A Primary Human *in vitro* Focal Segmental Glomerulosclerosis (FSGS) model for drug development applications

KIDNEY

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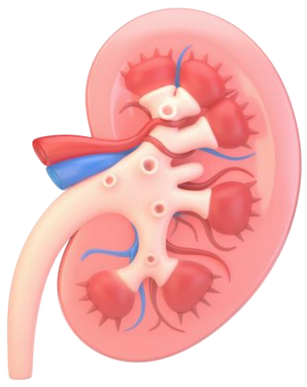

Introduction

Focal Segmental Glomerulosclerosis (FSGS) is a common feature of Chronic Kidney Disease and is characterised by proteinuria and the deposition of extracellular matrix (scarring) of glomeruli. Drug-induced FSGS can be caused by Adriamycin, Daunorubicin, Pamidronate, and others (ref. 1), and leads to podocyte death and loss (**Figure 1**), and a decrease in Glomerular Filtration Barrier integrity. The clinical consequence of this is proteinuria, whereby essential proteins, including albumin, are lost from the blood into the urine. FSGS is commonly studied using the Adriamycin Nephropathy mouse model (ref. 2); here we present an alternative *in vitro* model composed of freshly-derived human podocytes.

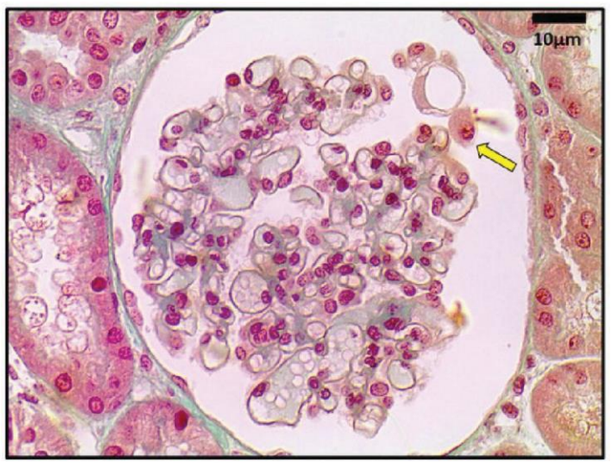

Figure 1: FSGS is characterised by detachment and decrease in podocyte number (image ref. 3).

Methods

Primary podocytes isolated from human kidneys were seeded onto 96-well Transwell inserts (Corning #3381) or black-walled 96-well imaging plates (Corning #3904). For cells grown in Transwell inserts, Transepithelial Electrical Resistance (TEER) was measured using an EVOM2 Voltohmmeter. Podocytes were treated with Adriamycin for 72 h when TEER >100 Ω.cm^2. FITC-dextran 4, 10, 20, 40, 70 kDa was applied to the upper compartments and rate of filtration through the podocyte monolayer assessed at 30, 60, 90 mins by performing linear regression analysis. Cell viability was determined using CellTiterGLO (Promega). For podocytes grown in black-walled plates, cells were stained with CellROX or CellEvent Caspase 3/7 dye (Thermo Fisher) or fixed following 80 mins or 72 h treatment (without or with 1 h pre-treatment with R-7050 TNFR1 blocker) and immunostained for IκBα, NFκB, or fibronectin, and imaged using the ImageXpress Pico high content imaging system. Images were analysed using Cell Reporter Xpress (CRX) software (Molecular Devices).

Results

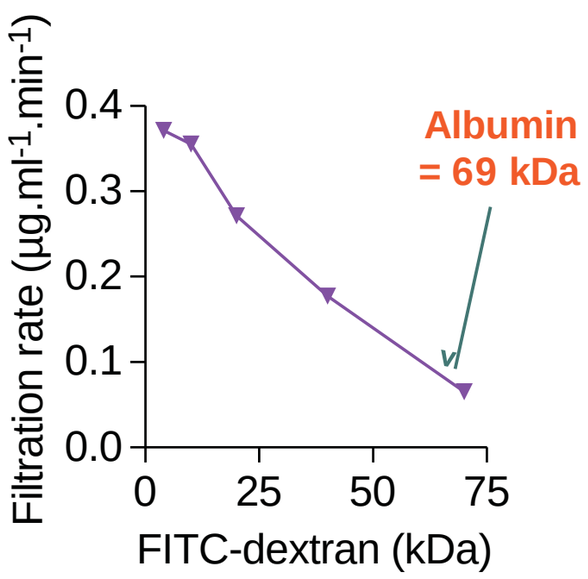

Figure 2: Primary human podocytes recapitulate the size selection properties of the Glomerular Filtration Barrier.

