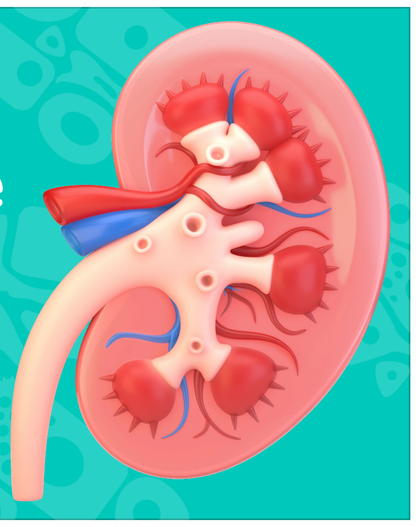
A Live Cell High Content Imaging Assay for Monitoring Large Molecule Uptake into aProximate™ Proximal Tubule Epithelial Cells via Megalin and Cubilin



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Introduction

The proximal tubules of the kidney nephron play a key role in the reabsorption of filtered amino acids, glucose, solutes, and low molecular weight proteins, such as albumin, from the tubular filtrate. Many xenobiotics are also reabsorbed here, which if not rapidly cleared from the cells, can cause nephrotoxicity.

The large number of drug transporters and receptors expressed in proximal tubule cells (PTCs) give them their absorptive and secretory functions. Two key endocytic receptors strongly expressed on the apical surface of these cells are megalin and cubilin, which play an important role in reabsorption of large molecules and glomerular-filtered proteins (Fig. 1).

We have recently developed a live cell high content imaging assay to monitor the uptake of fluorescently labelled proteins, as well as large molecules, including oligonucleotides, aminoglycosides, and antibody-drug conjugates via this endocytic uptake mechanism.

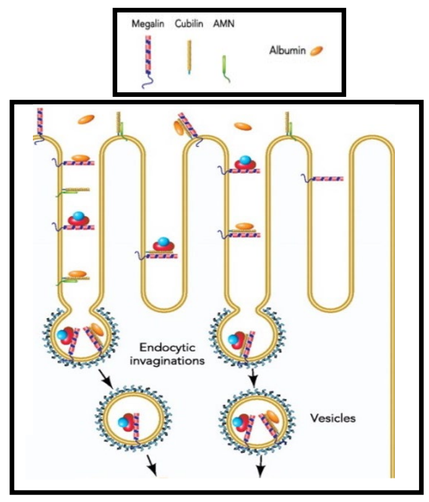

Figure 1: Albumin reabsorption by endocytosis via megalin and cubilin, key uptake transporters of large molecules in the proximal tubule

Methods

- High content imaging (HCI) method: Primary human and mouse PTCs (aProximate™)[1] were cultured on 96-well black-walled plates for up to 5 days, before being exposed to 50µg/mL FITC-albumin for 4 hours. Live cell imaging using the ImageXpress Pico high content imaging system (Molecular Devices), and subsequent analysis using Cell Reporter Xpress (CRX) software enabled uptake of FITC-albumin to be quantified. Expression of megalin and cubilin in these monolayers was confirmed by immunostaining.
- Plate reader method: Primary mouse PTCs were cultured on 24-well Transwell plates with semi-permeable membranes until confluent. Cells were pre-incubated in pH7.4 Krebs buffer +/- RAP, washed, and the apical chamber was then dosed with 25µg/mL FITC-albumin in Krebs for 60 minutes. To terminate the experiment, cells were washed in ice-cold Krebs, lysed in 0.1% SDS and transferred to a flat-bottomed plate before being read on a microplate reader (BMG Labtech) Ex/Em 490/525 nm.
- In both methods, pre-treatment and co-dosing mouse monolayers with 4µM (HCl method) or 5µM (plate reader method) RAP, a proposed inhibitor of megalin, was used to assess the ability to inhibit uptake of FITC-albumin. All data shown is from single biological donors.

