Synthetic EGF Receptor Drives Expansion of HPSC Derived Hepatocyte-Like Cells

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Abstract

The liver can regenerate after injury. However, this unique capacity fails in severe or chronic disease where the damaged organ becomes hostile to transplanted cells. To address this challenge, we aim to engineer hPSC-derived hepatocytes so they can better withstand this hostile environment and regenerate damaged liver tissue. Our approach combines stem cell biology with synthetic receptor engineering to create hepatocytes with externally controllable proliferation. First, we tested that hPSC-derived hepatocytes respond to epidermal growth factor (EGF). We then **engineered a synthetic inducible EGF receptor (iEGFR) that activates hepatocyte proliferation** in response to the small-molecule AP20187. This creates a tunable "on/off" system, where proliferation is triggered by drug treatment and halted upon withdrawal. This work addresses a critical need: liver failure is a leading cause of death in the United States, and donor organ shortages leave many patients without treatment. Engineered hPSC-derived hepatocytes could provide a scalable regenerative therapy for these patients, bridging the gap of limited supply of donor organs.

Introduction

hPSC to HLC Differentiation Timeline and Signaling Roadmap (Ang et al, 2018)

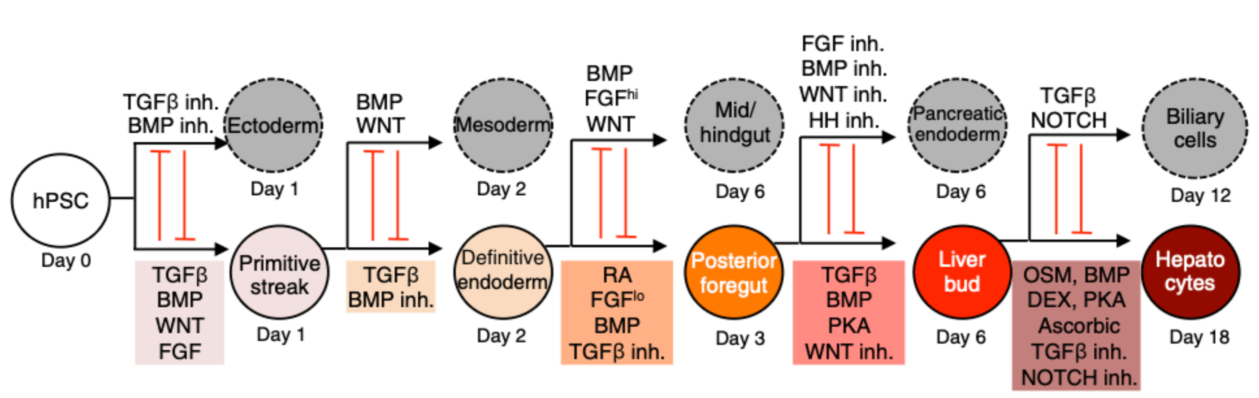


Figure 1: Hepatocyte differentiation from hPSCs requires dynamic modulation of signals

AP20187 induces dimerization of engineered receptors with FKBP domain, mimicking EGFR activation

