Vascular endothelial growth factor-C (VEGF-C) reduces cystogenesis in a human cellular model of polycystic kidney disease generated by CRISPR technology

Nuria Perretta-Tejedor, Mariam Seda, Dagan J Jenkins, Pierre Ronco, Adrian S Woolf, Paul J Winyard, David A Long

The majority of cases of autosomal dominant polycystic kidney disease (ADPKD) are caused by mutations in polycystin-1 (*PKD1*) which promote cyst formation and eventually lead to renal failure. New treatments are urgently needed for ADPKD and recently, our laboratory demonstrated that the vascular growth factor, VEGF-C reduces the severity of a mouse model of ADPKD (Huang JL et al *J Am Soc Nephrol* 27:69-77, 2016). However, the mechanisms underlying VEGFC's effects in PKD are currently unknown. One hypothesis is that VEGFC might act on the cyst epithelium to directly alter cystogenesis and we tested this idea in a novel cellular model of ADPKD using the CRISPR-Cas9 system.

We generated cell lines with mutations in exon 36 of *PKD1* in both human embryonic kidney (HEK) 293 cells and human collecting ducts (HCD). HEK and HCD cells with: (i) a homozygous mutation *(*c.10745delCC) or (ii) compound heterozygous mutations in *PKD1* (HEK - allele 1 c.10745delCC and allele 2 c.10732delCGAGCTTCCC and HCD allele 1 c.10745delC and allele 2 c.10745delCC) were established. All of the mutations lead to a frameshift deletion in *PKD1*. The mutated cells were subsequently embedded in Matrigel and compared with wild-type cells in 3-dimensional culture. Examination of the size of 180 cyst-like structures per condition found that both the wild-type and *PKD1* mutated cells formed cyst-like structures in culture, but the cysts from the cells with *PKD1* mutations were significantly larger with more prominent lumens. HEK and HCD cells both expressed VEGFR-3, the main receptor for VEGF-C. Addition of exogenous VEGF-C from 10 to 100 ng/mL reduced the area of cyst-like structures in both wild-type ($p<0.05$) and *PKD1* mutated cells ($p<0.05$ in both cases).

Collectively, we have generated a human cellular model using CRISPR-Cas9 technology which may be used to test the effect of different drugs in cystogenesis or examine different biological process involved in ADPKD. The model has been used to show that VEGF-C, a novel therapy for ADPKD may partly reduce PKD severity by having an effect on the cyst epithelium and directly modulating cystogenesis.