Induction of Hepatocyte Nuclear Factor 4 alpha (HNF4α) P1-isoforms Using a Selective Epigenomic Controller

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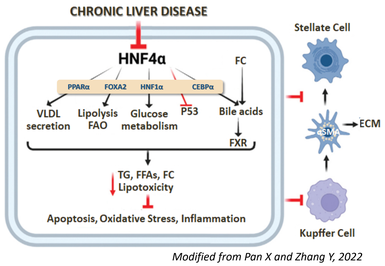
Background

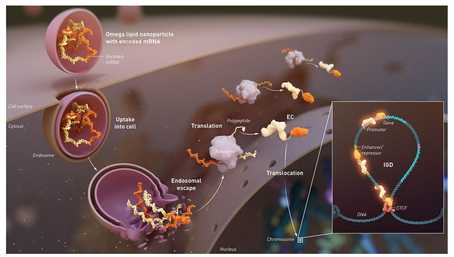
Hepatocyte nuclear factor 4 alpha (HNF4α) is a nuclear receptor and master regulator of liver development and function (Figure 1). HNF4α expression is dysregulated in fibrotic liver disease and upregulation of this key transcription factor has been shown to improve hepatocyte function. The HNF4α gene is driven by two promoters, with isoforms derived from the P1 promoter demonstrating enhanced transcriptional activity associated with therapeutic benefit.

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 HNF4α-EC to induce selective upregulation of the P1 isoform of HNF4α in a liver-specific manner. This 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. HNF4α 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.



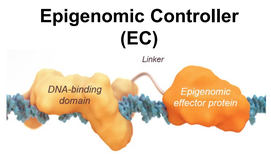


Figure 2. Structure, Mechanism of Action and Design of ECs. Representative Insulated Genomic Domain (IGD; left) and schematic of Epigenomic Controller (EC; above) with DNA-binding domain and effector domain.

ECs induce upregulation of HNF4a expression *in vitro*

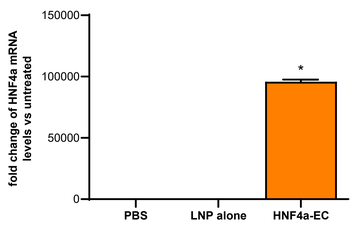


Figure 3. HNF4α mRNA expression levels following treatment with HNF4α-EC. Hepatic stellate LX-2 cells were transfected with HNF4α-EC and HNF4α mRNA levels were measured by qPCR 24 hours later. HNF4α mRNA was nearly undetectable in LX-2 cells treated with PBS or LNP alone but was substantially upregulated with HNF4α-EC treatment. *1-way ANOVA , p<0.001

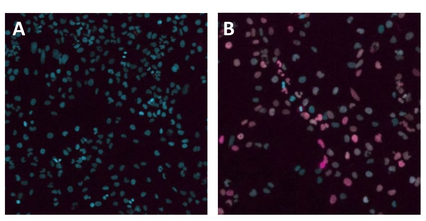


Figure 4. HNF4α protein expression in LX-2 cells following HNF4α-EC treatment. Representative fluorescence images of LX-2 cells treated with PBS (**A**) or HNF4α-EC (**B**) for 48 hours, followed by immunohistochemistry to detect HNF4α protein expression.

Durable HNF4a upregulation following single dose of EC

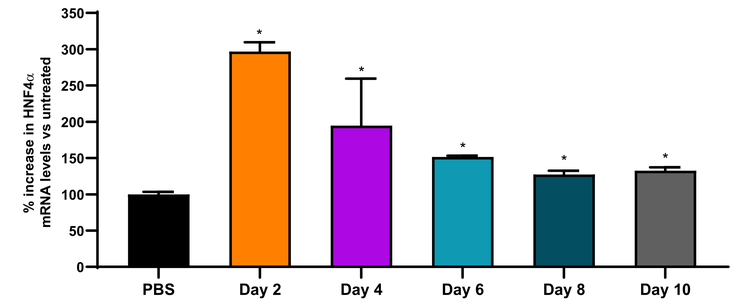


Figure 5. Time course of HNF4α mRNA expression levels following HNF4α-EC treatment. Primary human hepatocytes were transfected with HNF4α-EC. HNF4α mRNA levels were measured by qPCR at time points up to 10 days later. HNF4α expression remained above baseline for 8-10 days after transfection. Evidence suggests that inducing the HNF4α transcriptional network for as little as a week is sufficient to initiate a regenerative process, supporting the therapeutic potential for HNF4α-EC treatment. *1-way ANOVA, P<0.05

P1-targeting EC drives upregulation of P1 isoform

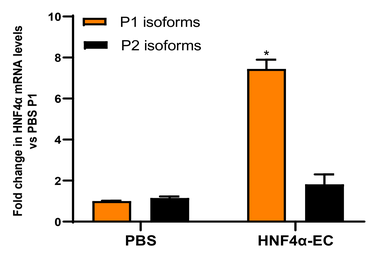


Figure 6. *In vitro* expression of P1 and P2 isoforms of HNF4α after HNF4α-EC treatment. Primary human hepatocytes were treated with single dose of LNP-encapsulated EC, and qPCR was performed 24 hours later to measure HNF4α expression. *T-test vs PBS , p<0.05

