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P341 -The role of the G-actin sequestering peptide Thymosin Beta-4 in renal podocyte function

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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-β4 (Tβ4) is a G-actin sequestering protein that regulates actin cytoskeleton assembly, cell morphology and motility. We have previously reported that downregulation of endogenous Tβ4 in podocytes in vitro increases migration and actin stress fibre formation. The aim of this study is to assess the effect of exogenous Tβ4 on healthy and injured podocytes.
Methods
To assess the effect of Tβ4 on healthy podocytes, differentiated immortalised mouse podocytes were treated with 0, 10, 100 or 1000 ng/ml Tβ4 (n=4-5). Co-treatment with Tβ4 (0, 10, 100 or 1000 ng/ml) and ADR (0, 0.0125 or 0.125 μg/ml) was used to investigate the effect of Tβ4 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 Tβ4 did not alter cell viability, migration, cell area, F-actin density, or stress fibre prevalence. ADR treated podocytes (0.125 μg/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 μg/ml at all time points ($p<0.05$). Migration was unchanged after 6 hours, but after 24 hours 0.0125 μg/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 μg/ml ADR; $p<0.05$) and increased prevalence of cytoplasmic stress fibres (0.0125-0.5 μg/ml ADR; $p<0.05$). Treatment with Tβ4 (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 Tβ4 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 Tβ4 may protect the ADR-induced changes on the podocyte cytoskeleton.