Developing podocyte-targeted gene therapy: the rise of vascular endothelial growth factor (VEGF)C!

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Background: Novel and efficient interventions in the treatment of diabetic kidney disease are required. We aim to use gene therapy in the kidney by inducing vascular endothelial growth factor (VEGF)C expression. VEGFC is a lymphagiogenic growth factor that, within the renal glomerulus, is expressed by podocytes and signals to glomerular endothelial cells (GEnCs). Importantly, we have previously shown that podocyte-specific transgenic expression of VEGFC can protect from early diabetic kidney disease. We hypothesise that VEGFC expression can be induced in podocytes using a viral approach, for future use in diabetic kidney disease.

Methods: Conditionally immortalised mouse podocyte cells were transduced with a novel adeno-associated virus (AAV), using a podocyte-specific promoter, to induce the expression of human VEGFC. Successful human VEGFC expression was investigated by RT-qPCR (using TaqMan gene expression assays and mouse GAPDH as housekeeping gene) and Western blotting. HEK293T, transfected with a plasmid expressing human VEGFC, under the CMV promoter, were used as controls. Conditioned media was collected from transduced podocytes and used to treat GEnC to investigate the functionality of the expressed transgenic VEGFC. A pilot in vivo study was carried out whereby male FVB mice (n=2) received AAV-VEGFC via tail vein injection. Six weeks post-injection, human VEGFC expression was assessed by RT-qPCR in glomeruli isolated by graded sieving, and by immunofluorescence in kidney sections.

Results: Mouse podocytes transduced with AAV-VEGFC using increasing amount of virus (MOI 10^5 - $3x10^6$) showed a significant dose-dependent increase in human VEGFC mRNA relative expression ($p<0.05$, n=3/group, one-way ANOVA, Bonferroni's multiple comparison test). Western blots clearly demonstrate expression of VEGFC by AAV VEGFC treated mouse podocytes. Additionally, treatment of GEnC with conditioned media from transduced podocytes led to significant phosphorylation of VEGFR2, revealed by VEGFR2 immunoprecipitation and probing with a pan anti phospho-tyrosine antibody ($p<0.05$, One-Way ANOVA). Preliminary *in vivo* data suggest that human VEGFC protein is expressed in podocytes of mice injected with AAV-VEGFC. In addition, human VEGFC mRNA relative expression was increased in glomeruli from mice injected with AAV-VEGF-C (14.79 ± 8.085, n=2) compared to glomeruli from non-treated littermate controls (1.095 ± 0.4350, n=2).

Summary: These data suggest that human VEGFC expression can be induced in podocytes in vitro and in vivo using our viral approach. In addition, the induced VEGFC can signal to GEnCs *in vitro* suggesting it is functional. Next, we will confirm whether this gene therapy approach can be used to prevent diabetic kidney disease.