Cystinosis Research Foundation Progress Report
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A. OBJECTIVE AND SPECIFIC AIMS

The objective of this work is to correct genetically encoded renal tubulopathies by using fusion with bone marrow-derived macrophages (BMM) to deliver a healthy genome to renal proximal tubular cells. The specific aims of the project are:

Aim 1. To induce fusion of donor BMM with renal proximal tubular cells in vivo.
a) Induced in vivo fusion of BMM with CD46 expressing renal proximal tubular cells using the measles virus H and F proteins.
b) Assessment of the extent of donor BMM reprogramming to renal tubular function as exemplified by de novo expression of the transcription factor HNF4α.
c) Temporal limitation of expression of fusogenic measles virus 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 measles virus H protein by tethering it to a single chain antibody 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

Low number of fusion events between bone marrow-derived macrophages expressing measles virus H and F protein and renal proximal tubular cells in *CD46$^{+/-}$, R26R$^{+/-}$* mice.

We have shown that the tropism of the measles virus (MV) H protein for CD46 in combination with the MV F protein can be used to induce fusion between cells of a human CD46 transgenic mouse model (CD46$^{+/-}$ mice) and cells expressing the MV proteins via plasmid transfection. We also demonstrated that bone marrow-derived nuclei are reprogrammed by fusion with renal proximal tubular cells to activate expression of the transcription factor HNF4α, suggesting that bone marrow-derived cells can be used to genetically and functionally correct a wide range of tubulopathies, including cystinosis.
In our last report, we showed that fusion can be induced between MV H/F expressing macrophages and CD46/R26R-expressing renal proximal tubular cells in vivo. In this same report, we also described experiments transplanting BMM armed with a bi-specific antibody (an antibody for the macrophage-specific F4/80 antigen linked to an antibody for the cell surface marker DPPIV present on

renal proximal tubular cells) into the kidney. However, we were not able to detect induced fusion events with this strategy. Flow cytometry of BMM mixed with CD46-expressing hepatocytes suggested that this could be due to loss of activity of the conjugated antibodies in the linking process (data not shown). This problem can likely be overcome by new linking attempts or the use of novel cell surface marker antibodies. However, in the interest of time, we focussed on establishing the key principles of targeted and induced cell fusion in the kidney in our model comprised of BMM expressing MV H/F proteins and *CD46*$^{+/-}$ mice.

The number of induced fusion events between MV H/F expressing macrophages and renal proximal tubular cells observed in *CD46*$^{+/-}$, *R26R*$^{+/-}$ mice was low (2 fused cells/100,000 renal proximal tubular cells). Although BMM and *CD46*$^{+/-}$, *R26R*$^{+/-}$ mice were on the same strain background, loss of fusion products may have occurred due to an immune response against the MV proteins. To rule out acute rejection of H/F expressing cells, we are currently comparing the number of engrafted GFP-expressing macrophages in kidneys of *CD46*$^{+/-}$, *R26R*$^{+/-}$ and immune-deficient mice (*Rag2*$^{-/-}$, $\gamma_c^{-/-}$). These analyses will reveal whether induction of fusion with H/F proteins will require transient immune suppression.

A mouse model of renal Fanconi syndrome.

In order to assess the efficacy and, to some extent, safety of cell therapies such as induced fusion for therapy of tubulopathies, a mouse model is needed. For this purpose, we proposed to use the fact that FAH, the last enzyme in the tyrosine degradation pathway, is only expressed in hepatocytes and renal proximal tubular cells and that its deficiency causes mild renal injury by an accumulation of the substrate fumarylacetoacetate (FAA). This renal injury aggravates when hepatocytes of mice lacking FAH (*Fah*$^{-/-}$ mice) are also deficient in homogentisic acid dioxygenase (HGD), an enzyme upstream of FAH (1). Typically, hepatocytes take up and degrade most tyrosine but it they are HGD deficient, the HGD substrate homogentisic acid (HGA) is shifted towards the kidney where it enters the tyrosine degradation pathway, thereby producing large amounts of the toxic metabolite FAA in proximal tubular cells. As a result, we hypothesized that *Fah*$^{-/-}$ mice repopulated with HGD deficient hepatocytes would develop renal Fanconi syndrome.
To investigate this, we transplanted *Hgd*$^{-/-}$, *Fah*$^{+/+}$ hepatocytes into the spleen of *Fah*$^{-/-}$ immune-deficient mice (*Fah*$^{-/-}$, *Rag2*$^{-/-}$, $\gamma_c^{-/-}$). Mice were taken off 2-(2-nitro-4-trifluoro-methylbenzyol)- 1,3 cyclohexanedione (NTBC), a drug protecting *Fah*$^{-/-}$ mice from FAH deficiency, 4 weeks after hepatocyte transplantation to allow their selective expansion. Two months after transplantation, mice showed repopulation with *Hgd*$^{-/-}$, *Fah*$^{+/+}$ hepatocytes in the liver (Fig. 1A). As expected, progressive repopulation was associated with development of kidney damage including massive enlargement (Fig. 1B), death of proximal tubular cells (Fig. 1 D) and proteinuria (Fig. 1D-E). Hence, we believe that we have generated a mouse model truthfully recapitulating the kidney injury underlying renal Fanconi

syndrome. Since this model can be established in a relatively short period of time and allows the use of NTBC for "tittering" the level of tubulopathy, we hope that it will be helpful for research on the pathology and therapy of cystinosis.