In vivo induction of HNF4α with EC treatment

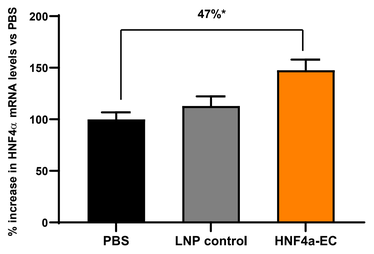
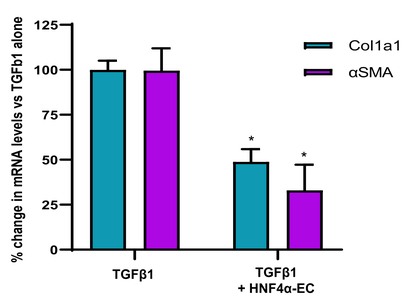


Figure 7. HNF4α expression in humanized liver following HNF4α-EC treatment *in vivo*. To measure *in vivo* induction of HNF4α, a single dose of LNP-encapsulated EC was administered intravenously to humanized Fah$^{-/-}$/Rag2$^{-/-}$/Il2rg$^{-/-}$ (FRG) mice and qPCR was performed 24 hours later to measure HNF4α expression. *T-test vs PBS, p<0.05

HNF4a upregulation alters fibrotic gene expression *in vitro*

Figure 8. TGFβ1-induced Col1a1 and αSMA gene expression is reduced with HNF4α EC treatment. Hepatic stellate LX-2 cells were treated with 10ng/ml TGFβ1, transfected with LNP-encapsulated HNF4α ECs, and changes in expression of pro-fibrotic markers were measured by qPCR at 24 hours. EC treatment reduced Col1a1 expression by 48% and αSMA expression by 70%, compared to TGFβ1-treatment alone. * T-test vs TGFβ1 , p<0.05



HNF4α induction confers protection in DDC fibrosis model

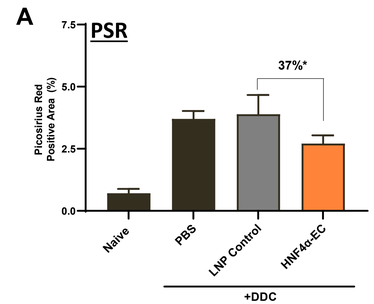
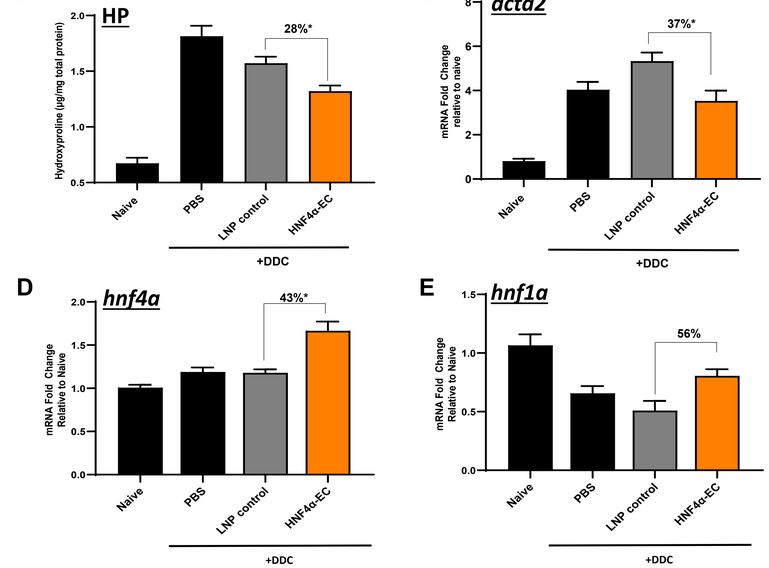


Figure 9. HNF4α-EC treatment upregulates HNF4α expression and reduces fibrosis in DDC mouse model. Mice fed the 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet develop liver injury with fibrosis. In our study, mice were placed on DDC diet and administered 1mpk murine HNF4α-EC Q5d for 42 days. (**A**) Picosirius red staining was performed on section of DDC mouse livers and staining was quantified to measure deposited collagen. (**B**) Hydroxyproline assay was conducted to measure collagen content of DDC mouse livers. The mRNA levels of αSMA (*acta2*) (**C**), HNF4α (**D**), and the HNF4α target gene, HNF1α (**E**) were measured in DDC mouse livers by qPCR. *1-way ANOVA, P<0.05



Summary and Conclusions

We demonstrate the ability of an Epigenomic Controller (EC) to durably increase HNF4α expression both *in vitro* and *in vivo*. In addition, this modulation preferentially induces the upregulation HNF4α P1 promoter isoforms, resulting in inhibition of fibrotic responses *in vitro*. Significant upregulation of HNF4α is observed following EC treatment in a humanized liver mouse model. We then tested a murine HNF4α-EC in the DDC fibrosis model and saw increased HNF4α mRNA levels, increased levels of a downstream target of HNF4α, and a decreased fibrotic signature, supporting the therapeutic potential of epigenetic targeting of HNF4α in fibrotic liver disease. (Omega thanks Acuitas for the preparation of LNPs utilized in the *in vivo* expression and efficacy studies.)

Contact Information

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