DEVELOPMENT OF OLIGONUCLEOTIDE-BASED MIR-132 ANTAGONISTS FOR THE TREATMENT OF NASH

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1 INTRODUCTION

MicroRNAs are small regulatory RNAs that play critical roles in animals and plants by regulating target gene expression at the post-transcription level[1,2]. Many miRNAs are dysregulated in disease states including cancers[3] and NASH[4], which indicates potential therapeutic targets for oligonucleotide-based drug development[5]. Previous studies have shown that miR-132 is upregulated in the livers of NASH patients and is involved in the development of NASH in rodent models[6]. This study sought to validate miR-132 as a potential therapeutic target and to develop antagonists of miR-132 for the treatment of NASH.

2 AIMS

1. Validate the dysregulation of miR-132 in the livers of NASH patients and various mouse models
2. Identify cell types that miR-132 is expressed and upregulated
3. Design, synthesize and optimize oligonucleotide lead antagonizing miR-132
4. Generate proof of efficacy for lead compound in multiple mouse models of NASH
5. Explore the mechanism of miR-132 involved in NASH

3 METHODS

1. qRT-PCR was used to quantify miR-132 levels in primary cells and liver tissues from NASH patients
2. Multiple diet-induced mouse models of NASH including diet-induced obesity (DIO), choline deficient high fat diet (CDHFD) and AMYLIN models were used for efficacy studies
3. Oligonucleotide antagonists of miR-132 were administered through subcutaneous (s.c.) injection once per week for four to eight weeks
4. The severity of NASH was assessed with biomarkers including liver triglyceride, serum liver enzymes (ALT and AST) and miRNAs (miR-122 and miR-132), liver histopathology and glucose tolerance
5. RNA-seq analysis for liver tissues was applied to study the underlying mechanism of the lead compound

4 RESULTS

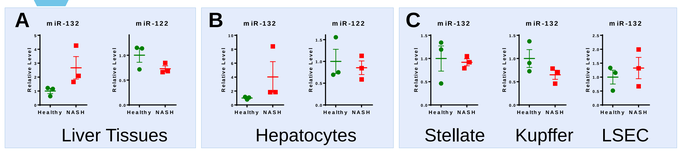


Fig. 1 miR-132 is up-regulated in Hepatocytes but not other liver cell types in NASH patients. qRT-PCR of miR-132 and miR-122 levels in (A) liver tissues, (B) isolated primary hepatocytes and (C) stellate cells, Kupffer cells and LSECs.

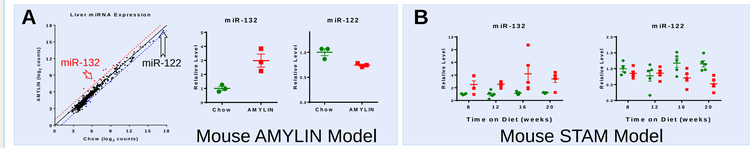


Fig. 2 miR-132 is up-regulated in the liver in multiple NASH mouse models. (A) miRNA-profiling by Nanostring (left, red doted line indicates two-fold increase) and qRT-PCR of miR-132 and miR-122 in AMYLIN model, (B) qRT-PCR of miR-132 and miR-122 in STAM model.

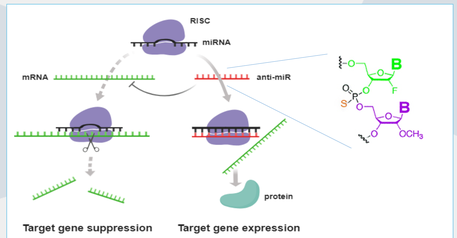


Fig. 3 Synthetic oligonucleotides antagonizing miRNA. Left, a cartoon depiction of how oligonucleotide miRNA antagonists work in regulating gene expression; right, chemical design of oligonucleotides including modification of the sugar ring (2'-**fluoro** and 2'-**O-methyl**) and the phosphodiester backbone (**phosphorothioate**).

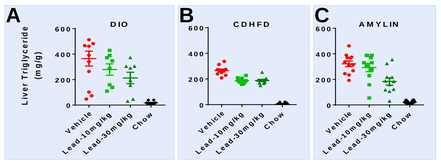


Fig. 4 miR-132 antagonizing lead compound reduced liver triglyceride levels in multiple mouse models of NASH. (A) Diet-induced obesity, (B) Choline deficient high fat diet, and (C) AMYLIN diet.