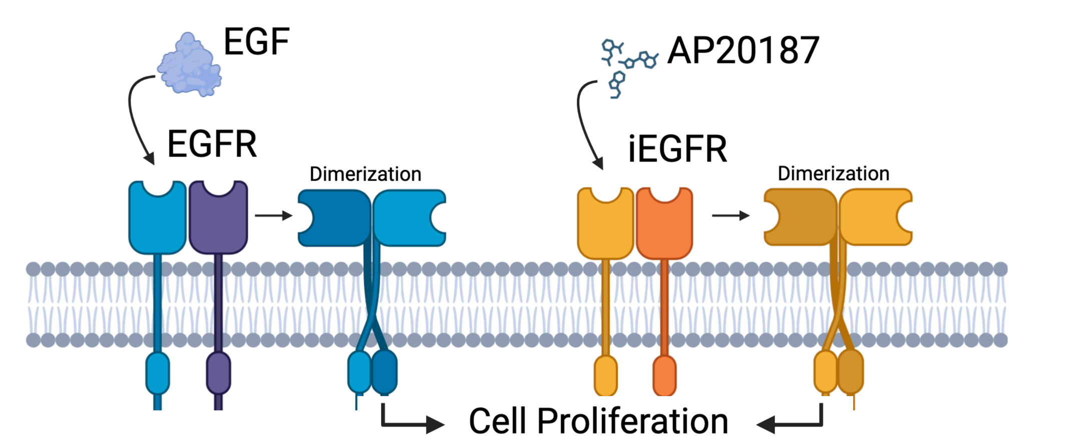
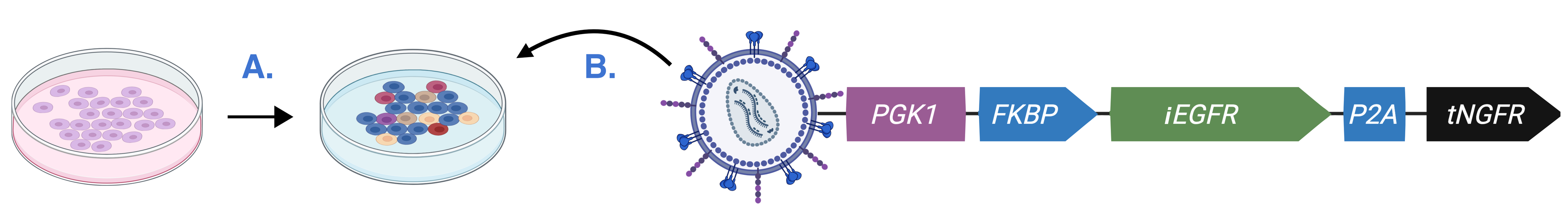
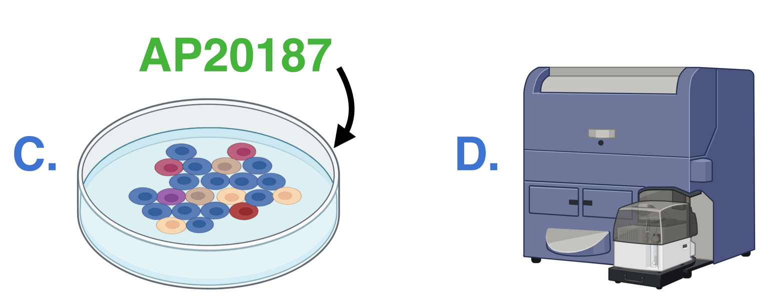


Figure 2: iEGFR is engineered with an extracellular FKBP domain that dimerizes in the presence of AP20187, mimicking downstream effects of native EGFR activation

Methods





A. hPSCs are differentiated into HLCs
B. HLCs are transduced with iEGFR lentivirus with a tNGFR tag
C. Transduced HLCs are treated with AP20187 for 5 days
D. Flow cytometry, qPCR, and cell counts conducted on D18

Results

hPSC-derived hepatocytes express EGFR, IL6R and HGFR (MET) as shown by single cell RNA-seq

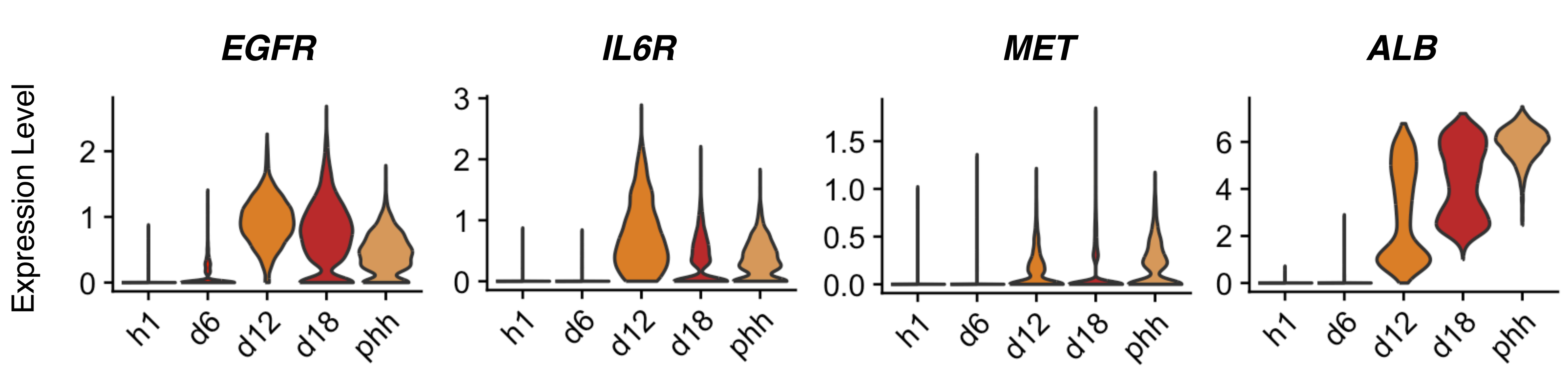


Figure 3: RNA-seq data comparing embryonic stem cells (H1), primary human hepatocytes (PHH), and hPSC derived HLCs at three different time points, D6, D12, and D18. Results show comparable expression of MET, IL6R, EGFR, and ALB for D18 hPSC derived HLCs and PHH.

