Epithelial-Mesenchymal Interactions in Multipotent Mesenchymal Stem Cells Isolated From Adult Mouse Kidney

Matthew Plotkin*

New York Medical College, Valhalla, NY

Using a method developed for isolation of adult mesenchymal stem cells from muscle and bone marrow, a cell line (4E) was isolated from adult FVB/NJ mouse kidney with similar characteristics including expression of Sca-1, CD34, and CD44 and the ability to differentiate into multiple mesodermal cell types. To determine if these cells maintained similarity with interstitial mesenchymal or stromal cells identified during kidney development, changes in 4E cell gene expression by semi-quantitative RT-PCR and western blots in response to growth factors and cytokines shown to be important in renal epithelial-mesenchymal interactions and cell phenotype expression following cell co-culture and transplantation were studied.

In co-culture with human umbilical vein endothelial cells or in subcutaneous Matrigel implants, an assay for angiogenesis, 4E cells formed PDGFβ receptor expressing pericytes. In co-culture with RUB1 cells, a ureteric bud cell line, 4E cells formed a tubular network with increased expression of tenascin, MECA32, Flk-1, and CD31, characteristic of vasculogenesis. By western blot analysis and RT-PCR, rFGF2 increased levels of endothelial markers and VEGF expression while TGFβ resulted in increased smooth muscle differentiation in cell culture.

4E cells expressed bHLH transcription factors shown to be important in epithelial-mesenchymal interactions during development, including POD-1 and SCL/Tal-1. FGF2 and Sonic Hedgehog increased SCL/Tal-1 expression, while TGFβ and TNFα increased and FGF2 decreased POD-1 levels. Reduction of POD-1 expression using siRNA resulted in decreased cell proliferation by BRDU ELISA in response to FGF2 and increased p21 expression together with increased expression of smooth muscle or endothelial differentiation markers in response to inducing cytokines. By immunohistochemistry, POD-1 was found to be located in pericytes and peritubular interstitial cells in the adult mouse kidney and was increased 4-fold in the inner cortex and outer medulla 7 days following ischemia/reperfusion, a renal injury associated with increased TNFα production by tubular epithelial cells.

Subcapsular injection of 4E cells following unilateral ischemia-reperfusion in adult mice resulted in migration of cells to a peritubular interstitial location within the inner cortex at 7 days following injury while cells injected in the contralateral control kidney remained underneath the capsule. Co-culture of 4E cells with MDCK cells on type 1 collagen resulted in formation of PNA lectin positive tubular structures, while MDCK cells alone maintained a monolayer appearance.

In summary, multipotent mesenchymal cells isolated from adult mouse kidney express transcription factors and receptors important for epithelial-mesenchymal interactions during development that may also have a role following injury in the adult kidney. Based on *in vitro* and *in vivo* evidence, these cells are likely to be involved in angiogenesis and support of epithelial repair following kidney injury.

*** Recipient of NIDDK Travel Award**