Mfap4: a promising target for enhanced liver regeneration and chronic liver disease treatment

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INTRODUCTION

Against the background of rising incidence of liver failure, there is a pertinent medical need to suppress disease progression and reverse end-stage liver disease. In spite of the liver's regenerative capacity, hepatocyte death and injuries are still imminent in chronic damaging conditions[1,2]. Existing literature showed that expression of major hepatic mitogens may promote liver restoration[3,4] and regeneration[5].

This study leveraged in vivo functional genetic RNA interference (RNAi)[6] screen and identified the role of Microfibril associated protein 4 (Mfap4) as a therapeutic target. This study aims to investigate the role of Mfap4 knockdown in accelerating liver regeneration and opening the potential for siRNA-based therapeutics in treating chronic liver disease.

METHODS

Screening for shRNAs

Cloned and excised shRNAs from the ROMA-amplicon library were injected by hydrodynamic tail vein into wild-type mice. Genomic DNA was extracted from mouse livers post-treatment and shRNA abundance was analyzed using sequencing.

Validation experiments

shRNAs with stable expression targeting Mfap4 were selected for further analysis.

Proliferation assays using wound healing, EdU incorporation and cell cycle assay were performed to assess hepatocyte growth upon Mfap4 knockdown.

In-vivo models

Liver regeneration was tested on FAH knockout mouse model with partial hepatectomy. TAA injections were introduced to induce chronic liver damage. Protein arrays and western blotting from mouse cells lines and immortalized human hepatocytes were used to investigate the signaling pathways affected by Mfap4 knockdown such as mTOR.

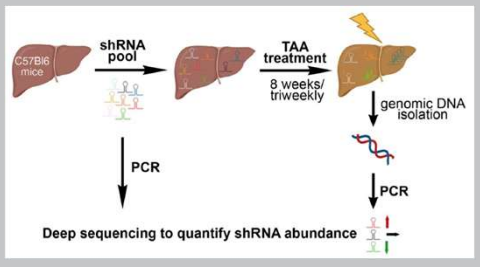
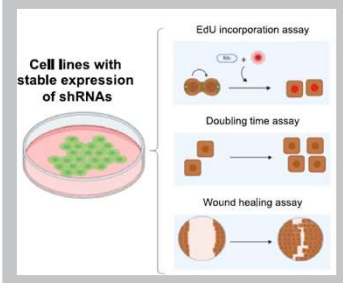


***Fig. 1**: Functional genetic in-vivo RNAi screen in mouse model to identify therapeutic targets that can enhance liver regeneration based on changes in shRNA abundance.*

RESULTS

Identification of Mfap4 as potential target for knockdown (see Fig 1): It was first found that within an in-vivo environment with TAA-induced liver damage, presence of shRNA targeting Mfap4 conferred hepatocytes a competitive advantage with increased abundance seen on deep sequencing.



Validation of result in various models was then pursued sequentially.

***Fig. 2**: Methods utilised for **in-vitro** validation of Mfap4 knockdown in accelerating hepatocyte proliferation. Mouse and human hepatocytes with shRNA targeting Mfap4 performed better universally across all these tests.*

Subsequent **in-vivo** validation in **mouse model** found the effect of Mfap4 knockdown to be both regenerative (Fig. 3) and protective (Fig. 4)

***Fig. 3** : PH = Partial hepatectomy, a liver damage model. Cells with Mfap4 knockdown had higher %Ki-67-positive correlating with rate of regeneration.*

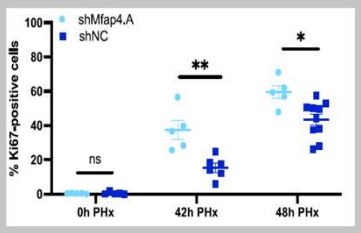
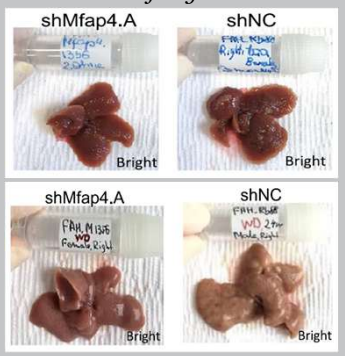


***Fig. 4**: Gross images of mouse liver showing protective effect. Notably, this effect was conserved in mice on WD ("Western Diet"), a contributor to non-alcoholic fatty liver disease.*



Finally, transferability of results to a **human model** was demonstrated (see Fig 2). Mfap4 was also found to be a potential biomarker of liver disease.

DISCUSSION AND CONCLUSION

Mfap4 is shown to be a promising target for enhancing liver regeneration. The knockdown of Mfap4 in vivo is shown to accelerate hepatocyte proliferation and reduce fibrosis. Importantly, these results carry over between mouse and human hepatocytes. Targeting Mfap4 also has potential beyond the liver, such as reducing renal fibrosis[7] and cardiac remodelling[8]. Results from these studies and our own suggest that targeting Mfap4 might be a viable therapy for many other different fibrotic diseases.

Liver regeneration in Mfap4 knockdown neither induces hepatomegaly nor promotes oncogenesis which is a legitimate concern for any therapy which stimulates cellular regeneration. This may be due to a hypothesized regenerative 'brake' which controls the proliferation.

Mfap4 knockdown plays a crucial role in promoting liver regeneration and slows the progression of chronic liver disease. Hence, clinical translation of the findings would be highly beneficial to patients living with the condition.

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