Results Continued

Epidermal Growth Factor increases HLC Proliferation and Confluency after Passaging

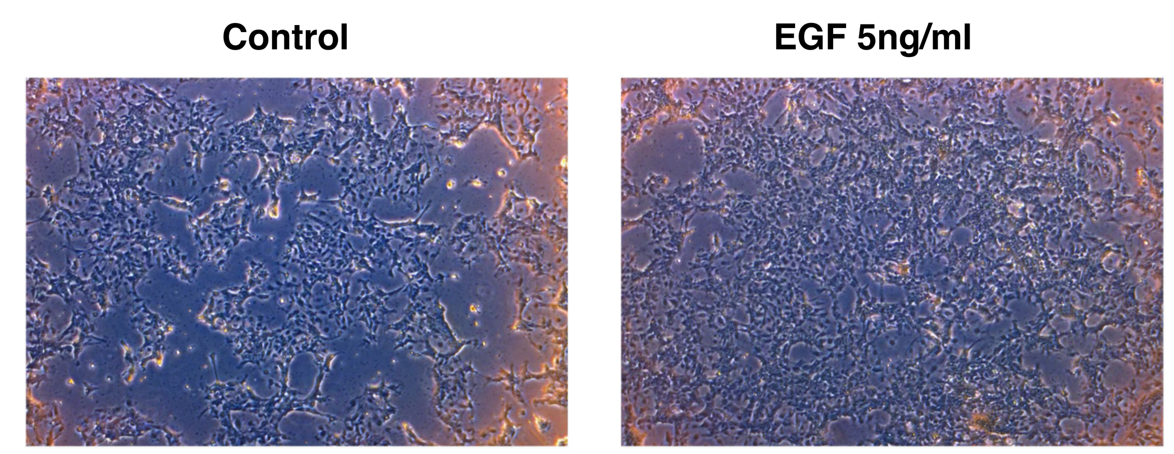


Figure 4: Passaged hepatocytes show increased survival and proliferation when treated with EGF

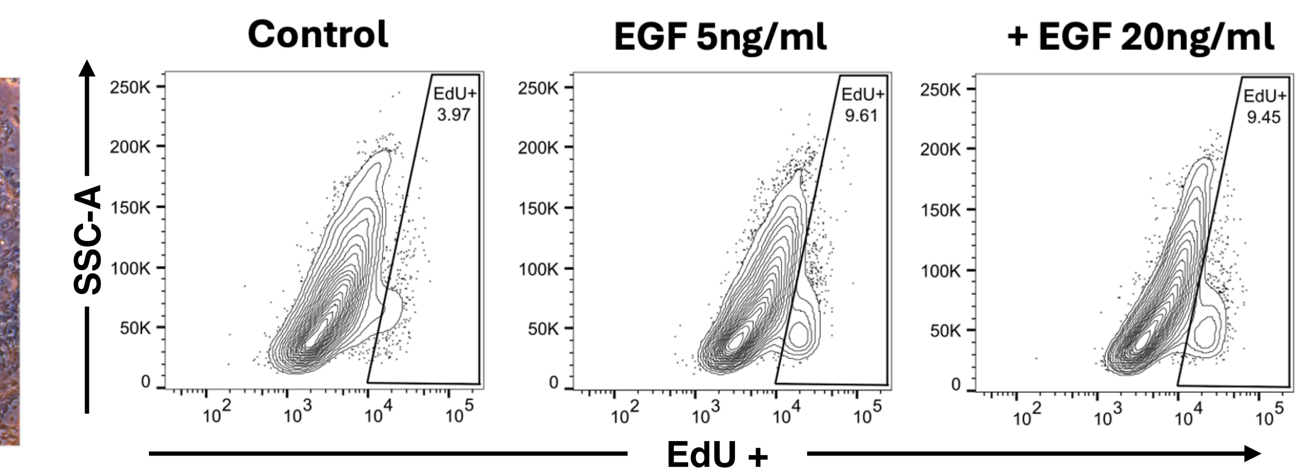


Figure 5: Flow cytometry analysis shows higher EdU incorporation in EGF treated cells, with the effect plateauing at 20 ng/ml

