Human pluripotent stem cells for modelling Alport disease

Summary

Chronic Kidney Disease (CKD) is characterised by the progressive loss of podocytes, the glomerular epithelial cells responsible for the filtering of the blood in the kidneys. Currently CKD affects a large proportion of the human population (12% approx.), and its incidence is likely to increase in the future as a result of ageing, the increase in type II diabetes and high blood pressure. CKD can lead to End Stage Renal Disease (ESRD) where the only available treatments are dialysis and kidney transplantation. The latter is insufficient to meet the world demand, while the former replaces only a small percentage of kidney function at the expense of a dramatic decline in patient's quality of life, and at a high cost for the healthcare system, making it unsustainable even in developed countries. Alport nephritis is a genetic form of CKD that has been shown to be particularly important in Cyprus, where several mutations in genes coding for collagen type IV have been identified. In order to understand Alport disease in more detail and to investigate new pharmacological agents, it is necessary to develop more accurate disease models that could be used for basic research and as drug screening platforms in a human context. In our work we aim to establish a 3D culture system of kidney organoids differentiated from human pluripotent stem cells. Once we optimize the differentiation conditions, podocytes and organoids derived from Alport and control patient stem cells will be compared to investigate the early features of the disease *in vitro*.

To facilitate better monitoring of the differentiation process and develop a protocol that can replicate the normal developmental niche of podocytes *in vitro*, we will insert a genetic tag in human induced pluripotent stem cells (hiPSCs) using a new genome editing technology. This is the first attempt in Cyprus and in the world to model Alport nephritis using human kidney organoids derived from hiPSCs. Apart from helping to understand the disease, we hope our work will awake the interest of researchers and students about the use of hiPSCs for studying kidney diseases at the University of Nicosia and in Cyprus in general.

The promise of human pluripotent stem cells in regenerative medicine of the kidney

The kidneys are the filtering units of the body that eliminate the waste products produced by the cells. Key players in this process are the podocytes, highly specialized cells located in the kidney glomerulus. Their function and complex structure composed of hundreds of "foot-like processes" make these cells unable to regenerate in the adult. Thus, the progressive loss of these cells can lead to CKD. As mentioned earlier, many underlying causes of CKD are on the rise which indicate that the problem will become worse in the future. This constitutes a major problem not only for the patient, who will end up with ESRD, but also for the health care system which will be affected by the economic burden. Alport nephritis is a genetic form of kidney disease caused by mutations in the *COL4A3, COL4A4* and *COL4A5* genes that codify for the collagen type IV matrix of the glomerular basement membrane (GBM), where the podocytes attach. How these mutations lead to the cell death of podocytes is not completely clear, therefore it is imperative to develop accurate models to investigate new therapeutic strategies for this and other forms of CKD. In Cyprus, many patients originally diagnosed with thin basement membrane nephropathy, a non-progressive disorder, were actually carriers of new mutations that cause Alport disease (Voskarides *et al* 2007; Demosthenous *et al* 2012), making the need to address this health problem at the national level. In this scenario,

human pluripotent stem cells are ideal for modelling these diseases because of their unlimited self-renewal and their ability to differentiate into any somatic cell type. There are two types of pluripotent stem cells: human embryonic stem cells (hESCs) derived from human embryos (Thomson *et al* 1998), and human induced pluripotent stem cells (hiPSCs) artificially reprogrammed from adult cells into an embryonic pluripotent state by the forced expression of a set of transcription factors (Takahashi and Yamanka 2006; Yamanaka *et al* 2007). hiPSCs derivation is a breakthrough in biomedical research because it overcomes the need for using human embryos for the isolation of pluripotent stem cells, meaning that these cells could be available to any lab in the world. Second, for cell replacement therapies, it is ideal to use the patient's own cells to avoid immune rejection of transplanted tissue. Finally, the reprogramming of somatic cells means that we can have as many hiPSC lines as there are different patients, and we will therefore be able to study a wide variety of disease genotypes *in vitro*, bringing closer the possibility of personalised medicine. Thus, in our work we will exploit this valuable tool to model a complex disease like Alport nephritis. It should be noted that so far no group in Cyprus has used hiPSCs to study kidney diseases, something that highlights the importance of our work even more.

Genome editing in human cells

One of the key milestones in our project is the genetic modification of hiPSCs. It is worth emphasising that one of the drawbacks when using hESCs and hiPSCs is the difficulty of permanently modifying their genome. The process of homologous recombination, by which an exogenous DNA is incorporated into a genomic region of sequence homology, is extremely rare in these cells compared with those of the mouse. In the last decade, there has been substantial progress in the delineation of refined growth conditions and in new genome editing technologies applied to hESCs and hiPSCs, expanding the horizons of stem cells research. One of these technologies, and the one we will be using in our project, is the CRISPR/Cas9 nuclease system. This gene editing technology is derived from a bacterial immune system mechanism against foreign viral RNA, which was adapted for the modification of mammalian cells in 2013 (Cong *et al* 2013; Mali *et al* 2013). In brief, the system requires a hybrid RNA sequence composed from bacterial RNA and a 20bp RNA sequence complementary to the target genomic location to be modified, and the Cas9 nuclease that will bind and cut the genomic DNA RNA complex. Once DNA is cut, the cell repair mechanisms stick them back together, but the process is highly prone to errors, making point mutations at the desired gene of interest. The CRISPR/Cas9 system has evolved to include variants for induced forced expression, repression and single-chain cuts. Together with stem cell culture techniques, the CRISPR/Cas9 repertoire is allowing researchers across the world to study gene function and "disease in a dish" in the human context. For our project, we aim to exploit this technology to insert genetic tags that will allows us to observe in real time when differentiation towards the podocytes is activated, thus helping us to improve the generation of podocytes with high efficiency. Only a few groups have generated these reporter hiPSC lines to study differentiation towards the kidney lineage (Araoka *et al* 2014; Takasato *et al* 2014; Sharmin *et al* 2016; Howden *et al* 2019), but the combination of two reporters has not yet been exploited. Thus, our project stands at the forefront of scientific research and the cell lines as well as the know-how to be generated will be shared with the scientific community in Cyprus.

Conclusion

What is the impact of our research? Until now, there have been only a few reports that use hiPSC to understand Alport disease (Chen *et al* 2015; Haynes *et al* 2018). Our innovative work will shed new

light upon the mechanisms of Alport disease. These insights could be also expanded to other forms of CKD in the longer term. What about the immediate impact? At the local level, this type of research is new to Cyprus. We expect that with our research we will foster interest in stem-cell research among students and Principle Investigators at UNIC and across Cyprus. In addition, we will generate tools useful for other research groups interested in studying kidney diseases. At a personal level, our aims are ambitious and will allow me to improve my expertise in emerging technologies. We are sure that you will find the research starting here at UNIC Medical School interesting and innovative, and we are looking forward to answering any questions you may have about our project.

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