Development of GalNAc-conjugated saRNA targeting HNF4A for treatment of metabolic disease

Jon Voutila[1], Karin Purdie[1], Alexandre Debacker[1], Vikash Reebye[1,2], Kai-Wen Huang[3], Matthew Catley[1], David Blakey[1], Robert Habib[1], Pal Saetrom[4], John Rossi[5], Nagy Habib[1,2]

[1]MiNA Therapeutics Limited, [2]Imperial College London, [3]National Taiwan University Hospital, [4]Norwegian University of Science and Technology, [5]Beckman Research Institute of City of Hope

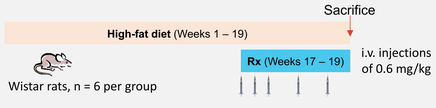
ABSTRACT

Hepatocyte nuclear factor 4 alpha (HNF4A) is a liver-enriched transcription factor and master regulator of hepatocyte function. HNF4A expression is downregulated in chronic liver disease, and the expression of HNF4A correlates with the level of liver dysfunction. We have developed a small activating RNAs (saRNAs) that upregulate the rodent HNF4A gene in primary hepatocytes in vitro and in vivo. In a rat high-fat diet model, treatment with HNF4A saRNA complexed with a PAMAM dendrimer significantly upregulated HNF4A mRNA expression in the liver. Intravenous injection of dendrimer-HNF4A saRNA significantly reduced liver cholesterol, glucose, and body weight, and increased the ratio of HDL to LDL in high-fat diet rats. Histological images showed reduced fat deposition in treated animals, and markers of inflammation such as IL-1β were significantly reduced.

For subcutaneous administration of HNF4A saRNA to treat chronic metabolic diseases such as non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH), we have developed GalNAc-conjugated HNF4A saRNAs for hepatocyte-specific delivery. Fully 2'OMe/2'F modified saRNA oligonucleotides activate HNF4A expression by transfection in primary rat hepatocytes in a dose-dependent manner. When conjugated to a triantennary GalNAc cluster, GalNAc-HNF4A saRNAs show dose-dependent upregulation of HNF4A by ASGR receptor-mediated uptake in primary rat hepatocytes.

When injected subcutaneously in normal mice, GalNAc-HNF4A saRNA conjugates significantly upregulate HNF4A expression in the liver. Downstream markers of lipid metabolism were significantly upregulated and expression of inflammatory IL1B mRNA was significantly reduced. Optimization of dose and schedule is ongoing, as well as efficacy in disease models. These data demonstrate that GalNAc-mediated delivery of saRNA is possible to upregulate hepatocyte-specific targets, and HNF4A is a promising target for treatment of NAFLD/NASH.

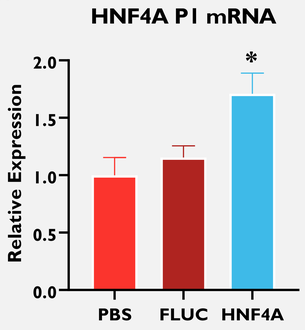
HIGH-FAT DIET STUDY DESIGN



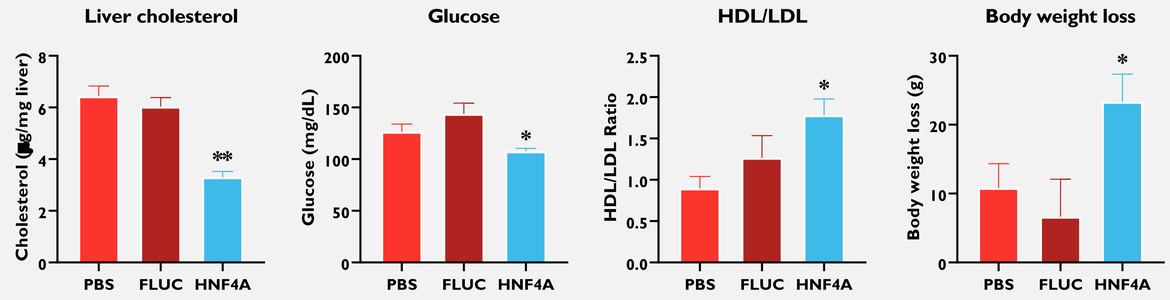
Wistar rats were fed a high-fat diet for 16 weeks to induce metabolic disease. At the beginning of the 17th week, animals were randomized into three groups of 6 and injected via tail vein with PBS, negative control FLUC siRNA, or HNF4A saRNA at 0.6 mg/kg. Oligonucleotides were complexed with a 5-(G_5)-triethanolamine-core PAMAM dendrimer. Animals were treated for three weeks and then sacrificed at the end of week 19. Treatment schedule was three injections in the first week followed by a single injection in the second and third week of treatment.

