Diabetic Complications Consortium

Application Title: Modeling the Renal Interstitium for Nephron Regeneration

Principal Investigator: Benjamin D. Humphreys MD, PhD

1. Project Accomplishments:

During this funding period, we successfully characterized the pro-angiogenic potential of Gli1+ MSC using an in vivo matrigel plug assay. We also performed co-culture studies that were limited by differential growth rates of the separate cell lines employed.

2. Specific Aims:

Specific Aim 1: To define the pro-angiogenic and vascular stabilizing properties of Gli1+ MSC. We have developed an *in vitro* tube formation assay using coculture of endothelial cells and Gli1+ MSC, and will measure the effects of MSC on vascular basement membrane matrix deposition, morphology, lumen diameter and endothelial-specific integrin upregulation.

Results: We made good progress on the coculture studies within this aim (Figure 1). We determined the endothelial growth media supported culture of pericytes and primary endothelial cells. A limiting factor was survival of endothelial cells, however, which generally was not longer

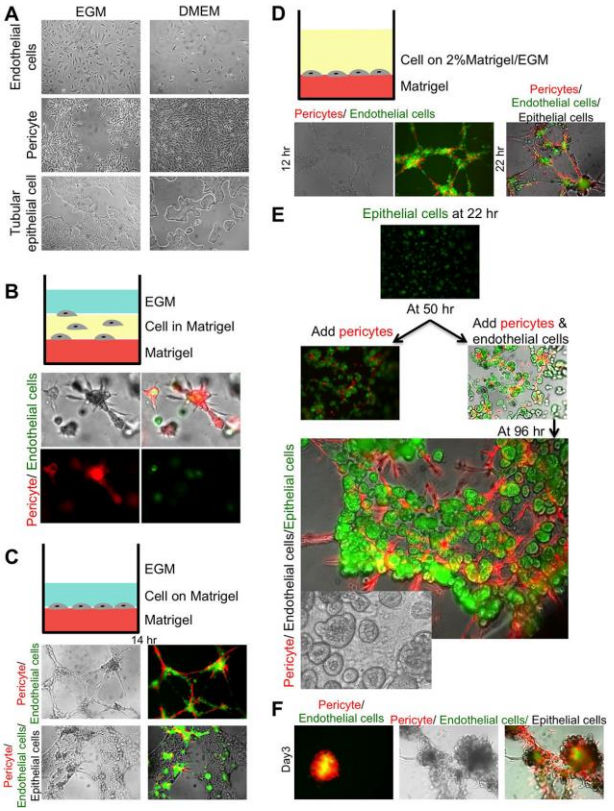

Fig1. Experiment result in three cell co-culture experiments. A. Endothelial growth media (EGM) can be used to culture three kind of cells without cell death, picture shown at day3 after new media. Endothelial cell line is human umbilical vein endothelial cells (HUVECs). Pericyte is Gli1+ heart pericyte, Tubular epithelial cell is Madin-Darby canine kidney (MDCK). **B.** Culturing pericytes and endothelial cells in 3D environment using Matrigel result in tube formation. **C.** Culturing pericytes and endothelial cells on Matrigel also result in tube formation. When culture with epithelial cells, vascular tube interspersed epithelial cell sheet. **D.** Culturing pericytes, endothelial cells and epithelial cells on Matrigel with 2%Matrigel supplement to EGM result in epithelial cells folding and integration with vascular tube. **E.** Sequential plating allow epithelial cells to form cyst induced by 2%Matrigel. After adding pericytes and endothelial cells, cellular tubes are formed connecting epithelial cell cysts together. At 96 hr, epithelial cells cyst are spread amongst pericyte and endothelial cell network. **F.** After 3 days of three cells coculture, without sequential plating, cells are pulled together and form cellular clump.s

than 72 hours. We stained endothelial cells with green dye to distinguish them from red pericytes, and we witnessed a propensity for the Gli1+ pericytes to make close contacts with endothelial cells (Figure 1B, C).

Specific Aim 2: To investigate the ability of novel co-culture of Gli1+ MSC, vascular endothelial cells and epithelial cells to recapitulate the spatial architecture and cell relationships within the kidney interstium. We will establish this model, and use it to define the potential of Gli1+ MSC to differentiate into fibroblasts, pericytes and vascular smooth muscle cells using genetic lineage analysis and high-resolution imaging.

Results: We coculture of endothelial, pericyte and epithelial cells (Figure 1 E,F). This was largely successful, but limited by differential growth rates of immortalized (Gli1+ pericytes, MDCK) and primary (HUVEC) cells. We established an in vivo assay, the matrigel plug assay,

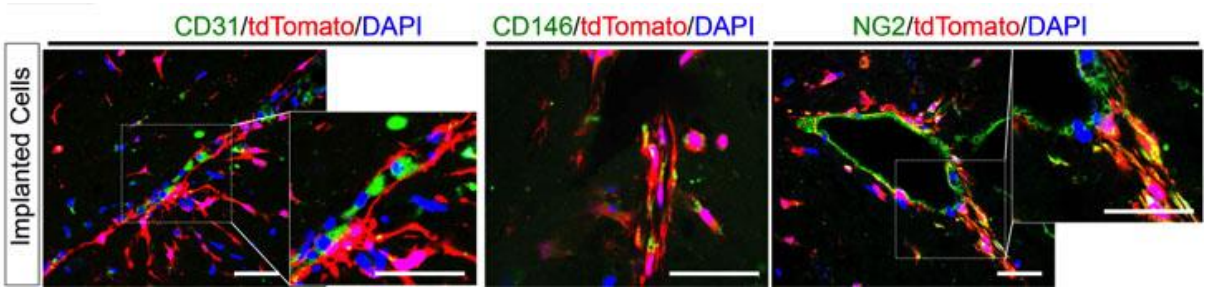

Figure 2. Gli1+ cells mediate angiogenesis in vivo. Matrigel plugs with Gli1$^+$ cells, 4 weeks after implantation in the *in vivo* angiogenesis model. Co-staining for the endothelial cell marker CD31 indicates sprouting endothelial cells with Gli1$^+$ cells associated in a pericyte-like distribution around CD31$^+$ endothelial cells. NG2 and CD146 co-staining indicates that Gli1$^+$ cells acquire expression of these mature pericyte markers during angiogenesis. Scale bars 50µm

to assess the angiogenic properties of Gli1 MSC. What we find is that Gli1+ cells localaize adjacent to CD31+ endothelium, and acquire markers of differentiated pericytes (Fig. 2).

3. Publications:

R. Kramann, R.K. Schneider, D.P. DiRocco, F. Machado, S.V. Fleig, P.A. Bondzie, J.M. Henderson, B.L. Ebert and B.D. Humphreys. Perivascular Gli1+ progenitors are key contributors to injury-induced organ fibrosis. *Cell Stem Cell*, 16(1):51-66, 2015.

- This article, published in *Cell Stem Cell* (Impact factor 26), ranks in the 95th percentile for quality and quantity of online attention compared to all articles ever published in Cell Stem Cell (altmetric). Funding from the DCCT was acknowledged in the published manuscript.

E. Ó hAinmhire and B.D. Humphreys. A plumbing solution for stem cell-derived kidneys. Transplantation. *In press*.