Targeting CXCL9/CXCL10/CXCL11 Using Novel Epigenomic Controllers for the Treatment of Inflammatory Liver Disease

Christopher E. Pedigo, Wanzhu Zhao, Yoseph Kassa, Justin Chen, Jeremiah Farelli, Amy McCurley, Joseph Newman, Charles O'Donnell, Thomas McCauley

Omega Therapeutics, Cambridge, MA, USA

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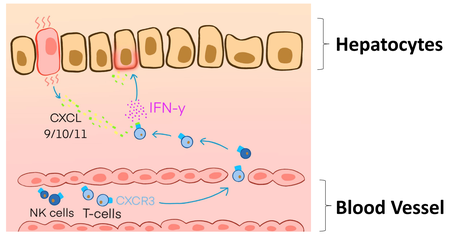
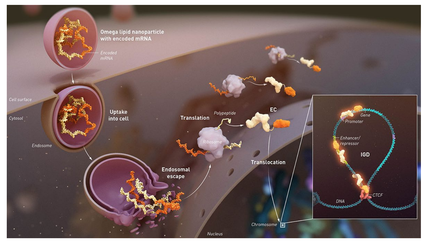
Background

Hepatitis is a broad pathophysiological feature common to acute and chronic liver diseases characterized by activation of inflammatory mechanisms, including increased interferon gamma (IFNG) expression. IFNG is a pleotropic proinflammatory cytokine released by immune cells that contributes to liver injury by stimulating the release of the chemokines CXCL9, CXCL10 and CXCL11 from hepatocytes (**Figure 1**). CXCL9, CXCL10 and CXCL11 bind to the CXCR3 receptor on T cells, promoting T cell recruitment to the site of inflammation. Through our OMEGA platform, we are able to rapidly develop programmable mRNA medicines called Epigenomic Controllers (ECs) to precisely tune gene expression at the pre-transcriptional level (**Figure 2**). ECs are mRNA therapeutics delivered in tissue-specific lipid nanoparticles (LNPs) that site-specifically modulate epigenetic state. Using ECs to simultaneously target all three *CXCL9, CXCl10 and CXCL11* genes in a liver-specific manner represents a novel and promising strategy for treatment of inflammatory liver disease.

Figure 1. Proposed mechanism of hepatitis. Graphical representation of IFNG stimulated hepatocytes releasing CXCL 9, CXCL10 and CXCL11 leading to recruitment of immune cells to the liver, a common process in inflammatory liver diseases.





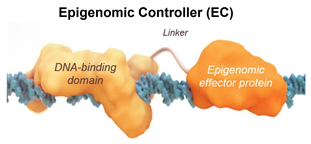


Figure 2. Structure, mechanism of action and design of ECs.

The multigenic Insulated Genomic Domain (IGD) and component sequences driving epigenetic control of the *CXCL9, CXCL10 and CXCL11* genes were interrogated and ECs were designed to modulate their expression simultaneously in a multiplexed fashion.

IFNG treatment increased CXCL9, CXCL10 and CXCL11 mRNA expression in hepatocyte cell lines

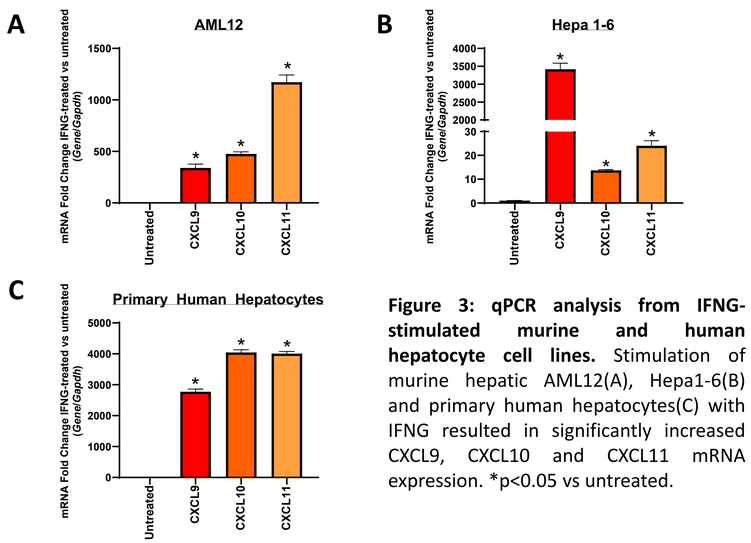


Figure 3: qPCR analysis from IFNG-stimulated murine and human hepatocyte cell lines. Stimulation of murine hepatic AML12(A), Hepa1-6(B) and primary human hepatocytes(C) with IFNG resulted in significantly increased CXCL9, CXCL10 and CXCL11 mRNA expression. *p<0.05 vs untreated.

