

Cystinosis Research Foundation Progress Report
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A. Objective and Specific Aims

We have recently shown that cell fusion of transplanted hematopoietic cells with kidney proximal tubular cells in vivo leads to their functional reprogramming which results in the loss of hematopoietic and activation of renal proximal tubular gene expression¹. Studies from our lab suggest that bone marrow-derived macrophages (BMM) could be the hematopoietic cells that fuse with renal proximal tubular cells. These findings highlight an opportunity for using readily available BMM for the correction of genetically encoded tubulopathies including cystinosis. The objective of this proposal is to investigate whether BMM can be efficiently used to deliver a healthy genome, including the *CTNS* gene and its regulatory sequences to renal proximal tubular cells affected by cystinosis.

The specific aims are as follows:

Aim 1. To induce fusion of donor BMM with renal proximal tubular cells in vivo.

- a) Induced in vivo fusion of BMM to CD46 expressing renal proximal tubular cells using standard MV H and F proteins.
- b) Assessment of the extent of donor BMM reprogramming to renal tubular function as exemplified by de novo expression of HNF4 α .
- c) Temporal limitation of expression of fusogenic MV proteins by means of an estrogen receptor specific for a synthetic ligand.

Aim 2: Targeting of donor BMM fusion specifically to renal proximal tubular cells.

- a) Retargeting of the MV H protein by tethering it to a scFv for DPPIV.
- b) Assessment of efficiency and safety of specific induction of fusion between BMM and renal proximal tubular cells in vivo.

B. Studies and Results

Mouse hepatocytes expressing CD46 fuse in vitro with mouse embryonic stem cells that express the hemagglutinin and fusion proteins of measles virus. We co-cultured ESC that express Hemagglutinin (H) and Fusion (F) proteins of the measles virus as well as a red fluorescent protein (Tomato) with mouse hepatocytes that express CD46 and that were nuclear stained with Hoechst before co-culturing. A few hours after co-culture, we observed Tomato⁺ syncytia. The nuclei of these cells were both Hoechst negative and positive suggesting that they derived from fusion between embryonic stem cells and hepatocytes (Fig. 1). These results demonstrate that cell fusion between cells derived from the *CD46*^{+/-} mouse model and cells expressing the H and F proteins of measles virus can be induced in vitro, supporting the usefulness of the CD46 mouse model for our future studies of induced cell fusion in vivo.

Bone marrow-derived nuclei are reprogrammed to express the renal tubular specific transcription factor HNF4 α . We analyzed fused renal tubular cells in *Fah*^{-/-}, *R26R*^{+/-} mice which is a Cre reporter mouse model that develops renal proximal tubular injury. These mice were lethally irradiated and transplanted with bone marrow cells from *EGFP*^{+/-}, *HNF4 α -Cre*^{+/-} mice, a model that expresses Cre under the transcriptional control of the endogenous HNF4 α promoter and therefore in renal proximal tubular cells. We found a significant number of β -gal⁺ renal tubular cells reflecting activation of the

R26R reporter gene by Cre and therefore cell fusion between a host and a donor cell (Fig. 2). Since hematopoietic cells fail to express HNF4 α , this result indicates that the gene expression in the transplanted cell was altered to a proximal tubular cell program. Moreover, the observed proliferation of the fused cells demonstrates normal renal proximal tubular cell capabilities. These results show that the reprogramming of bone-marrow derived nuclei after fusion with proximal tubular cells is not limited to the *Fah* gene. Since HNF4 α regulates a large number of genes in proximal tubular cells, its activation suggests that bone marrow-derived cells can be used to correct a wide range of genetically encoded tubulopathies including cystinosis.

C. Plans

In preliminary studies using Amaxa's nucleofection protocol, we found that 50% of BMM were successfully transfected with a Tomato plasmid. Recent work using a lentivirus demonstrated that the efficiency of bone marrow-derived myeloid cell transduction was >90%². We are currently generating a lentivirus that expresses H and F measles virus proteins to infect BMM and determine the efficiency of fusion between BMM and CD46 positive renal proximal tubular cells in vivo.

We are in the process of quantifying the number of EGFP, *Fah* and β -gal positive renal tubular cells in the *Fah*^{-/-}, *R26R*^{+/-} mice that were transplanted with bone marrow cells from *EGFP*^{+/-}, *HNF4 α -Cre*^{+/-} mice to determine the overall efficiency of reprogramming and consequently the therapeutic potential of tubular fusion products.

D. References

1. **Held PK**, Al-Dhalimy M, Willenbring H, Akkari Y, Jiang S, Torimaru Y, Olson S, et al. In vivo genetic selection of renal proximal tubules. *Mol Ther* 2006;13:49-58.
2. **Takahashi K**, Prinz M, Stagi M, Chechneva O, Neumann H. TREM2-transduced myeloid precursors mediate nervous tissue debris clearance and facilitate recovery in an animal model of multiple sclerosis. *PLoS Med* 2007; Apr;4:e124. **Error!**

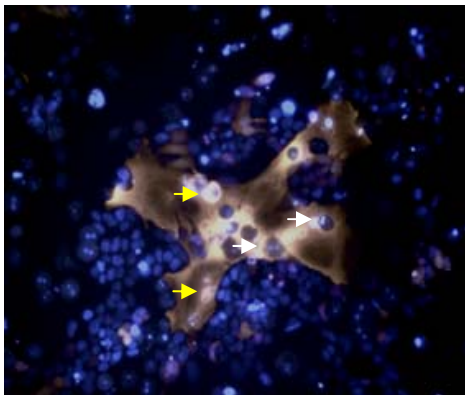


Fig. 1: Tomato positive syncytium showing Hoechst negative (yellow arrows) and positive (white arrows) nuclei.

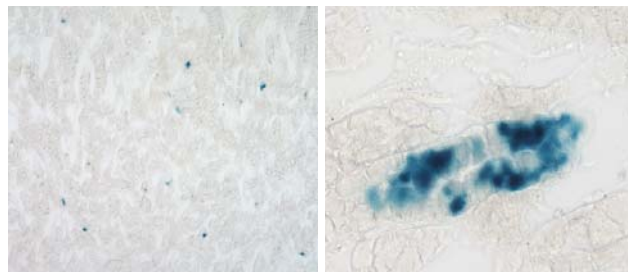


Fig. 2: β -gal positive proximal tubular cells in kidney sections from a *Fah*^{-/-}, *R26R*^{+/-} mouse that was transplanted with bone marrow from *EGFP*^{+/-}, *HNF4 α -Cre*^{+/-} mouse. 10x (left panel), 40x (right panel)