Growing kidneys: a party trick or reality?

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Kidney disease is a major and pervasive health problem that affects millions of individuals worldwide. Severe renal illness requires permanent dialysis treatment or a kidney transplant. Unfortunately, the average life expectancy of dialysis patients is frequently lower than that of cancer patients and renal transplants are in short supply. The problem is even more severe in countries with limited access to dialysis or transplantation. Therefore, the use of human pluripotent stem cells (hPSCs) has emerged as a potential field of research for addressing this issue.

The promise of human pluripotent stem cell technology

Pluripotent stem cells have the potential to differentiate into any type of somatic cell and so we can envisage two primary purposes: 1. to create normal kidney cells to be used in regenerative medicine therapies; or 2. to create in vitro models of kidney diseases to explore disease mechanisms and test novel therapies. Previously, researchers have used mutant mice to study the role of different genes in kidney development, and they have identified two key components of the metanephric embryonic kidney: the ureteric bud, which forms the ureter and collecting ducts, and the metanephric mesenchyme, which forms nephrons and stromal cells. These components communicate via the release of growth factors, the expression of which is regulated by transcription factors.

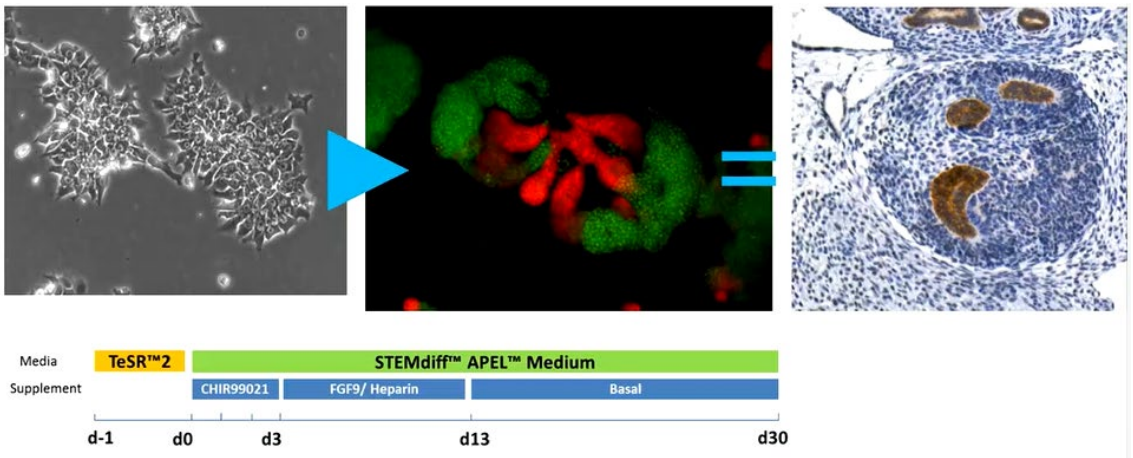

Figure 1. Inducing primitive kidney-like tissues from human pluripotent stem cells in vitro. Adapted from Bantounas I et al 2018 and 2021

The two main methods to obtain hPSCs are from embryonic stem cells derived from early human fertilized embryos that have not been used in *in vitro* fertilization, or from induced pluripotent stem cells, created by reprogramming adult white blood cells, or other somatic cells, back to a pluripotent state. These pluripotent stem cells can then be directed towards kidney nephron or collecting duct lineages, and these resulting cells can then be grown in 2D culture or 3D culture as 'organoids', or implanted into an immunocompromised experimental animal host where they differentiate further.

