Hepatodifferentiation Of Cadherin Expressing Embryonic Stem Cells Within A Microengineered Organotypic Model

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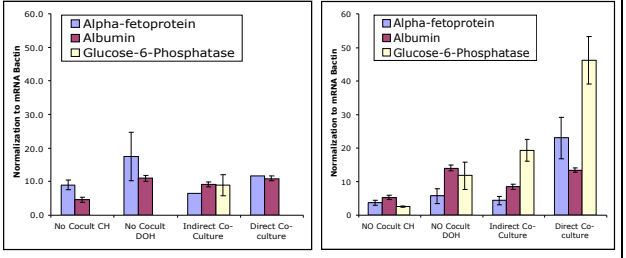
Statement of Purpose: Since optimally primed ES cells may potentially be further differentiated when transplanted into the liver, the overall goals of this study are to derive insights into the ability of cadherin-engineered ES cells[1] to maintain and further mature hepatospecific differentiation under co-culture treatment with mature hepatocytes. The underlying hypothesis is that adult hepatocytes can further augment the maturation of primed cadherin-expressing (CE-ES) cells via juxtacrine (local contact-driven) signaling as well as basal levels of paracrine (non-contact driven) signaling. We will test this hypothesis in co-cultures of rat adult hepatocytes and CE-ES cells (or cadherin-deficient: CD-ES control cells) using contact-permissive co-cultures and contact-inhibitory co-cultures. Based on the outcomes of this aim, we will establish the *base-line responsiveness* of CE-ES cells to contact-mediated and non-contact mediated stimulation via adult hepatocytes.

Specific Aim: To investigate the hepatodifferentiation behavior of cadherin expressing ES cells within a microengineered organotypic model comprising co-cultures of ES cells and adult hepatocytes.

Methods: Elastomeric stencils are dip-dried in acetone, then ethanol, allowed to air dry, and placed on an adsorbed collagen (0.13 mg/mL) surface and incubated for 24 hours to enhance contact adhesion between the stencil and surface. Rat adult hepatocytes are seeded ($6.25x10^4$ cells/cm^2) with in each well. Subsequently, the cell suspension is removed and cells are reseeded to ameliorate uniform cell attachment. Cells are gently rocked every 15 minutes for another 1 hour and then allowed to adhere and spread overnight. The next day, unattached cells are removed and media is placed in the stencil. Forceps are used to gently remove the stencil while it immersed in media. Once the hepatocytes are patterned, ES cells are seeded ($9.25x10^4$ cells/cm^2) in hepatocyte growth medium (C+H medium) and allowed to attach/grow. Subsequent cell morphology and production of liver specific protein (albumin) was assessed.

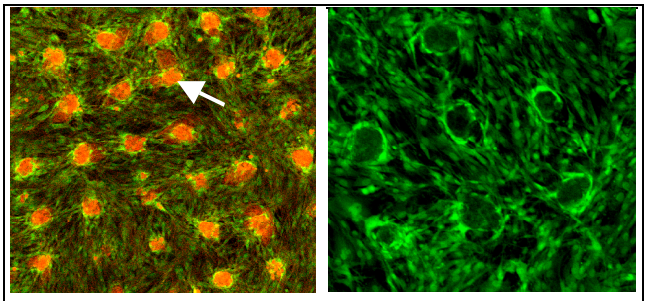
Results / Discussion: Our major hypothesis is that mature hepatocytes can induce hepatospecific maturation of primed ES cells via two primary mechanisms related to cadherin signaling: juxtacrine signaling (initiated through cadherin-cadherin contacts between CE-ES and hepatocytes) and paracrine signaling (distally initiated by hepatocytes; mediated by cadherin-growth factor signaling pathways). In our earlier coculture studies, we showed that both of these mechanisms were important for the differentiation of hepatocytes. As shown in Figure 1, the presence of the late hepatic marker, glucose-6-phosphatase (G6P), was elevated under direct co-culture treatments compared to no co-cultures in CE-ES cells.



A Cadherin Deficient ES Cells **B** Cadherin Expressing Cells

Figure 1: Effect of Direct Co-culture on the Hepatic Maturation of ES cells. The presence of direct cell-cell contact augmented the presence of the late hepatic marker, G6P in the cadherin expressing (CE-ES) cells

Contacting cocultures that have coupled juxtacrine-paracrine signaling have been successfully established with adult rat hepatocytes. We have successfully and reproducibly micropatterned hepatocytes within the micro-islands utilizing the microfabricated stencils. Microengineered configurations will allow us to quantify the expression of ES hepatospecific maturation markers via immunolocalization and DNA microarray studies. We seek to elucidate (in conjunction with results from non-contacting cocultures) the role of direct cadherin-mediated contact in directing hepatic differentiation in ES-cells. A significant increase in liver-specific albumin was observed within the CE-ES cells at the heterotypic junctions with adult hepatocytes.



A) Micropatterned Co-culture B) Albumin in ES cells

Figure 2: Micropatterned Hepatocytes (orange) and ES cells. Prominent albumin staining (green) was observed at heterotypic (hepatocyte: ES-cell) interface (see arrow).

Conclusions: The presence of direct cell-cell contact augmented the presence of the late hepatic marker, glucose-6-phosphatase in cadherin-expressing cells. Also, there was enhanced albumin-based differentiation at the direct stem cell/hepatocyte interface, indicating that murine ES cells derived differentiating cues from micro-scale islands of mature liver cells. In the future, these cadherin based interactions will be promoted via optimized microscale coculture configurations.

References: [1]Dasgupta A., Hughey R., Lancin, P., Larue L., and Moghe P.V. E-cadherin synergistically enhances liver-specific phenotype and maturation of ES cells. *Biotechnol. Bioeng*. 92: 257, 2005