Mouse ECs individually reduce CXCL9, CXCL10 and CXCL11 expression in mouse hepatocyte cell lines

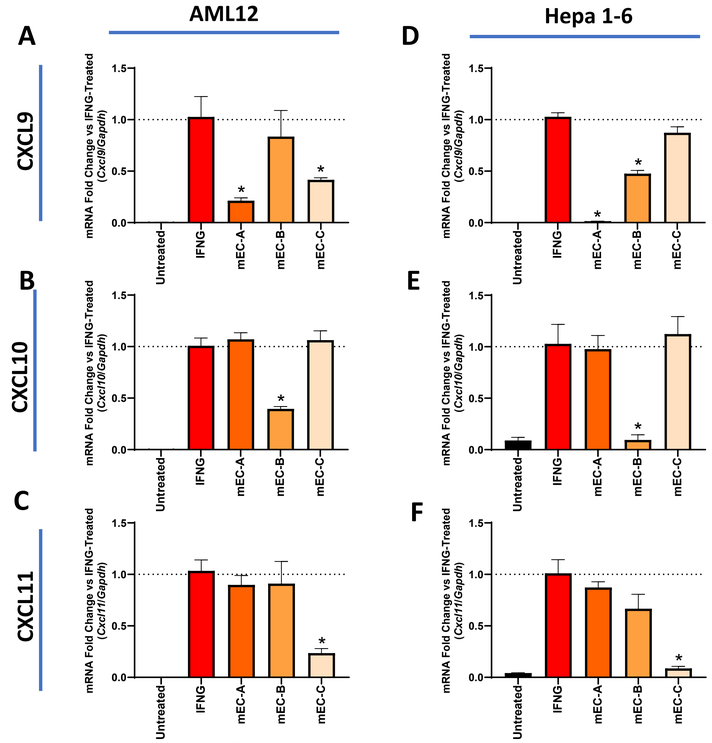


Figure 4: Development of mouse ECs targeting CXCL9, CXCL10 and CXCL11. Mouse ECs (mEC) reduced CXCL9, CXCL10 and CXCL11 mRNA expression respectively in AML12 (A,B,C) and Hepa 1-6(D,E,F) following IFNG stimulation. *p<0.05 vs IFNG.

Human ECs reduce CXCL9, CXCL10 and CXCL11 expression in primary human hepatocytes

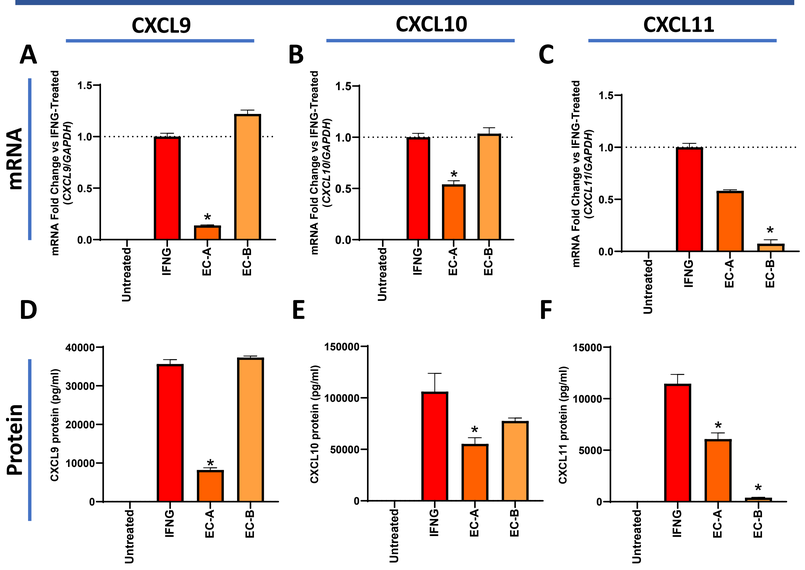


Figure 5: Development of human ECs targeting CXCL9, CXCL10 and CXCL11. Human ECs reduced CXCL9, CXCL10 and CXCL11 mRNA expression (A) and protein levels in cell culture supernatants (B) following IFNG stimulation. *p<0.05.