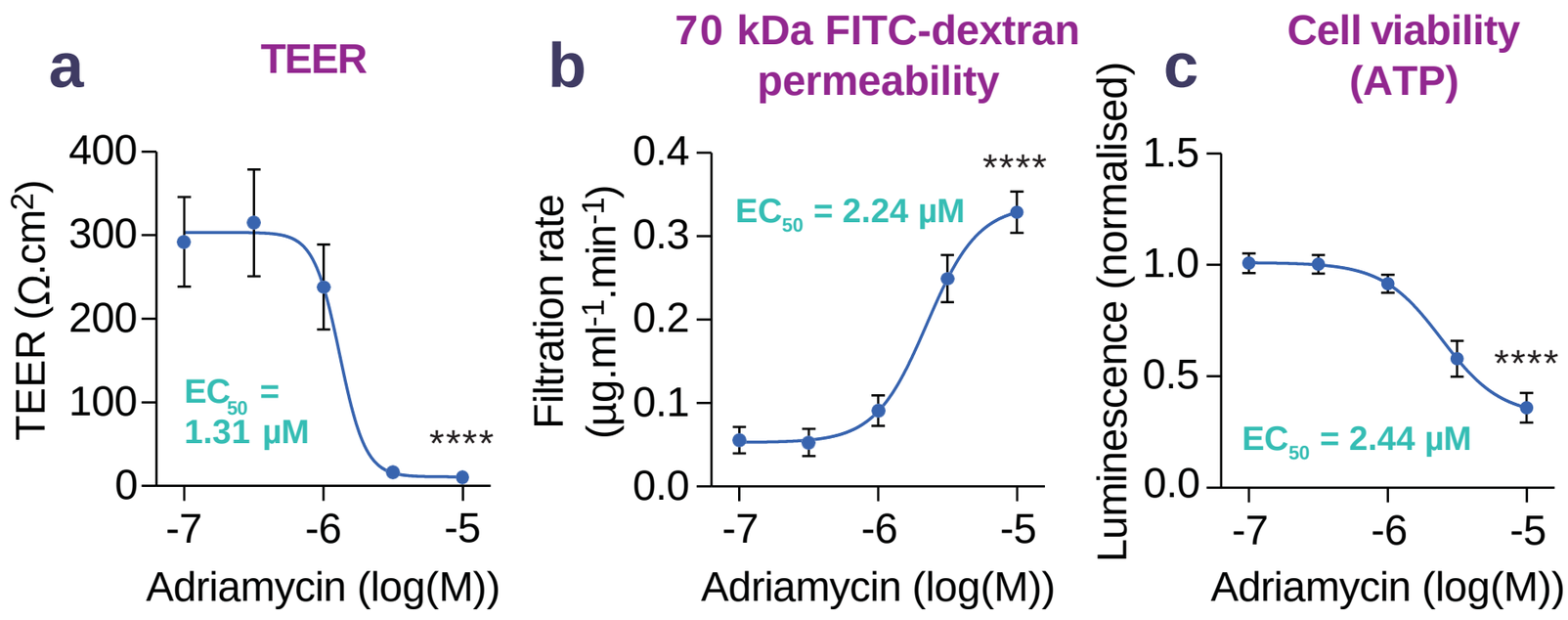

Figure 3: Adriamycin disrupts filtration barrier integrity. (a) Adriamycin induces a dose-dependent decline in TEER. **(b)** Permeability to 70 kDa FITC-dextran increases in response to Adriamycin. **(c)** Cell viability decreases in response to Adriamycin (72 h treatments, *N*=4 biological repeats (separate kidney donors), **** p<0.0001).

