Genome Engineering of Renal Epithelial Cells with the Goal of Improved Function in an Implantable Artificial Kidney

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Background:
Development of an implantable artificial kidney (IAK) will require renal epithelial cells capable of reabsorption of salt and water. We are using genome engineering to bioengineer cells for improved Na+/H+ exchange and H20 reabsorption. The piggyBac transposon system offers a simple but highly efficient non-viral strategy for genome engineering cells to stably overexpress one or more transgenes simultaneously. The piggyBac transposase enzyme integrates transposon DNA containing one or more transgenes into the genomic DNA of cells via a cut-and-paste mechanism.

Methods:
Standard molecular biology techniques were used to subclone the human sodium hydrogen exchanger 3 (NHE3) and aquaporin-1 (AQP1) cDNAs into piggyback transposon vectors. The NHE3 transgene was engineered with a C-terminal hemagglutinin (HA) epitope tag. The AQP1 transgene contained flag and myc epitope tags. The piggyback transposase was then co-delivered with these transposons to stably integrate and overexpress NHE3 or AQP1 in cultured renal epithelial cells.

Results:
We generated MDCK cells stably expressing a cumate inducible NHE3 and confirmed cumate-induced overexpression via Western blot and immunofluorescence analysis of the HA tag. Confocal microscopy confirmed apical expression of NHE3 in polarized MDCK cells. We also generated MDCK cells stably overexpressing AQP1. Overexpression of AQP1 was confirmed using Western blot and immunofluorescence assays of the flag and myc tags.

Conclusions:
We are currently also overexpressing NHE3 and AQP1 in cultured human renal proximal tubule cells (RPTECc). Cellular transport assays are ongoing to evaluate for increased capability in moving salt and water across a genome engineered cellular monolayer. These studies will allow us to determine the optimally.