SPATIAL PATTERNING OF LIVER PROGENITOR CELL DIFFERENTIATION MEDIATED BY CELL CONTRACTILITY AND NOTCH SIGNALING

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Liver progenitor cell differentiation and bile duct formation are driven by spatially-dependent and temporally-sequenced cell–cell and cell–factor interactions coordinated by several biochemical signaling pathways, namely Notch and TGFβ. The regionalization of biliary differentiation and morphogenesis near the portal region of the liver has suggested that spatially segregated microenvironmental signals govern this process. Our recent work utilizing biomaterial substrates of defined stiffness suggests that mechanical cues play a previously-unrecognized role in liver progenitor differentiation. Here, we used a cell microarray platform that enables the simultaneous analysis of these biochemical and biomechanical microenvironmental cues to define the mechanisms of action and functional overlap of these pathways.

We used bipotential mouse embryonic liver (BMEL) progenitor cells cultured by standard techniques (M. Weiss and H. Strick-Marchand, Institut Pasteur). To present Notch ligand to cells, we printed Fc-chimeric recombinant DLL4 on a polyacrylamide hydrogel substrate together with collagen I and Protein A/G. We integrated this cell microarray platform with traction force microscopy (TFM) by adding fiducial beads to the polyacrylamide hydrogel and imaging bead displacement before and after cell dissociation.

In order to determine the effect of activating Notch signaling on liver progenitor differentiation, we presented Fc-DLL4 to cells in arrays, inducing biliary differentiation restricted to the edges of patterns as measured by expression of OPN (Figure 1A). Addition of an inhibitor of Notch signaling prevented peripheral biliary differentiation (Figure 1A). Immunofluorescence analysis of expression of the biliary transcription factor SOX9 showed restriction to the island periphery while expression of the hepatocyte transcription factor HFN4A was central (Figure 1B). Further, we observed that SOX9 expression increased on stiff substrates while that of HNF4A increased on soft substrates (Figure 1B), which implicated biomechanical stimulation as the gradient-forming cue. Finite element modeling (FEM) simulations suggested a radial gradient of mechanical stresses in the circular patterns, which we confirmed experimentally using TFM (Figure 1C). We next sought to modulate cell contractility and measure the resulting change in fate trajectory. Peripheral biliary differentiation intensified by viral transduction with constitutively-active $RhoA^{L63}$ (MOI=200), an inducer of cell contractility (Figures 1D and 1E). Conversely, treatment with blebbistatin (25 μM), which inhibits phosphorylation of myosin II, abrogated peripheral differentiation (Figures 1D and 1E).Liver progenitors require both Notch signaling and sufficient cell contractility in order to differentiate into bile duct cells. Ongoing work is focused on characterizing Notch family signaling using *in situ* hybridization as well as examining the mechanistic links between Notch and mechanotransduction pathways.

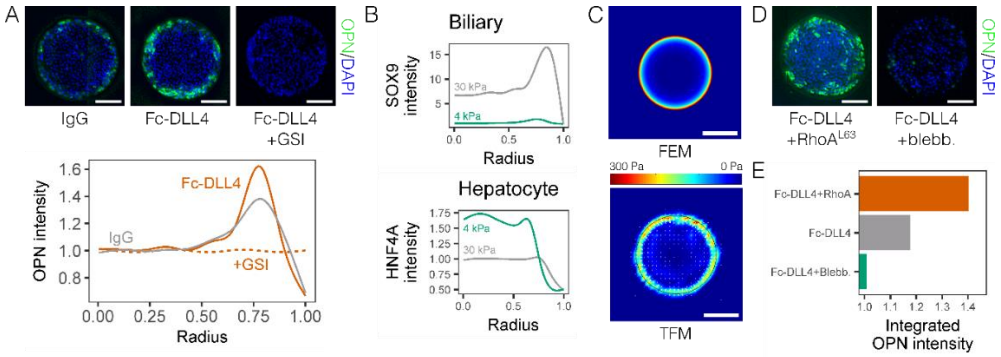

Figure 1: Biliary differentiation of liver progenitors requires both Notch signaling and cell contractility. (A) Liver progenitors presented with ligand (Fc-DLL4) express OPN peripherally while those treated with Notch inhibitor (γ-secretase inhibitor X, GSI) do not. n=6 islands per condition. (B) Expression of the hepatocyte transcription factor HNF4A is central while that of the biliary transcription factor SOX9 is peripheral. Polyacrylamide substrate stiffness was either 4 kPa or 30 kPa. n=6 islands per condition. (C) Both FEM simulations and experimental TFM of liver progenitor islands show high cell-generated stresses at the periphery. (D) Induction ($RhoA^{L63}$) and inhibition (blebbistatin) of contractility show that it is both necessary and sufficient for biliary differentiation. (E) Means of data from (D) with n=6 islands per condition. Scale bars are 200 μm