Single EC product reduces CXCL9, CXCL10 and CXCL11 in IFNG-stimulated primary human hepatocytes

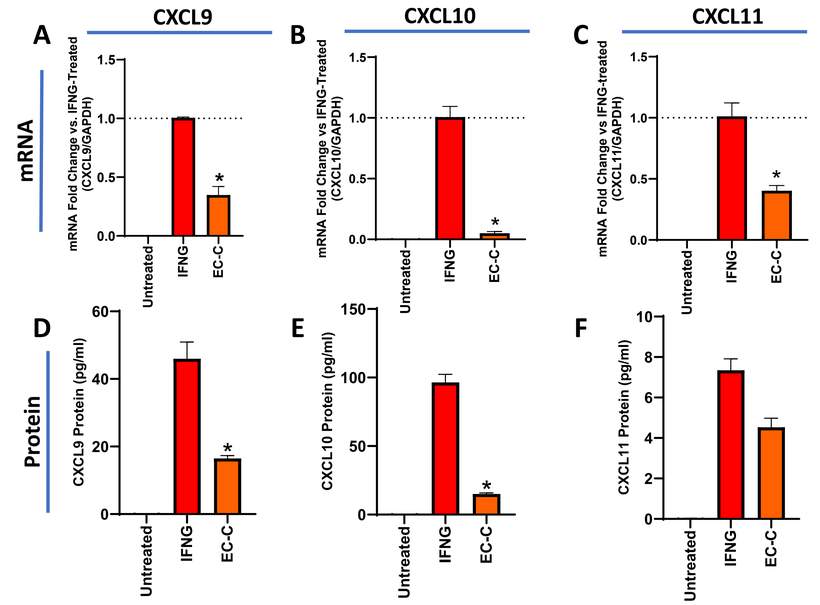


Figure 6: Development of a single EC product that simultaneously targets CXCL9, CXCL10 and CXCL11. Human EC product reduced CXCL9, CXCL10 and CXCL11 mRNA expression (A) and protein levels in cell culture supernatants (B) of IFNG-stimulated primary human hepatocytes. *p<0.05.

Novel ex vivo assay demonstrates reduced T-cell migration due to EC-treated human hepatocyte media

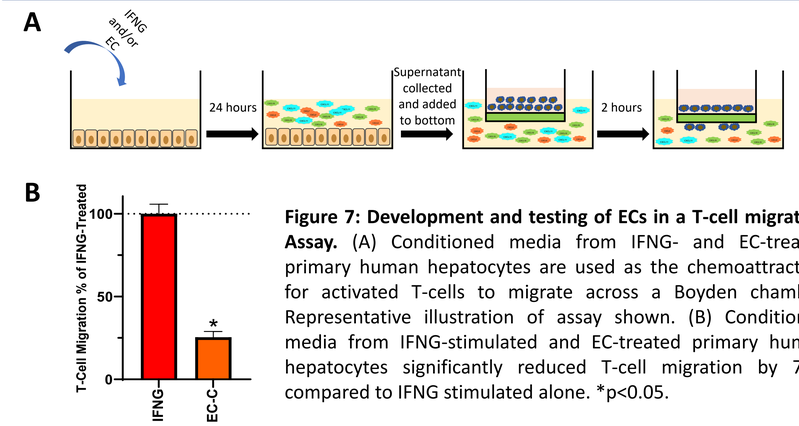


Figure 7: Development and testing of ECs in a T-cell migration Assay. (A) Conditioned media from IFNG- and EC-treated primary human hepatocytes are used as the chemoattractant for activated T-cells to migrate across a Boyden chamber. Representative illustration of assay shown. (B) Conditioned media from IFNG-stimulated and EC-treated primary human hepatocytes significantly reduced T-cell migration by 75% compared to IFNG stimulated alone. *p<0.05.

Summary

In summary, we demonstrate development of a rationally designed and engineered epigenomic controller (EC) which potently reduces *CXCL9, CXCL10* and *CXCL11* expression in hepatocytes leading to a significant reduction in T-cell migration. Mechanistically, our data demonstrate that we can reduce CXCL9, CXCL10 and CXCL11 expression following IFNG stimulation in multiple mouse liver cell lines and in primary human hepatocytes, showing our platform approach can target mouse and human IGDs. Further, we developed a single EC that was able to potently downregulate all three chemokines (CXCL9, CXCL10 and CXCL11) in the IGD simultaneously. Robust, multiplexed downregulation of this IGD with an EC significantly reduced T-cell migration, a critical driver of inflammation-induced liver injury. Overall, our data support that liver-specific multiplexed targeting of *CXCL9, CXCL10* and *CXCL11* via a programmable epigenomic mRNA therapy may offer a novel approach for treatment of inflammatory liver diseases.