**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 actuating 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. Intra-venuous 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 diseases such as non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NASH), we have developed GalNAc-conjugated HNF4A saRNAs for hepatocyte-specific delivery. Fully 2′OMe/2′F modified saRNAs oligonucleotides activate HNF4A expression by transfection in primary rat hepatocytes in a dose-dependent manner. When conjugated to a trisaccharide 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 upregulated 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 target hepatocyte-specific targets, and HNF4A is a promising target for treatment of NASH/Diabetes.

**RESULTS**

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

![Graph showing increased HNF4A mRNA expression](image)

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

![Graph showing decreased liver cholesterol and glucose](image)

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

![Graph showing reduced fat deposition and IL-1β](image)

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

![Graph showing dose-response increase in HNF4A mRNA](image)

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

![Graph showing dose-response increase in HNF4A mRNA](image)

**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**