RESULTS

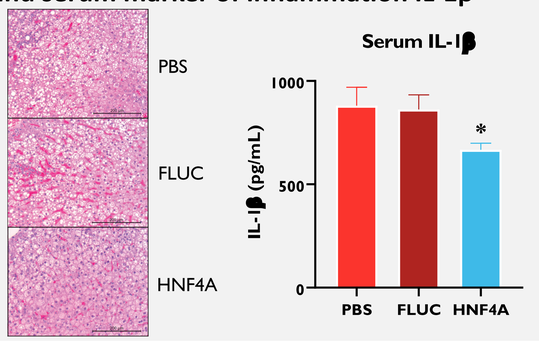
1. High-fat diet rats treated with HNF4A saRNA show significantly increased liver HNF4A mRNA expression



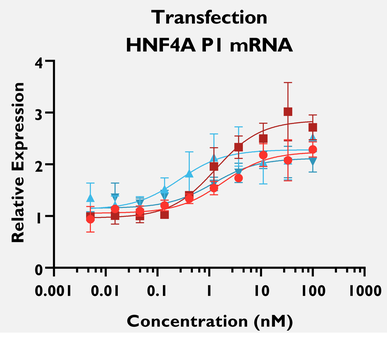
2. High-fat diet rats treated with HNF4A saRNA have significant decreases in liver cholesterol and blood glucose, significant increase in the ratio of HDL to LDL, and lost weight during treatment



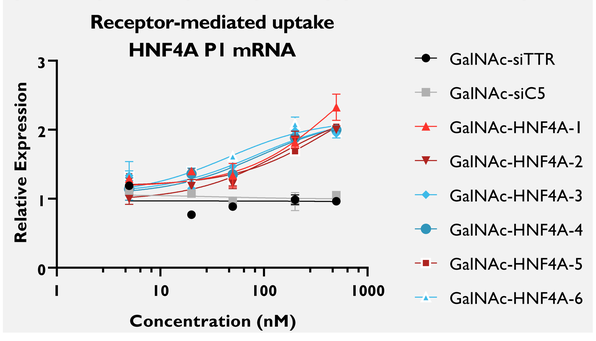
3. High-fat diet rats treated with HNF4A saRNA have reduced fat deposition in the liver and serum marker of inflammation IL-1β



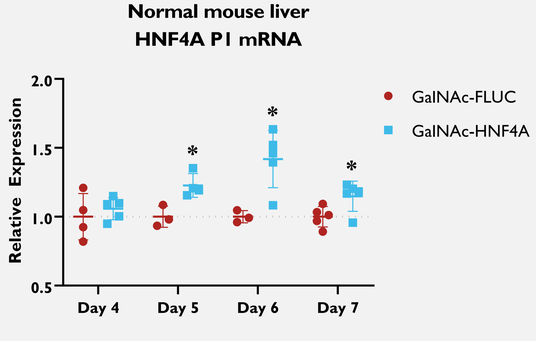
4. Fully 2'OMe/2'F modified HNF4A saRNAs show a dose-response increase in HNF4A mRNA after transfection in rat hepatocytes



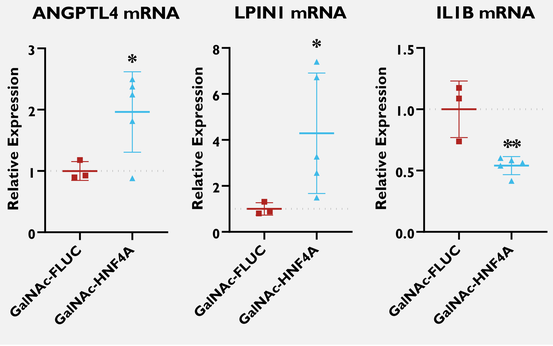
5. GalNAc-conjugated HNF4A saRNAs show a dose-response increase in HNF4A mRNA in primary rat hepatocytes by passive delivery



6. GalNAc-HNF4A saRNA increases liver HNF4A mRNA expression after subcutaneous injection in normal mice



7. GalNAc-HNF4A saRNA increases downstream HNF4A targets of lipid metabolism and decreases inflammatory IL1B mRNA expression in the liver of normal mice



SUMMARY

- saRNA-mediated upregulation of HNF4A mRNA improves the metabolic profile of high-fat diet rats, including reduction of lipids in the liver and inflammatory cytokines in circulation
- Fully 2'OMe/2'F modified saRNAs retain activity, and when conjugated to a GalNAc cluster upregulate HNF4A mRNA in primary rat hepatocytes by ASGR receptor-mediated uptake
- GalNAc-HNF4A conjugates upregulate HNF4A mRNA in the liver in normal mice after subcutaneous administration, and downstream markers of lipid metabolism and inflammation are also regulated

REFERENCE

Huang KW & Reebye V, et al., Liver activation of Hepatocellular Nuclear Factor-4α by small activating RNA rescues dyslipidemia and improves metabolic profile. Molecular Therapy – Nucleic Acids (in press).