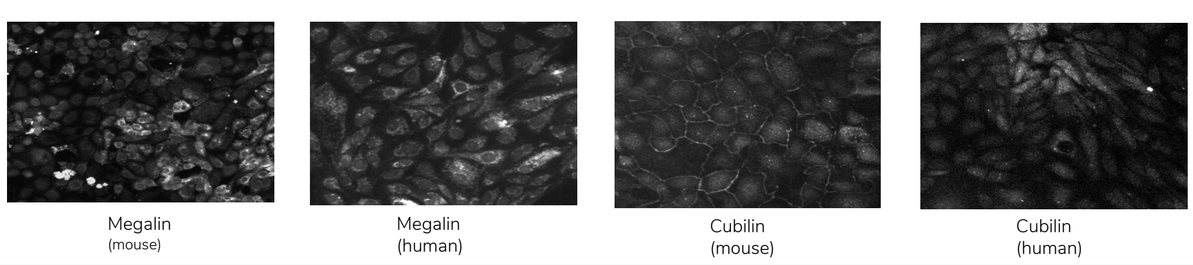

Figure 2: The monolayers of mouse and human PTCs express the endocytic receptors megalin and cubilin.

Results

A FITC-albumin uptake curve was generated (Fig. 3), which allowed for the K_m, the FITC-albumin concentration at half receptor saturation, to be determined (19.43 µg/mL for mouse and 13.57 µg/mL for human monolayers). Uptake of FITC-albumin in mouse monolayers was then assessed in the presence and absence of RAP; pre-treatment and co-dosing with RAP resulted in significant inhibition of uptake of FITC-albumin (Figs. 4, 5).

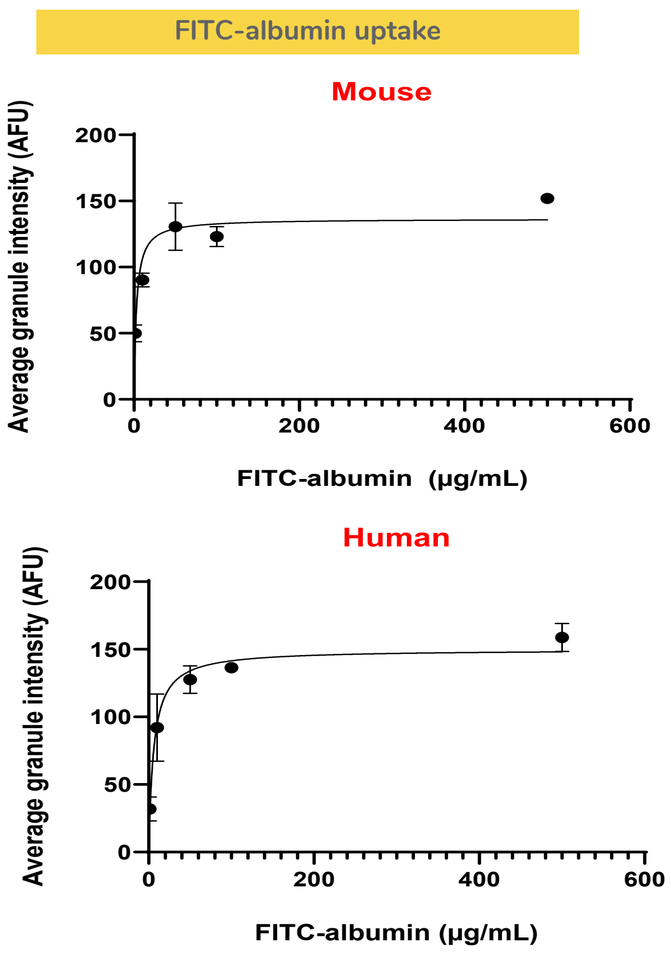

Figure 3: FITC-albumin uptake in mouse and human monolayers. K_m denoted by dotted line. Physiological albumin concentration in rodent filtrate (~25µg/mL) shown by the blue arrow.