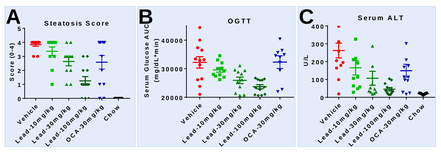


Fig. 5 Lead compound improved various parameters in AMYLIN NASH model. (A) Steatosis histopathology score, (B) oral glucose tolerance test (OGTT), and (C) serum level of liver enzyme ALT (AST levels exhibited similar trend, data not shown). OCA was used as reference for comparison purpose.

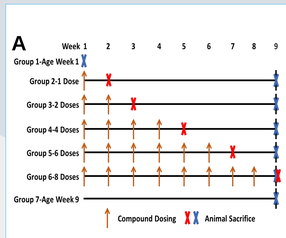
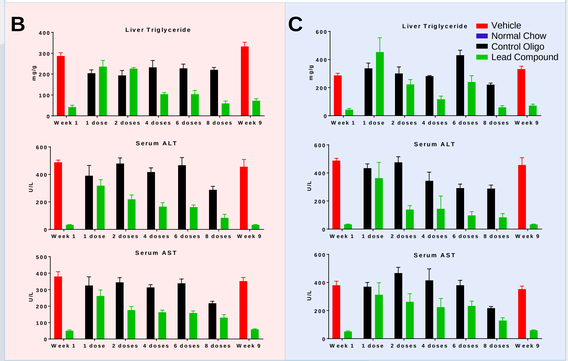


Fig. 6 The effects of the lead compound were fast and long-lasting in AMYLIN NASH model. (A) The design of a time course study of the lead compound in AMYLIN NASH model, (B) cohort 1, the effects were observed after 2-4 weekly s.c. dosing, and (C) cohort 2, the effects lasted for up to 7 weeks after the last dosing. Liver triglyceride and serum liver enzymes (ALT and AST) were used as efficacy readout.



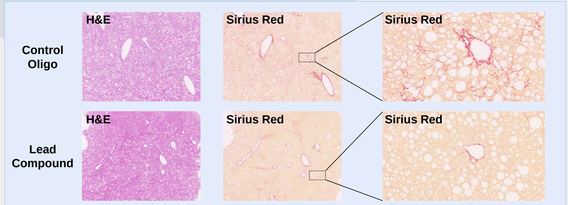


Fig. 7 Lead compound improved histopathology of the liver in AMYLIN NASH model. Liver tissue slides were stained with H&E (for steatosis) and Sirius Red (for fibrosis).

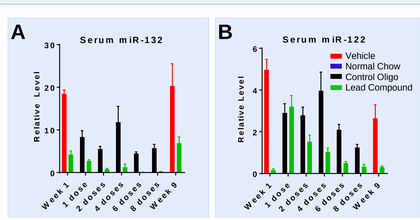


Fig. 8 Serum miRNAs are potential biomarkers for the effects of the lead compound. (A) Liver specific miR-122 and (B) disease-related miR-132.

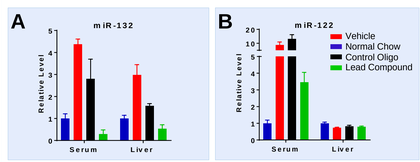


Fig. 9 Lead compound selectively down-regulated miR-132 in the livers in AMYLIN NASH model. (A) miR-132 levels in the serum and liver and (B) miR-122 levels in the serum and liver.

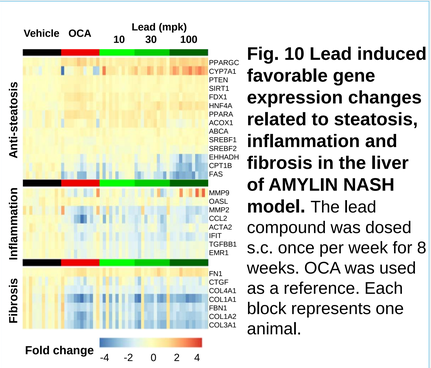


Fig. 10 Lead induced favorable gene expression changes related to steatosis, inflammation and fibrosis in the liver of AMYLIN NASH model. The lead compound was dosed s.c. once per week for 8 weeks. OCA was used as a reference. Each block represents one animal.

5 CONCLUSIONS

miR-132 is up-regulated in the hepatocytes from NASH patients and in the livers of multiple diet-induced NASH mouse models and is involved in the pathogenesis of the disease, presenting a promising therapeutic target for the treatment of NASH. Oligonucleotide-based antagonists of miR-132 exhibited excellent efficacies and pharmacological properties in multiple diet-induced mouse models of NASH, which warrants their further development. Lead optimization is currently ongoing to generate a drug candidate for first-in-human clinical studies.

6 REFERENCES

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