Durable Upregulation of P1-isoform Hepatocyte Nuclear Factor alpha (HNF4A) Using Novel Epigenomic Controllers

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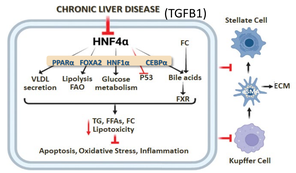
Background

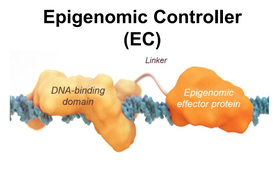
Hepatocyte nuclear factor 4 alpha (HNF4A) is a nuclear receptor and master regulator of liver development and function. The HNF4A gene is driven by two promoters with isoforms derived from the P1 promoter demonstrating enhanced transcriptional activity associated with therapeutic benefit. HNF4α expression is dysregulated in fibrotic liver disease, with an HNF4A P1-P2 expression imbalance in favor of P2, potentially driven by TGFβ signaling. Omega Therapeutics has developed a modular platform for design of programmable mRNA medicines, termed epigenomic controllers (ECs), that precisely tune gene expression by site-specifically modulating epigenetic state (Figure 2). Using an EC to induce selective upregulation of the HNF4A-P1 isoform to correct the disease state dysregulation represents a novel therapeutic strategy for the treatment of fibrotic liver disease.



Modified from Pan X and Zhang Y, 2022

Figure 1. HNF4α role in liver disease. HNF4A regulates numerous genes, including a network of transcription factors that control cholesterol, lipid, and glucose metabolism and homeostasis. Dysregulation of this signaling leads to apoptosis, oxidative stress, inflammation and fibrosis.

Figure 2. The OMEGA platform. The OMEGA platform develops programmable mRNA medicines called Epigenomic Controllers (ECs) that precisely tune gene expression by site-specifically modulating epigenetic state and can be delivered using tissue-specific lipid nanoparticles (LNPs; Figure 2). We designed an HNF4A-EC to induce selective upregulation of the P1 isoform of HNF4A in a liver-specific manner. This represents a novel therapeutic strategy for the treatment of fibrotic liver disease.



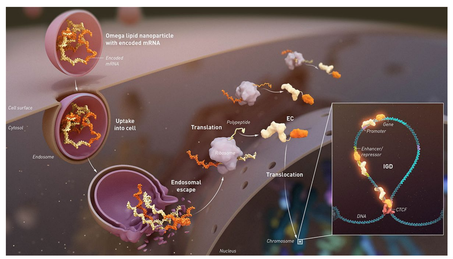


Figure 3. Structure, Mechanism of Action of ECs. Representative Insulated Genomic Domain (IGD; left) and schematic of Epigenomic Controller (EC; above) with DNA-binding domain and effector domain.

HNF4A-EC is designed to induce isoform specific increases of the HNF4A-P1 mRNA

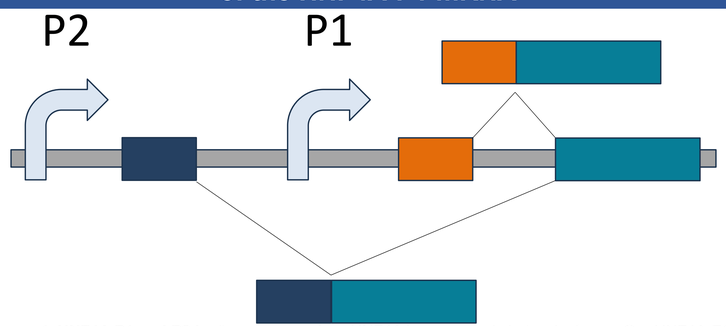


Figure 4. HNF4A P1 and P2 isoform expression. HNF4A is expressed via two isoforms. Our HNF4A-EC is designed to specifically and durably upregulate the P1 isoform of HNF4A(HNF4A-P1, orange) while not altering the expression of the P2 isoform of HNF4A (HNF4A-P2, navy) .