Along these lines, to use this model to test the therapeutic potential of induced fusion with BMM, we will transplant HGD deficient hepatocytes into *CD46*$^{+/-}$, *Fah*$^{-/-}$ (immunodeficient) mice to repopulate their livers and induce chronic renal tubular damage. Then, we will inject BMM expressing both GFP and the measles virus proteins into the kidneys of these mice to assess the regenerative capabilities of renal proximal tubular cells corrected by fusion. We expect that fused FAH expressing renal tubular cells will have a growth advantage over the FAH deficient cells, and therefore, will be able to repopulate and regenerate the damaged proximal tubular epithelium of the recipient mice.

D. REFERENCES

1. Held P.K., 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

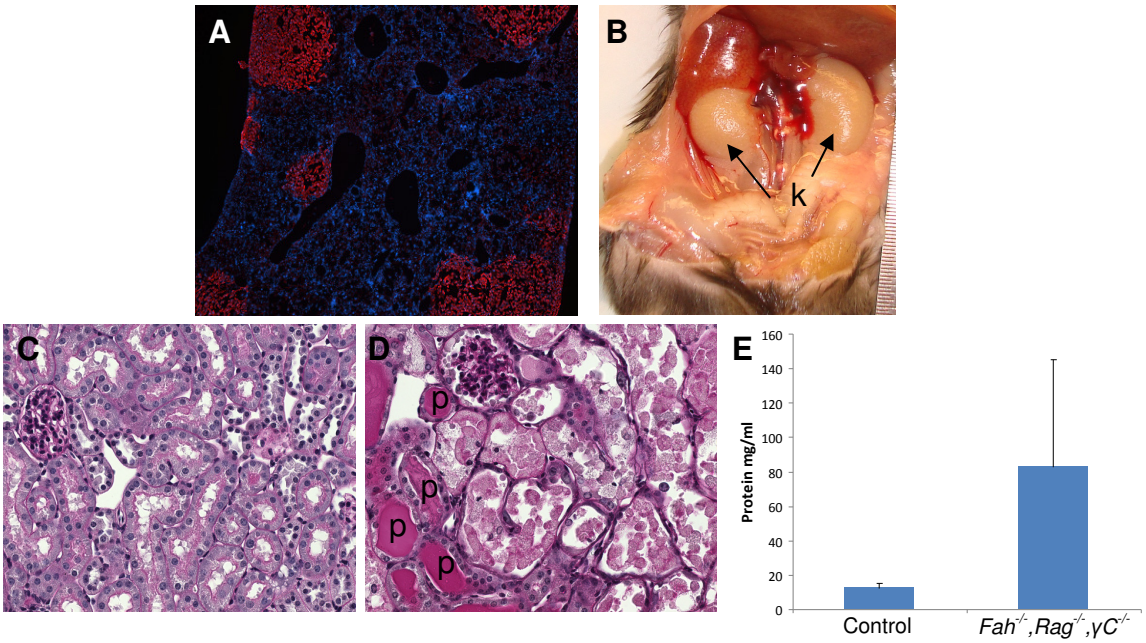

Fig 1 Kidney damage in *Fah*$^{-/-}$,*Rag2*$^{-/-}$,$\gamma_C$$^{-/-}$ mice transplanted with *Hgd*$^{-/-}$,*Fah*$^{+/+}$ hepatocytes. **A.** Clusters of FAH-expressing hepatocytes (red) in liver two months after transplantation. **B.** Picture showing massive enlargement and pale appearance of kidneys (k) in the same mouse. **C-D.** Sections of kidney tissue stained with Periodic-Acid-Schiff (PAS) from control healthy mouse (C) and *Fah*$^{-/-}$,*Rag2*$^{-/-}$,$\gamma_C$$^{-/-}$ mice with liver repopulation by *Hgd*$^{-/-}$,*Fah*$^{+/+}$ hepatocytes (D). Note the injured epithelial cells in cortical tubules and protein deposits (p) caused by proteinuria. **E**. Comparison of total urine protein levels in control healthy mice and *Fah*$^{-/-}$,*Rag*$^{-/-}$,*γC*$^{-/-}$ mice with liver repopulation by *Hgd*$^{-/-}$,*Fah*$^{+/+}$ hepatocytes.