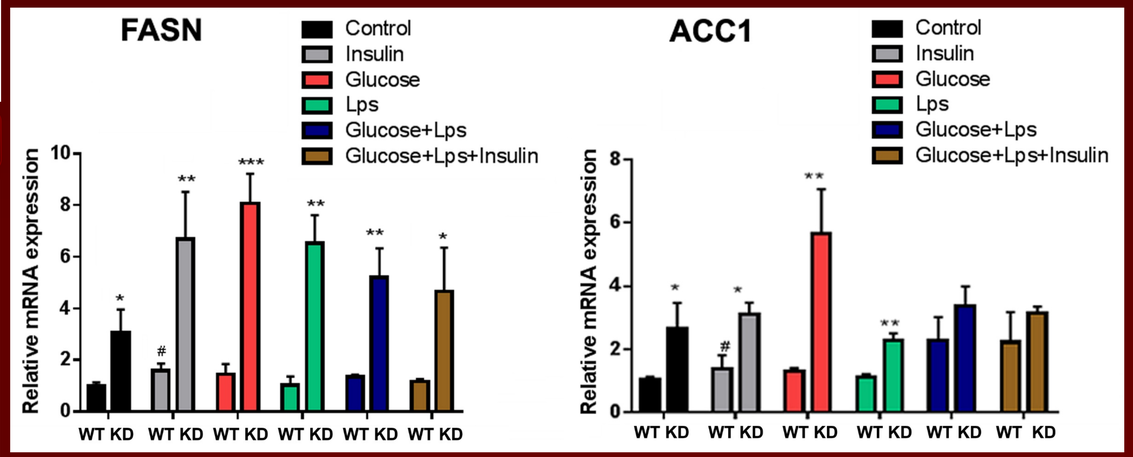


Figure 5&6: qPCR to detect mRNA levels of FASN and ACC1 in FL83B and FL83B-FGF21KD cells treated with glucose, insulin, and LPS. WT: wild type; KD: knock down. *, p<0.05 vs WT; **, p<0.01 vs WT; **, p<0.01 vs Control-WT.

Conclusion

- FGF21KD causes lipid accumulation in hepatocytes challenged by FFA and IL-17A.
- rhFGF21 may alleviate IR via restoring p-HSL protein and decreasing IL-17A production in hepatocytes.
- Lack of FGF21 contributes to DNL, which may further worsen lipid accumulation and IR in hepatocytes.

Future Direction

Further studies are needed to investigate the accurate mechanism of FGF21 negative feedback on DNL contributing to lipid accumulation and IR during the NASH-HCC transition.

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Conflict of Interest: Authors have no conflict of interest to disclose.