

## The role of the actin-sequestering protein, thymosin- $\beta$ 4, on renal podocyte function

W. Mason<sup>1</sup>, C. M. Peppiatt-Wildman<sup>2</sup>, D. A. Long<sup>3</sup>, E. Vasilopoulou<sup>1,3</sup>

<sup>1</sup>Medway School of Pharmacy, University of Kent, Chatham, UK

<sup>2</sup>School of Sport and Exercise Science, University of Kent, Chatham, UK

<sup>3</sup>Developmental Biology and Cancer Programme, UCL Great Ormond Street Institute of Child Health, London, UK

### Introduction

End stage renal disease (ESRD) is a condition that requires life-long dialysis or transplantation and is associated with increased risk for all-cause and cardiovascular mortality. A major cause of ESRD is damage of the glomerulus, the filtration unit of the kidney. Podocytes, epithelial cells located in the glomerulus, are responsible for the ultrafiltration of blood into urine. In glomerular disease, podocytes lose their morphology and glomerular filtration is disrupted. Thymosin- $\beta$ 4 (T $\beta$ 4) is a G-actin sequestering protein that regulates actin cytoskeleton assembly, cell morphology and motility. We have previously reported that downregulation of endogenous T $\beta$ 4 in podocytes *in vitro* increases migration and actin stress fibre formation. The aim of this study is to assess the effect of exogenous T $\beta$ 4 on healthy and injured podocytes.

### Methods

To assess the effect of T $\beta$ 4 on healthy podocytes, differentiated immortalised mouse podocytes were treated with 0, 10, 100 or 1000 ng/ml T $\beta$ 4 (n=4-5). Co-treatment with T $\beta$ 4 (0, 10, 100 or 1000 ng/ml) and ADR (0, 0.0125 or 0.125  $\mu$ g/ml) was used to investigate the effect of T $\beta$ 4 on injured podocytes (n=4). Podocyte mRNA levels were assessed by qPCR. Cell viability (MTT assay) was assessed 24, 48 and 72 hours post treatment. Podocyte migration was analysed by a scratch wound assay at 6 and 24 hours post wound formation. Actin filaments were visualised 24 hours post treatment with Acti-stain 488 phalloidin and cell area, F-actin density and stress fibre prevalence were assessed.

### Results

Treatment of healthy podocytes with T $\beta$ 4 did not alter cell viability, migration, cell area, F-actin density, or stress fibre prevalence. ADR treated podocytes (0.125  $\mu$ g/ml) showed a decrease in mRNA levels of TB4 (p<0.005), Schip1 (p<0.05) and cofilin 1 (p<0.05), however TB4 was unable to prevent this. ADR treatment reduced cell viability by >60% at doses higher than 0.125  $\mu$ g/ml at all time points (p<0.05). Migration was unchanged after 6 hours, but after 24 hours 0.0125  $\mu$ g/ml ADR increased migration (40%, p<0.05), whereas decreased migration and cell detachment was observed for the rest of the doses (p<0.05). Changes in the podocyte cytoskeleton were also observed with decreased cell area and F-actin density (0.125 and 1  $\mu$ g/ml ADR; p<0.05) and increased prevalence of cytoplasmic stress fibres (0.0125-0.5  $\mu$ g/ml ADR; p<0.05). Treatment with T $\beta$ 4 (100 ng/ml) significantly reduced the ADR-induced increase in cytoplasmic stress fibre prevalence (p<0.05) but did not modify the effects of ADR on cell viability and migration.

### Conclusion

Exogenous T $\beta$ 4 has no effect on healthy podocytes *in vitro*. ADR injury results in pronounced reduction of actin-associated protein mRNA levels, cell death and changes in the podocyte cytoskeleton *in vitro* in a dose-dependent manner. Our data indicates that treatment with T $\beta$ 4 may protect the ADR-induced changes on the podocyte cytoskeleton.