HNF4A-EC induced durable transcriptional changes in LX-2 cells

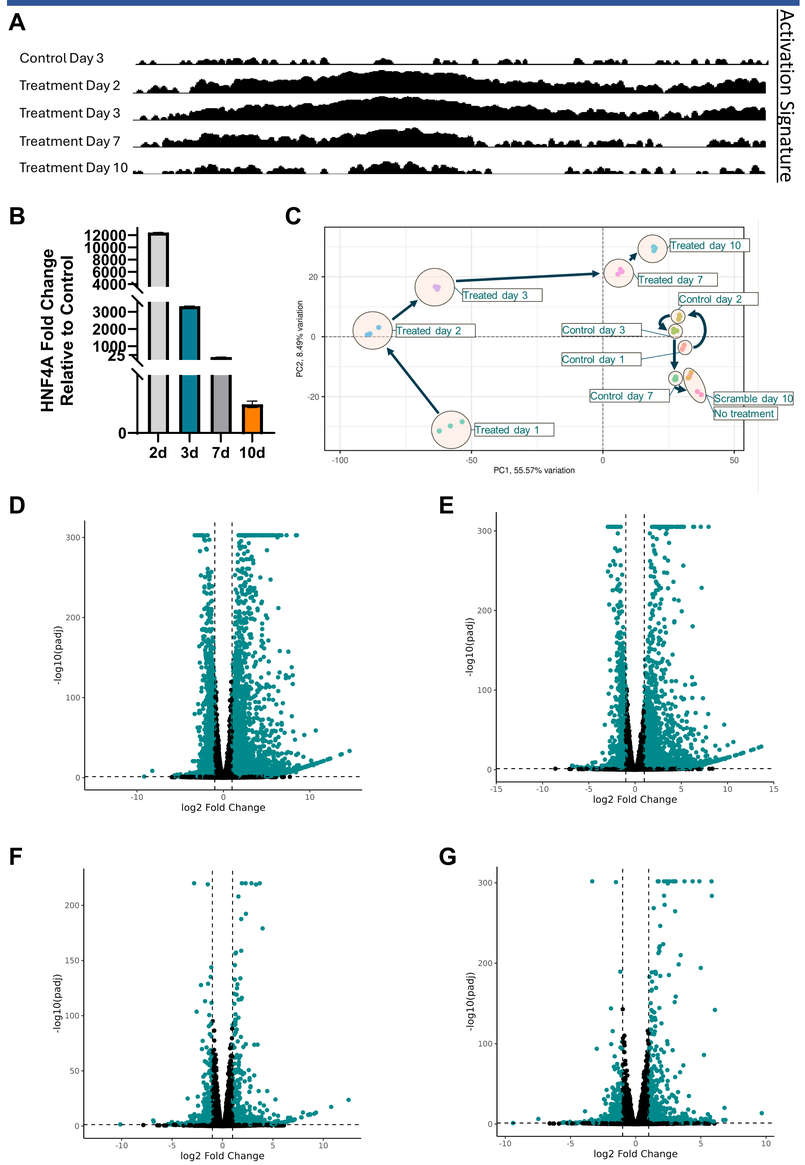


Figure 5. Analysis of HNF4A-EC in LX-2 cells. RNASeq and ChIPseq were performed in LX-2 cells treated with HNF4A-EC or controls for 2, 3, 7, or 10 days. **A.** On-target enrichment of epigenetic modification in response to HNF4A-EC treatment with detectable signal through day 10. Data are visualized on a uniform log-transformed scale. **B.** HNF4A expression is significantly increased (padj < 0.01) at 2 days (FC=12416), 3 days (FC=3326), 7days (FC=359) and 10 days (FC=25) post-transfection. **C.** PCA plot showing distinct clusters defined by transfection status and time projected onto the first 2 PCs, accounting for ~64% of the total observed variance. **D-G.** Volcano plots demonstrating changes in gene expression at 2, 3, 7, and 10 days respectively (teal dots: padj < 0.05, log2FC >= 1).

Durable HNF4α upregulation following a single dose of EC for 35 days in primary human hepatocytes

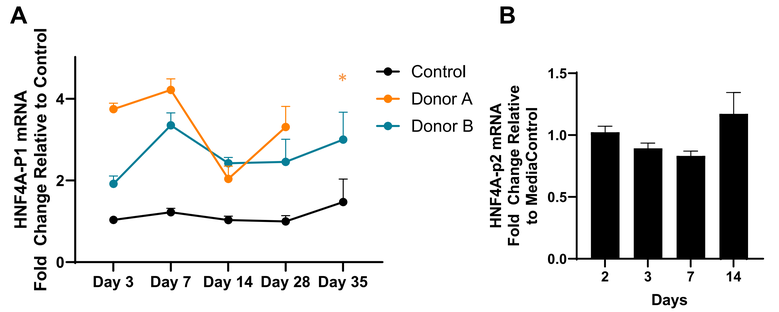


Figure 6. HNF4A-EC treatment increases HNF4A-P1 while leaving HNF4A-P2 expression unaltered for 35 days (**A**) HNF4A-EC treatment led to durable HNF4A-P1 mRNA upregulation up to 28 days* in Donor A (orange) and 35 days in donor B(teal). *Donor A cells fail to thrive at day 35 under all conditions, including healthy controls. **(B)** Cultured human hepatocytes treated with HNF4A-EC does not alter HNF4A-P2 mRNA expression.

HNF4A-EC treatment rescued TGFB1 induced HNF4A-P1 downregulation

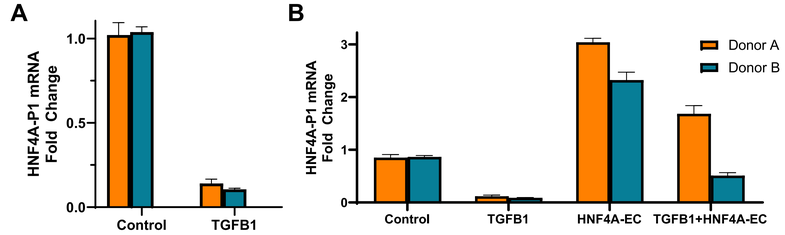


Figure 7. HNF4A-EC treatment restores HNF4A expression following TGFB1 treatment in primary human hepatocytes. (**A**) TGFB1 treatment of primary human hepatocytes for 72 hours reduced HNF4A-P1 expression in multiple donors. (**B**) Co-treatment of TGFB1 and HNF4A-EC for 72h was performed in cultured primary human hepatocytes revealing that HNF4A-EC can rescue HNF4A-P1 expression levels. Fold Change TGFB1+HNF4A-EC versus TGFB1: Donor A – 15, Donor B – 6.

Summary and Conclusions

We demonstrate the ability to durably increase the expression of HNF4A with EC treatment in a P1 isoform-specific manner. We showed that increasing HNF4A expression in LX-2 cells causes transcriptional changes. TGFB1 treatment induced a dysregulation of HNF4A-P1 expression and treatment with the HNF4A-EC was able to restore HNF4A-P1 expression levels. Future studies will test the efficacy of this treatment in liver disease models and further explore the therapeutic potential of epigenomic targeting and control of HNF4A in fibrotic liver disease.

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