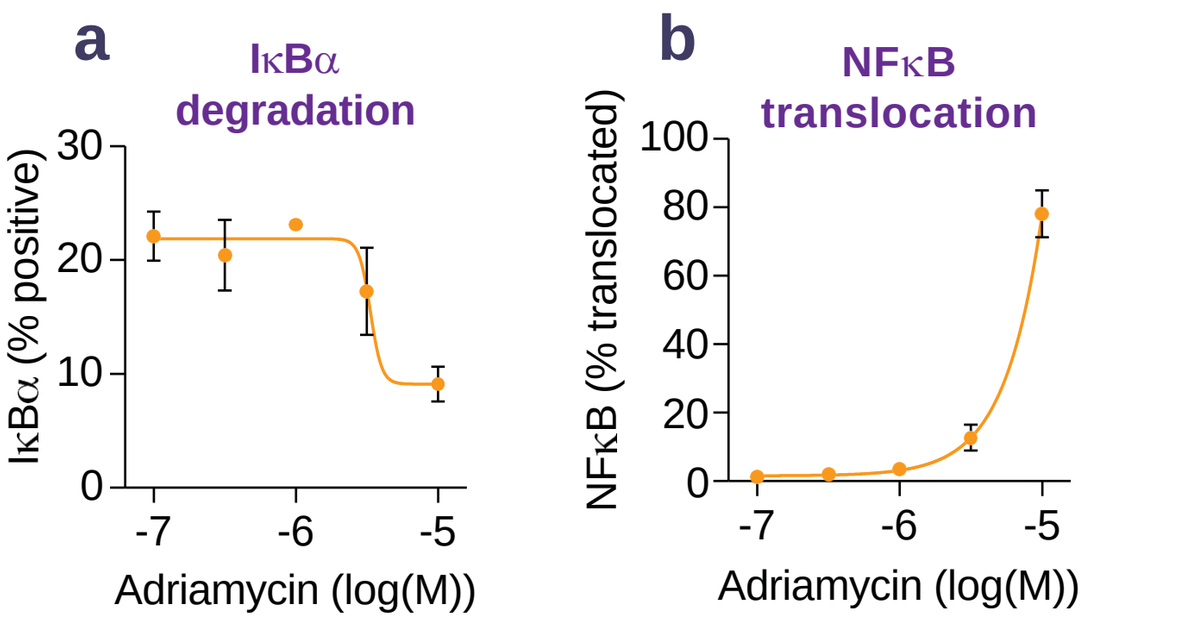

Figure 4: Adriamycin activates IkBa/NF-kB signalling pathway. Podocytes were treated with Adriamycin for 80 mins before being fixed and immunostained for **(a)** IκBα, and **(b)** NF-κB signalling components.