Pluripotent stem cells to generate normal kidney

Several in vitro studies have demonstrated that a defined cocktail of growth factors and small molecules applied in a timed sequence to hPSCs can result in primitive kidney morphogenesis in 2D. In 3D formats, kidney structures progress further giving some regional organization, but the kidney progenitors are necessarily limited in their growth and functional differentiation because, for example, they lack a blood supply. In research on mice, performed by Bantounas et al (2018), three genetically diverse wild-type hPSC lines were differentiated into kidney precursors that underwent in vitro primitive morphogenesis. They expressed nephron and collecting duct lineage marker genes, several of which are mutated in human kidney disease. In vitro differentiation of lentiviral-transduced hPSCs expressing reporter genes was comparable to controls. Immuno-deficient mice received subcutaneous implantation of kidney progenitors. They developed organ-like masses by 12 weeks that could be detected using bioluminescence imaging. The implants contained human-derived mesangial cells, podocytes with regions of the mature basement membrane, and perfused glomeruli containing human capillaries. A signal was detected in the tubules following intravenous injection of fluorescent low-molecular-weight dextran, suggesting uptake from the glomerular filtrate.

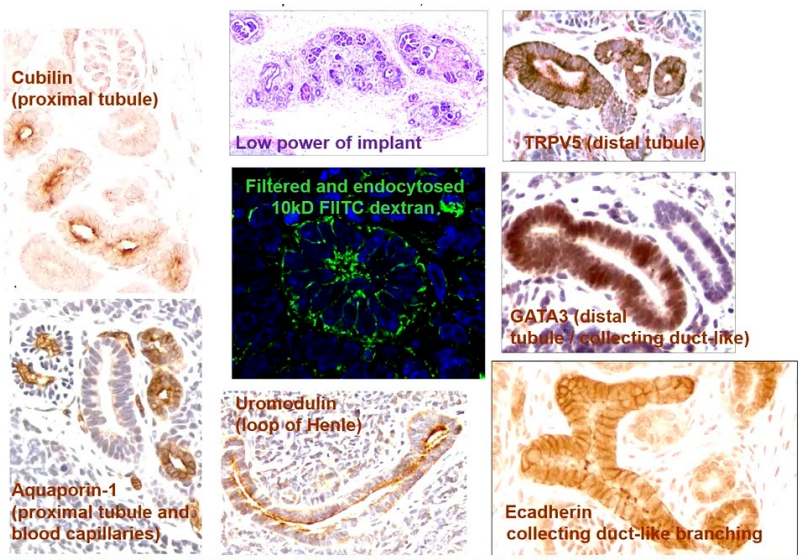

Figure 2. Diverse tubules differentiated from hPSC-derived kidney precursors that had been implanted into mice. Adapted from Bantounas I et al 2018.

Compared to typical adult kidneys, implants face additional challenges due to their reduced size. Firstly, they are connected to the host organism via small capillaries and arterioles, resulting in insufficient pressure for glomerular filtration. Secondly, implants lack ureters, thus hampering the creation of both a functional ureter and a nephron-rich kidney.

The implantation of precursor cells into the thighs of mice next to the femoral artery and vein is one of the novel methods developed by researchers to resolve the lack of an artery. This technique produces a more vascularized and larger implant, but no renal artery growth within the implant. The problem of the ureter is being addressed by producing ‘chimeric’ human nephron organoids in 3D culture alongside embryonic mouse ureters. In this context, the mouse ureter formed a urothelium and smooth muscle, and human nephrons differentiated around the ureter. Interestingly, in this

system, the ureter appeared to stimulate the expansion of human nephrons, although there was no apparent connection between the human nephrons and the mouse ureter.

Pluripotent stem cells to understand the mechanisms of disease and test novel therapies

Novel research in the field of pluripotent cells in nephrology focuses on unraveling kidney disease mechanisms and reproducing disease models in a laboratory setting. One area of study is the prevalence of dysplastic kidneys among children with severe kidney failure. It appears that as many as half of all children with severe kidney failure were originally born with malformed kidneys in which multiple immature tubules and glomeruli formed during further development. Studies have shown that gene mutations, particularly related to transcription factors, that are expressed during normal kidney development, can, under certain circumstances, lead to the formation of dysplastic kidneys.

