Actions of the protease Fibroblast Activation Protein alpha (FAP) in chronic liver injury

H Zhang[1,2], S Chowdhury[1], M Xiang[1,2], A Lay[1,2], E Hamson[1,2], S Wetzel[1], D Yu[1,2], Y Liu[1], O Schilling[3], B Yau[2], M Kebede[2], M Eslam[2,4], J George[2,4], A Majumdar[2,5], G McCaughan[1,2,5], M Gorrell[1,2]

1 Centenary Institute, Newtown, New South Wales, Sydney, Australia. 2 University of Sydney, NSW, Australia. 3 University of Freiberg, Germany. 4 Storr Liver Centre, Westmead Institute for Medical Research, NSW, Australia. 5 Royal Prince Alfred Hospital, Camperdown, NSW, Australia.

INTRODUCTION

FAP is a unique post-proline peptidase that is greatly upregulated in activated stellate cells in human liver fibrosis. Circulating FAP rises and falls with liver fibrosis severity in humans, where major FAP substrates are collagens, the starvation hepatokine FGF-21 and neuropeptide Y [2,4,5]. Whether FAP enzyme activity drives the liver towards or away from pathogenic progression was investigated in mouse models.

AIM

- To better understand NAFLD pathogenesis by examining outcomes of FAP enzyme depletion in mice.
- To seek associations between circulating FAP (cFAP) and disease in NAFLD and NASH patients.

METHOD

- Our FAP gene knock-in (gki) mouse lacks FAP enzyme activity by point mutation of the catalytic serine in FAP.
- To model diet-induced obesity (DIO), mice were fed atherogenic high fat and high sucrose diet (HFD) for 17 weeks, or provided thioacetamide in the drinking water to induce fibrosis. Data analysis used Mann-Whitney U test.
- Embryonic fibroblasts (MEF) were analysed in SILAC and TAILS proteomics methods [5].
- FAP enzyme activity was measured in sera (cFAP) from NAFLD & NASH patients, using an in-house assay [1].

RESULTS

FAP gene knock-in (gki) mice were:

- showing improved glucose tolerance
- protected against hyperinsulinemia
- protected against insulin resistance
- resistant to liver steatosis

MEF analyses identified many novel potential FAP substrates [5], notably CSF1, lysyl oxidases, several collagen subtypes and other ECM proteins.

In multivariate analyses, human cFAP was associated with (n=150; p<0.001):

- Fibrosis stage
- Insulin resistance
- Gender

RESULTS

- With HFD, compared to WT mice, FAP gki mice had less insulin resistance, pancreatic and plasma insulin, glucose intolerance, micro-vesicular steatosis, liver lipid, circulating LDL cholesterol and islet area (Fig 1-7). FAP deficiency increased p-ACC (Fig 3), indicative of increased lipolysis and β-oxidation. CD36 immunostaining was lower in FAP gki hepatocytes (Fig 6). Concordantly, lipogenic genes and hepatic triglyceride and fatty acid uptake genes were downregulated in FAP gki livers (Fig 1). FAP action may involve intrahepatic FGF-21, as it was increased in FAP deficient DIO compared to WT DIO mice (Fig 3).
- In the fibrosis model, significantly less Sirius red staining (Fig 8) and leukocyte clusters were evident in FAP gki livers.
- In MEF cultures, FAP modulated the abundance of intact forms of proteins involved in ECM remodelling, including collagens 1, 3 and 5, ECM-1, lysyl oxidases, CXCL5 and fibronectin. CCL2 and CXCL5 were shown to retain their functions after exposure to FAP. FAP appears to be a sheddase for CSF1 from fibroblasts. Cleavage of substrates generally occurred after Gly-Pro.
- FAP cleaved human but not mouse FGF21.
- Human cFAP was not associated with inflammation (biopsy), BMI, T2D or GGT.

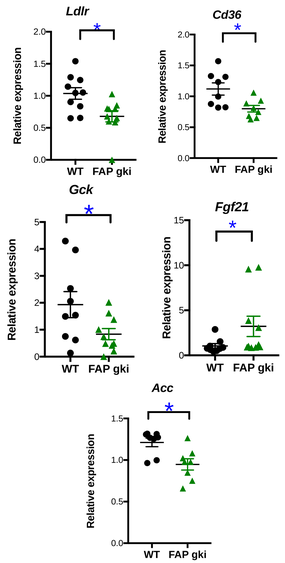


*Fig 1. Intrahepatic mRNA expression of energy metabolism genes. Downregulation of lipogenesis (Gck) and triglyceride uptake (Cd36, Ldlr). Transcripts relative to housekeeper gene beta-actin. *p<0.05.*

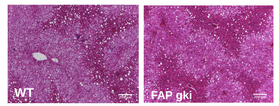


Fig 2. Liver H & E showing less microsteatosis in FAP gki (right panel). 17 weeks HFD.