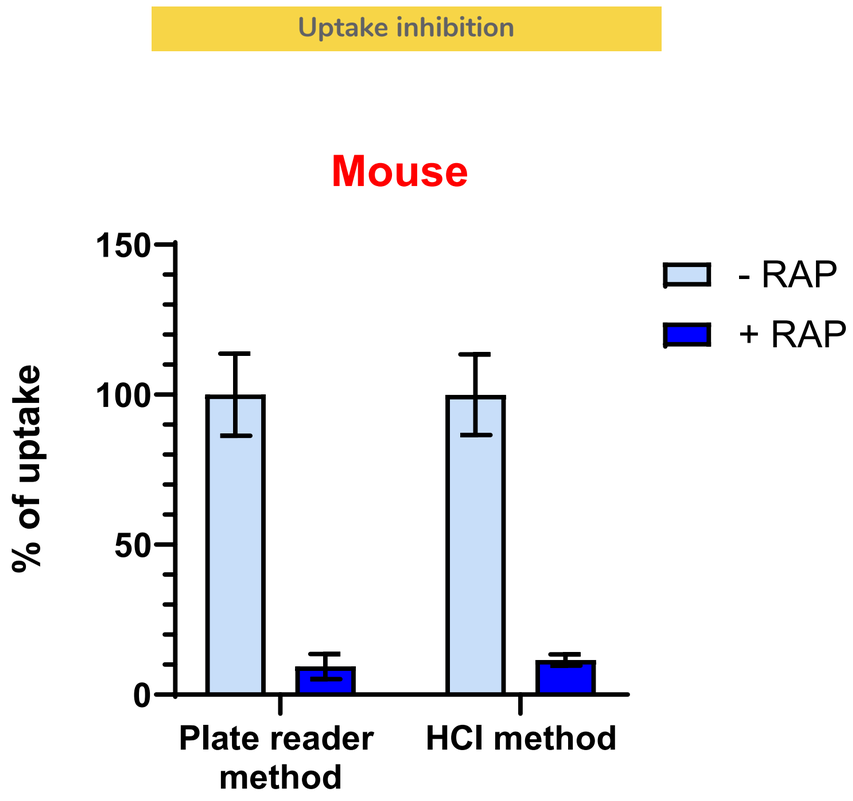

Figure 4: % of FITC-albumin uptake, compared to control, in the plate reader (9.4%) and HCl method (11.5%)

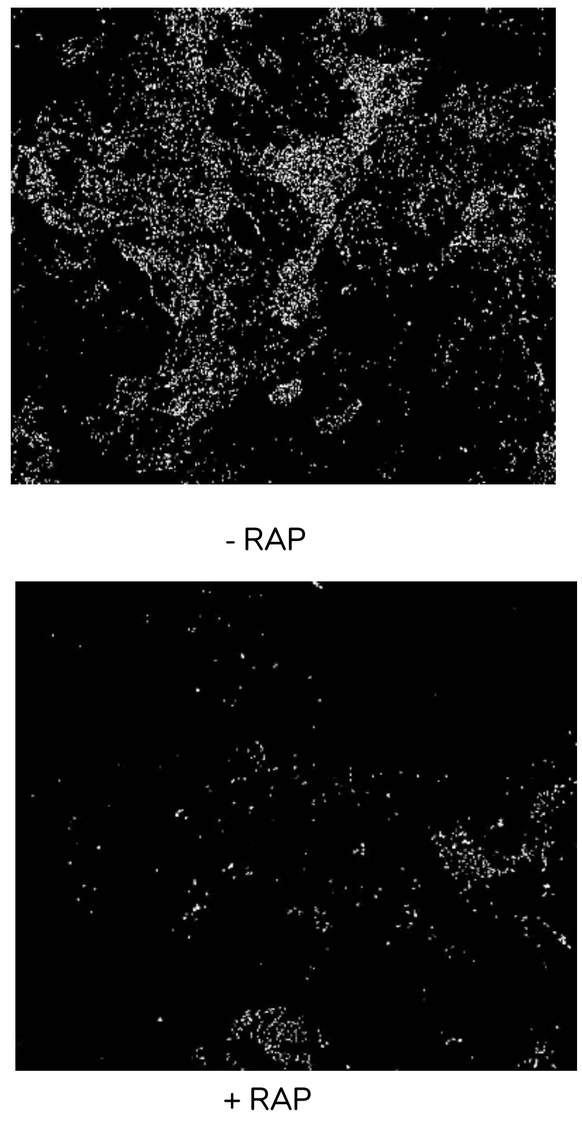

Figure 5: The CRX software highlighting granules that fit within user-defined parameters. After co-dosing mouse monolayers with RAP (right), less granules are positively selected than in the RAP-free condition (left).

References

1. Brown, C. D. A., et *al. Toxicol. Appl. Pharmacol.* 2008; 233(3):428-38

Summary

- We have developed an assay to monitor the uptake of fluorescently labelled proteins and large molecules into cultured PTCs using high content imaging.
- This new assay format could be used as an *in vitro* tool for assessing drug transport and toxicity.