Using CRISPR technology to generate models of polycystic kidney disease

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Polycystic kidney diseases (PKD) are characterised by the formation and progressive growth of renal cysts. Autosomal dominant (AD)PKD, the commonest PKD, affects 1 in 800 people and is caused by mutations in either *PKD1 (85 %)* or *PKD2* (15%) which encode for polycystin-1 and -2 respectively. These two proteins regulate proliferation, fluid secretion, ciliary function, cell–cell adhesion, and cell–matrix interaction of renal epithelial cells. Dysfunction of PKD1 and PKD2 results in aberrant cyclic AMP signalling which promote cyst formation. Understanding the molecular basis of PKD is essential for developing more effective therapies for patients and this requires clinical relevant cellular models. To do this, we employed the CRISPR-Cas9 system to generate mutations in exon 36 of *PKD1* in human embryonic kidney (HEK) 293 cells. Two different cell lines were subsequently generated: 1 with a homozygous mutation in *PKD1 (*c.10744delCC) and 1 with a compound heterozygous mutation (allele 1 c.10744delCC and allele 2 c.10732delCGAGCTTCCC), both of which lead to a frameshift deletion in *PKD1*. These cells were subsequently embedded in Matrigel and compared with HEK293 wild-type cells in 3-dimensional culture. We found that both the wild-type and *PKD1* mutated cells formed cyst-like structures in culture. Examination of the size of 180 cyst-like structures found that wild-type cells were smaller than both homozygous and compound heterozygous *PKD1* knock-down cells ($p<0.05$ in both cases). Next, we exposed the cells to forsolkin, a cyclic AMP agonist. In WT cells, forskolin increased the area of the cyst-like structures ($p<0.05$). In contrast, in both of the *PKD1* knock-down cells, forskolin did not cause any significant change in the cyst size. Collectively, we have generated a 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 such as cell proliferation or migration.