AP20187 increased NGFR+ population in 1% iEGFR HLCs in a dose dependent manner

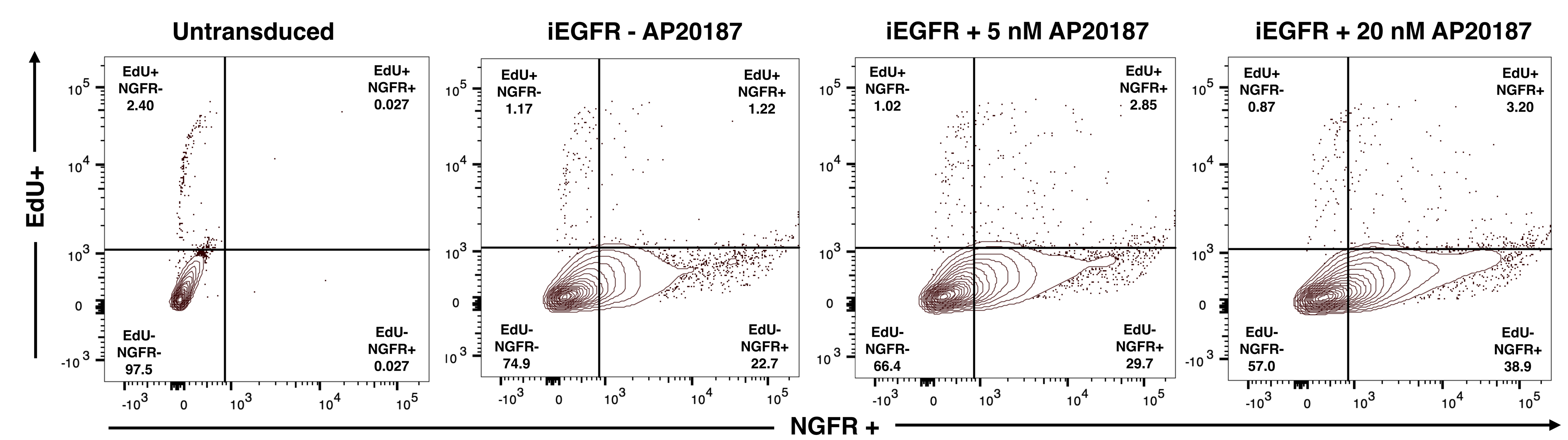


Figure 6: Flow cytometry analysis shows a dose dependent increase in NGFR+ hepatocyte population when treated with varying AP20187 concentrations, suggesting a competitive growth advantage for transduced cells in the presence of homodimerizer.

Confocal image of hepatocytes stained for NGFR, EdU, and DAPI

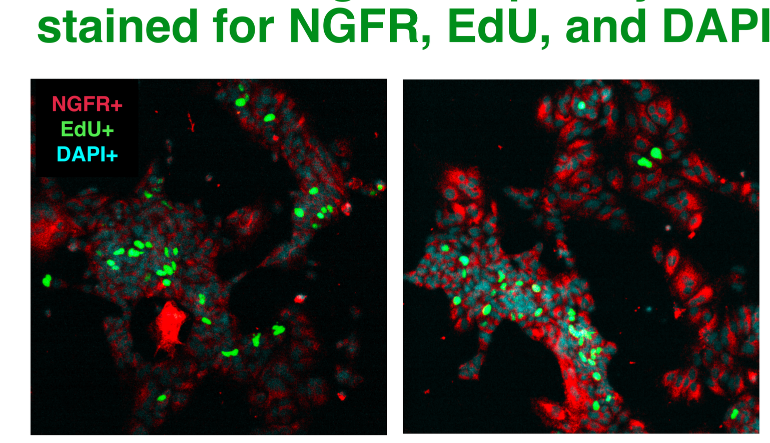


Figure 7: Passaged hepatocytes stained for NGFR, EdU, and DAPI. Left: 0.5% iEGFR transduced without AP2017 treatment. Right: 0.5% iEGFR with 1 nM AP20187 treatment for 5 days.

Discussion and Future Directions

In this project, we demonstrated that EGF treatment increases HLC proliferation and confluency after passaging. In our engineered iEGFR HLCs, treatment with AP20187 increased the transduced population in a dose dependent manner, suggesting that iEGFR confers a competitive growth advantage. We propose that employing small molecule + engineered receptor combos provide a feasible, potent, and cost effective strategy for hepatocyte expansion. Work is also underway to develop analogous inducible receptors for HGF and IL-6 signaling pathways. In future studies, we will transplant engineered hepatocytes into murine models of liver injury to test whether controlled activation of regenerative signaling improves cell survival, expansion, and liver repair.

References

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