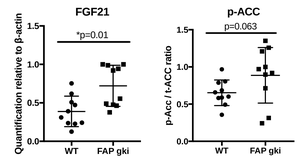


Fig 3. Liver immunoblots. 17 weeks HFD.

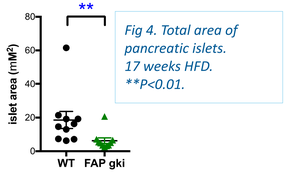


*Fig 4. Total area of pancreatic islets. 17 weeks HFD. **P<0.01.*

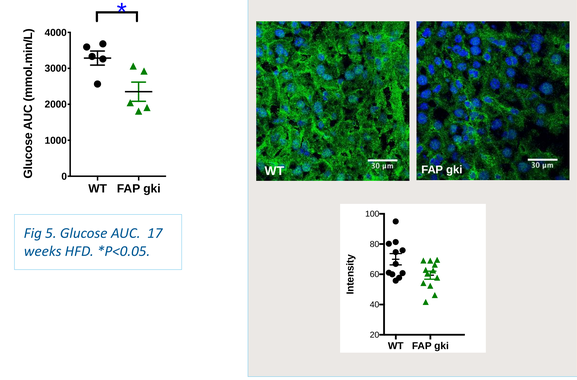


*Fig 5. Glucose AUC. 17 weeks HFD. *P<0.05.*

Fig 6. CD36 immunostain of WT and FAP gki livers and quantitation. 17 weeks HFD. P<0.05.

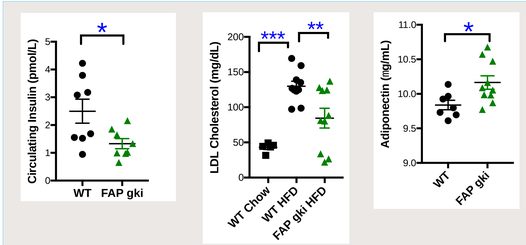


*Fig 7. Assays of sera of WT and FAP gki mice. 17 weeks HFD. *P<0.05. **P<0.01. ***P<0.001.*

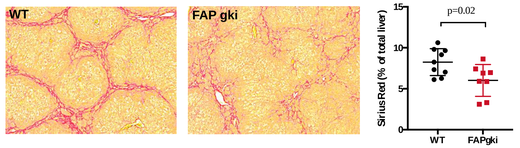
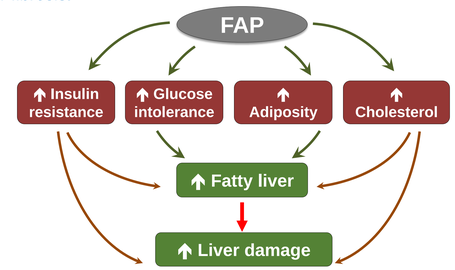


Fig 8. Intrahepatic collagen following thioacetamide ingestion for 16 weeks. Sirius red stain of liver was quantified.

CONCLUSIONS

This study is the first to demonstrate that specific genetic ablation of FAP activity, which mimics a specific potent inhibitor, is protective of diet-driven metabolic defects and fibrosis in mice. These mice had no adverse consequences of this enzyme deletion. The FAP gki phenotype has similarities to FGF-21 transgenic mice. Moreover, FAP was associated with fibrosis in human NAFLD/NASH. Our data indicates that a suitable FAP inhibitor may have potential as a therapy for insulin resistance, steatosis and liver fibrosis.



ACKNOWLEDGEMENTS

Funding from NHMRC and Rebecca L Cooper Foundation (Australia), and DAAD and DFG (Germany).

REFERENCES

1. Williams KH, et al. Lower serum fibroblast activation protein shows promise in the exclusion of clinically significant liver fibrosis due to non-alcoholic fatty liver disease in diabetes and obesity. *Diabetes Res Clin Pract.* 2015;**108**:466-72.
2. Wong PF, Gall MG, Bachovchin WW, McCaughan GW, Keane FM, Gorrell MD. Neuropeptide Y is a physiological substrate of fibroblast activation protein: Enzyme kinetics in blood plasma and expression of Y2R and Y5R in human liver cirrhosis and hepatocellular carcinoma. *Peptides*. 2016;**75**:80-95.
3. Majumdar A, et al. A sequential circulating fibroblast activation protein-based model is superior to Hepascore alone in excluding significant fibrosis in patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol.* 2018;**33**(S2):56.
4. Lay AJ, Zhang HE, McCaughan GW, Gorrell MD. Fibroblast activation protein in liver fibrosis. *Frontiers In Bioscience, Landmark*, 24, 1-17, 2019. 2019;**24**:1-17.
5. Zhang HE, Hamson EJ, et al. Identification of novel natural substrates of fibroblast activation protein - alpha by differential degradomics and proteomics. *Molecular and Cellular Proteomics*. 2019;**18**:65-85.

CONTACT INFORMATION

Professor Mark Gorrell, Australia. m.gorrell@centenary.usyd.edu.au