Towards functional kidney organoids

Chapter 8

Impact

Social and economic relevance and target groups

The aim of this thesis was to create further understanding of human fetal kidney development and to use this knowledge to better understand and improve kidney organoids. Developments in kidney organoid culture are relevant to researchers and clinicians aiming to model diseases, test drugs and develop renal replacement therapies.[1] Yet, to be functional replicates of adult human tissue, kidney organoids still have to mature further in terms of their cellular and structural complexity and functionality. The knowledge generated in this thesis supports these aims by highlighting important differences between kidney organoid and fetal kidney development and providing suggestions for culture improvement.

In the long term, kidney organoids might be an alternative treatment for patients with kidney failure and could therefore mitigate the donor tissue shortage and the need for dialysis. Both dialysis and donor kidney transplantation are not long-term solutions for patients since they do not prevent the progression of chronic kidney disease. Accordingly, a high risk of death remains. The 10-year graft failure from deceased donors is 49.7% and for grafts from living donors, it is 34.1%.[2] At the same time, the donor shortage remains an unresolved issue and the high prevalence of patients with chronic kidney disease (>10% worldwide[3]) drives research for an alternative treatment. Self-organizing kidney organoids produce nephrons of micro-anatomic detail similar to human nephrons and therefore show unprecedented potential for transplantation to restore kidney function. The work of this thesis has brought more insight into the functional ultrastructure, cellular organization and importance of the culture environment in kidney organoids, thereby helping to progress the field further towards a functional graft.

Importantly, the target groups eligible for receiving organoids as a tissue graft still need to be better defined. Chronic kidney disease is a multifactorial disease in which high age, cardiovascular disease, uremia, diabetes and chronic inflammation can co-exist and this might affect the functionality and lifetime of transplanted grafts.[4] Accordingly, animal studies will be needed that validate organoid survival and functionality in hosts with comparable co-factors.

Products

Adapting the kidney organoid culture protocol according to suggestions made in this thesis will likely lead to organoids with more physiological morphology and may thereby increase nephron functionality. For instance, the reduction of glucose in the culture medium (**Chapter 4**) may prevent the emergence of features of diabetic nephropathy and result in cell types with physiological metabolism. In contrast, due to the identification of features of diabetic nephropathy in this thesis, the current hyperglycemic culture could be implemented as a model of early diabetic nephropathy to increase our understanding of the development of renal pathologies and allow *in vitro* testing of reno-protective treatments in diabetic patients.

Innovation

The research of this thesis focused mainly on the development of human fetal kidneys and human kidney organoids and is innovative in a variety of approaches. To date, kidney morphology has been mainly studied in rodents or adult human tissues. However, given the fact that stem cell–derived organoids are immature and the field focused on gene expression analyses, we provided deeper insights into the ultrastructural development of human fetal kidneys in **Chapter 3**. This knowledge is applied in **Chapter 4**, showing that the comparison to fetal tissue is essential to validate whether organoids develop correctly. As such, we could find that kidney organoids possess features of hyperglycemic cultures and early diabetic nephropathy, which the field has not found with different approaches over the course of the past decade. **Chapter 5** explains the technical details of tissue clearing on kidney organoids, a state-of-the-art method for large organoid models that enables cell type identification and assessment in 3D. Following up on this, **Chapter 6** uses this technology to quantify the irregular endothelial network in 3D, which to date succeeded in such detail only in actual vascular networks. Furthermore, the research of **Chapter 6** was innovative in that the impact of a lower oxygen concentration in kidney organoids had not been suggested or studied. In contrast, many organoid cultures struggle with a pathological hypoxic and necrotic core and we show that culture of kidney organoids in physiological hypoxia improves the vascular network.

Overall, these findings show that detailed microscopic assessment of organoids should be a mandatory part of organoid assessment and that age-matched controls (such as human fetal kidneys) can support the creation of better organoid models.

Implementation

There is an urgent need for alternative treatments for patients with chronic kidney disease since it affects more than 10% of the global population and is the leading cause of mortality worldwide.[3] Clearly, current treatments are not a long-term solution. This gap creates great opportunities for tissue-engineered products. The implementation of kidney organoids as a renal replacement therapy is therefore promising. However, extensive research is still required to translate into the clinic.

