Evaluation of Renal Cells for In Vitro Modeling of Proximal Tubule Drug Transport

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Background:

Active transport by renal proximal tubules plays a significant role in human drug disposition and is therefore important to model when developing drugs. Although several proximal tubule cell lines exist, limited data are available regarding their ability to act as acceptable models of the tubule. Here, several cell lines are compared with respect to monolayer formation, drug transporter expression and function, and cilia function.

Methods:

hOCT2/hMATE1 double transfected (DT) MDCK cells, SV40 immortalized Human Proximal Convoluted Tubule (HPCT) cells, and hTERT immortalized Renal Proximal Tubule Epithelial Cells (RPTEC) were cultured on transwell inserts. Tight junctions and cilia were confirmed by immunofluorescence, monolayer tightness was measured by quantifying inulin leak through cells and RNA expression was measured by qRT-PCR. Transporter function was measured by quantifying cellular uptake and transport of substrates (+/- inhibitor). Cells were deciliated with 30 mM ammonium sulfate.

Results:

All cells formed tight junctions as demonstrated by ZO-1 expression, but showed varying levels of monolayer tightness with MDCKs at ~1%, HPCTs at ~7%, and RPTECs at ~10% inulin leak. Both human cell types tested had comparable RNA expression levels of OCT2, MATE1, OAT3 and MRP4 (~ΔCT of: 14-15, 12-13, 15 and 5-9 respectively), while double transfected MDCK cells had high expression levels of OCT2 and MATE1 (~ΔCT of -2 and -4 respectively). Both DT-MDCK cells and HPCT cells showed inhibitable uptake and transport of ASP+ and Metformin. Lastly, both MDCK and HPCT cells grew cilia that could be removed without effects on tight junction formation, RNA expression of transporters and transporter function.

Conclusions:

While overexpressing MDCK cells exhibit the best performance, both human proximal tubule cell lines show promise as potential models of human tubular function. Studies are ongoing.