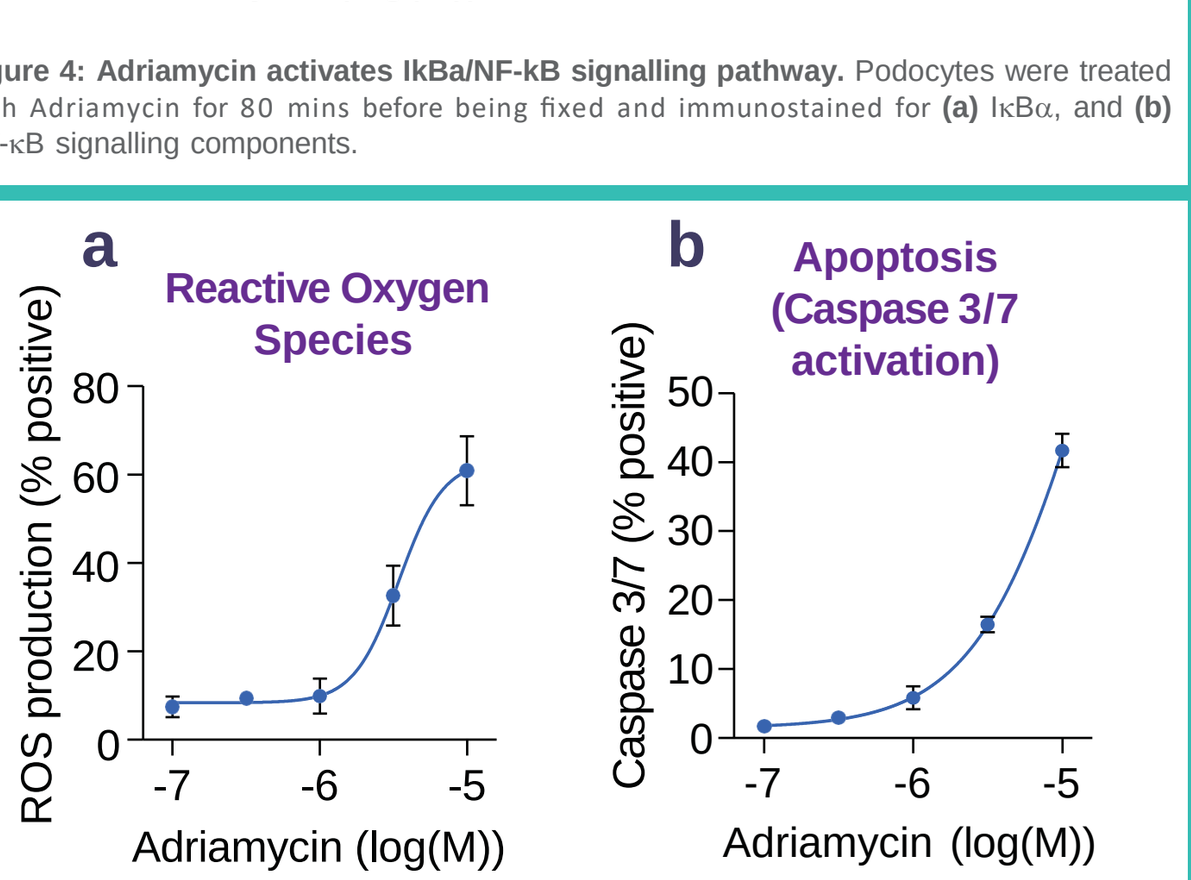

Figure 5: Adriamycin reveals its toxicity by (a) increasing Reactive Oxygen Species (ROS) production, and (b) promoting Caspase 3/7 cleavage (apoptosis effector molecules). Podocytes were treated for 72 h prior to staining and imaging.

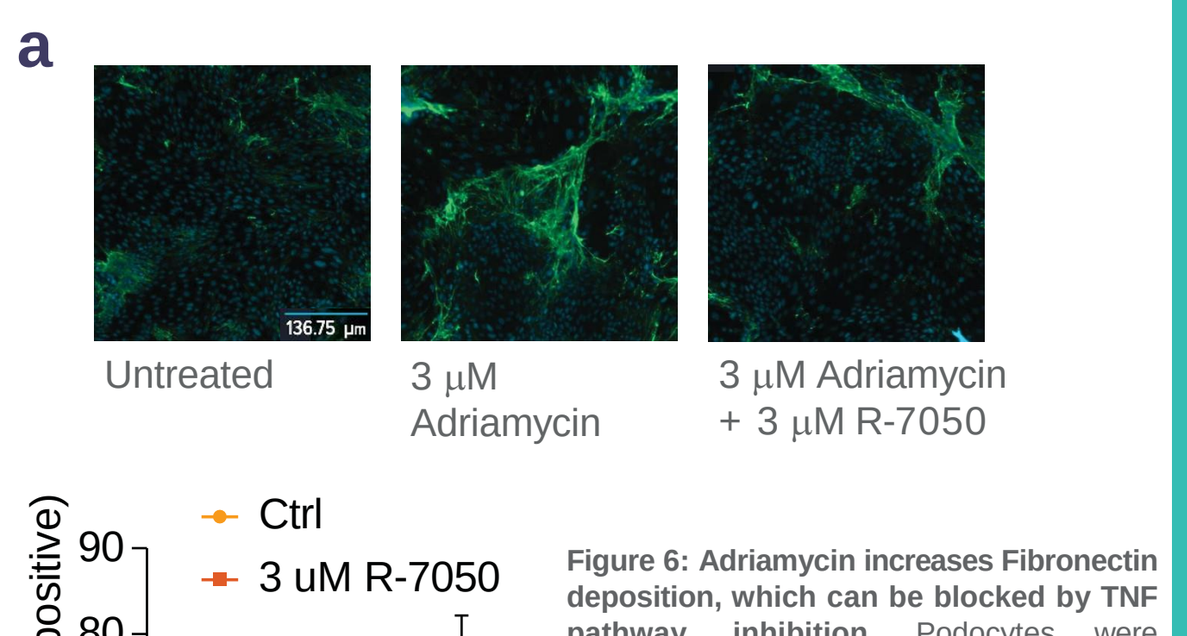

Figure 6: Adriamycin increases Fibronectin deposition, which can be blocked by TNF pathway inhibition. Podocytes were treated for 72 h with Adriamycin without or with 1 h R-7050 TNFR1 blocker pre-treatment, followed by fixing and immunostaining for Fibronectin. **(a)** Example immunofluorescent images. Blue, DAPI; green, fibronectin. **(b)** Quantification of fluorescent images.

Summary

- **Newcells' primary podocyte model can be used as an Adriamycin Nephropathy model to study FSGS *in vitro*.**
- **Podocytes are grown in 96-well plates making the model amenable to high throughput drug development applications.**

References

1. Guruswamy Sangameswaran *et al.*, 2023, PMID: 30335305.
2. Lee & Harris, 2010, Nephrol **16** (1): 30.
3. Cutrim *et al.*, 2022, Front Med (Lausanne) **9:** 846173.

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