One challenge for clinical translation is related to the dimension of kidney organoids compared to human kidneys. An adult human kidney contains from 200,000 to 2.5 million nephrons[5] and, together with a second kidney, filter an average of 200 liters of blood every day. A kidney organoid is estimated to contain approximately 100 nephrons.[6] Consequently, 2,000–25,000 organoids with nephrons of equal functionality are required to fully replace one kidney, though some patients may benefit still from the equivalent of a partial kidney. To date, the engineering of human adult-sized kidneys *in vitro,* to achieve equal functionality, has not succeeded. Generally speaking, culturing tissues of centimeter-scale dimensions is hindered by the lack of nutrient and oxygen delivery through blood vessels. On top of that, the kidney is one of the most complex organs owing to its hierarchical anatomy, complex ultrastructure, and large number of distinct cell types. It is imaginable that researchers will need to develop ways to recreate kidney grafts of larger dimensions, such as bioengineering a framework into which kidney organoids can be integrated as functional units, thereby overcoming the struggle of culturing tissue with large dimensions. Indeed, recent research shows promise in the upscaling of organoids for clinical translation and automization of the culture. These are essential steps to reduce costs and increase culture robustness.

Another factor that is both an opportunity and a challenge is the cell source of kidney organoids. IPSC–derived kidney organoids generate the best replication of kidney

tissue today in terms of cellular and structural complexity. IPSCs also hold the promise to be applied as an autologous therapy, since they can be fabricated from patient's healthy somatic cells. The first clinical trial involving iPSCs was in 2014 and since then numerous trials are ongoing, showing the potential of this cell source.[7] However, chronic kidney diseases often affect the elderly. It therefore can be expected that the creation of reliable and safe iPSCs from these patients is not as straightforward as an age-dependent linear increase in mutations is known to occur throughout the culture.[8] Additionally, organoids successfully replicate genetic mutations limiting their applications for patients with hereditary kidney diseases. Furthermore, two of the Yamanaka-factors (*c-Myc* and *Klf-4*) used for iPSC generation are potent oncogenes[9] and cancer-associated mutations, such as in the *TP53* gene, have been shown to accumulate during iPSC culture[10,11]. IPSCs are also being investigated for their potential to serve as an allogeneic, off-the-shelf cell source for tissue-engineered grafts.[12] This will allow a quicker, more standardized and therefore more cost-effective treatment. Personalized cell therapies are extremely expensive, in the range of hundreds of thousands of dollars.[12] Therefore, off-the-shelf therapy is highly desired. Yet, additional modifications could be needed to avoid rejection of autologous grafts. It may require donor patient matching and the detection of major histocompatibility complex (MHC)-specific neoepitopes that are currently known to occur due to mutations of mitochondrial DNA during iPSC culture and differentiation[13], and might be an additional risk for rejection. Yet, results are conflicting regard the long-term protection from rejection when MHC-specific matching of iPSC–derived cells was performed in non-human primates.[14,15] Additional CRISPR-mediated gene editing could be desired to create hypoimmune iPSCs, for instance by inactivating MHC class I and class II genes whilst over-expressing CD47[16], to evade allogeneic immune responses. Clearly, iPSC–derived grafts in general are close to be used as a therapy, however iPSC–derived kidney organoid cultures still require improvements.

Regarding the two main culture modifications suggested in this thesis, their implementation is expected to be feasible in clinical and research laboratories. The suggested reduction in glucose from **Chapter 4** will require additional research to confirm the ideal concentration, after which the current medium can be exchanged.

The same is the case for the oxygen concentration as suggested in **Chapter 6.** Consequently, laboratories will need oxygen-adjustable cell culture incubators.

Overall, this thesis provides deeper insights into human nephrogenesis and, in comparison with kidney organoids, revealed challenges of the culture. While these challenges need to be addressed to generate functional grafts, considering the progress made since the invention of kidney organoids, and the body of knowledge building this field, the future developments hold great promise.

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