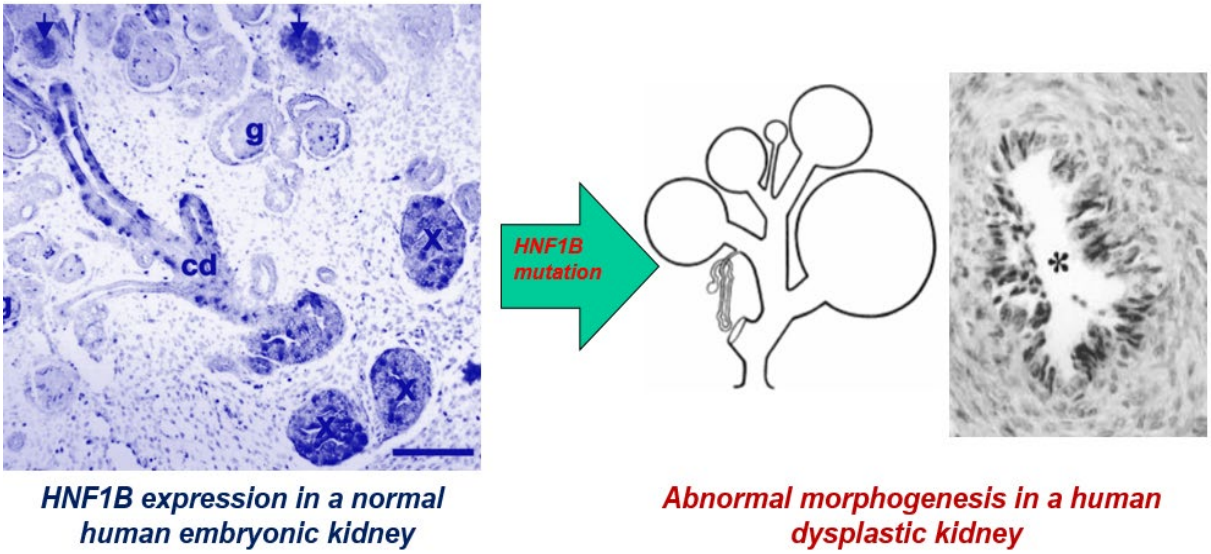

Figure 3. HNF1B mutation causing dysmorphic kidney tubules.
Adapted from Kolatsi-Joannou et al 2001 and Bingham C et al 2002.

A specific example of this is seen in families with an autosomal dominant inheritance of kidney dysplasia, where mutations in the transcription factor hepatocyte nuclear factor-1beta (HNF1β) have been identified as the most common. Affected children are born with renal cysts and may later develop diabetes mellitus, tubulopathy with renal magnesium wasting, and acute gout. HNF1β is expressed in many tubules during normal embryonic kidney development in humans, and the focus is on utilizing human stem cell technology to elucidate the association between HNF1β expression and normal kidney development. Research to create induced pluripotent stem cells from families with HNF1β mutations has revealed that HNF1β heterozygous mutant organoids have fewer but larger internal components compared to organoids derived from unaffected relatives. To achieve the goal of driving dysplastic tissue towards a more normal differentiation pathway, studies are comparing RNA panoramas of mutant organoids to wild-type organoids and identifying differentially regulated genes.

Key points

1. Human pluripotent stem cells (hPSCs) can be utilized to make kidney cells (that may in future be used for regenerative renal therapies) and to create models of kidney disorders to study disease causes and test new treatments.
2. The development of methods to trace hPSC-derived kidney precursors that formed functioning nephrons in vivo is critical step toward using hPSCs to model and treat kidney diseases.
3. The small scale, the lack of renal artery, and lack of an integrated ureter are potential problems to be tackled in relation to creating a holistic functioning kidney and lower urinary tract.
4. New technologies show promising results in the analysis of several genetic diseases. Future investigation should explore whether acquired renal diseases could be modeled as well.

Further reading

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